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ABSTRACT BOOK

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ABOUT EVER

EVER is a non-profit organisation. The aims of the Association are to encourage research and the dissemination of knowledge concerning the eye and vision by means of meetings, publications and exchange activities.

Membership is open to individuals of any nationality engaging in or having an interest in ophthalmic and vision research who meet the requirements established by the EVER Board. To become a member, a candidate shall apply to the General Secretary on the membership form. The application must be endorsed by one member and the applicant shall pay an application fee. If the candidate does not know any member who can endorse his/her application, he/she should attach a letter explaining the situation. The candidate becomes a member upon recommendation by the EVER Board and upon payment of the dues.

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The abstracts are arranged in order of their numbers in the programmebook. Abstracts of posters have three-digit numbers. They are grouped according to subject. Abstracts of oral presentations have four-digit numbers and are arranged per session. The authors are sole responsible for their contents.

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Cataract patients may be pre-operatively classified according to their risk of intra-operative complications

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Purpose: To devise a robust and reproducible system for pre-operatively assessing the risk of intra-operative complications in patients undergoing cataract surgery.

Methods: We prospectively assessed the risk of all patients undergoing phacoemulsification cataract surgery according to a newly devised system. The patients were pre-operatively allocated to one of four Risk Group according to the points of risk they accumulated using this system. Post-operatively, data were collected indicating the grade of the surgeon, whether a complication had occurred and, if so, its nature.

Results: 900 patients were assessed. The results confirm that the intra-operative complication rate is lowest in Risk Group 1 (4.65%) and increases progressively through Risk Group 2 (7.33%) and Risk Group 3 (13.85%) to peak in Risk Group 3 (31.82%), $p < 0.001$.

Conclusions: Our results suggest that candidates for cataract surgery can be assessed pre-operatively and allocated to a Risk Group. This allows for: 1) individualised counselling on the chances of operative complications, 2) meaningful comparisons between national complication rates and those of individual surgeons and 3) better selection of cases suitable for instruction.

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Spectral analysis of sclera using hyperspectral imaging

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Purpose: To use hyperspectral imaging to map spatial changes in the haemoglobin spectrum correlated with oxygen levels in the human sclera.

Methods: A xenon lamp coupled with a tuneable liquid crystal filter (VariSpec, VS-VIS2-10HC-35-SQ Cambridge Research Instrumentation) was used to illuminate the sclera with monochromatic light with 10 nm bandwidth. The sclera was imaged with a 12-bit digital camera with spatial resolution of 1344*1024 pixels (ORCA-ER, Hamamatsu). The system performed a spectral scan in the region 540-580 nm in steps of 5 nm. The optics was such that each individual pixel element corresponded to approximately 25 micron in the sclera. The relative reflectance spectrum of each individual pixel element was estimated against a standard white reference. The sclera of 10 human subjects were measured both using a telespectroradiometer (Photo Research, PR-650) and the hyperspectral system. The results were analysed in relation to the oxy-haemoglobin spectrum to distinguish oxy-haemoglobin from haemoglobin.

Results: It was found that a map of the oxygenation levels could be established and that blood vessels could be clearly identified on the basis of its spectral content.

Conclusions: Hyperspectral imaging can be useful to visualise and map oxygenation levels in the sclera.

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Chalazion therapy: Intralesional steroids versus incision and curettage in African black patients

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Purpose: To compare two methods of chalazion treatment in African black patients, namely intralesional triamcinolone acetonide injection and incision and curettage.

Methods: Fifty African black patients with chalazions were randomly divided in two groups. The first group (25 patients, 29 chalazions) received intralesional triamcinolone acetonide 5 mg/ml injection; the second group (25 patients, 33 chalazions) was treated by simple incision and curettage.

Results: Incision and curettage showed a higher success rate (100%) compared to the intralesional triamcinolone acetonide injection group (76%). In 44% of cases, two intralesional injections were necessary to obtain those results in the first group. Recurrence rate was 17% in the first group and 7% in the second group. During a mean of five months of follow-up, no post-injection ocular complication occurred in the first group while fistula was noted in the second group.

Conclusions: Intralesional corticosteroid injection and incision and curettage appear to be effective in managing chalazion in black African patients as reported in previous studies in Caucasian and Asian patients.

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Localization of endostatin in the human eye

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Purpose: For optimal light perception the eye has to meet some requirements. Light needs to reach the retina undisturbed through structures that have to remain avascular throughout life, while sufficient blood supply is necessary for nutrition and cooling. Angiogenesis and its inhibition is regulated by many growth factors. Recently endostatin, the NC1 fragment of collagen XVIII has been described as a potent inhibitor of angiogenesis. The purpose of this study was to investigate the distribution of endostatin in the normal human eye.

Methods: Twelve eyes of 10 human donors have been investigated by immunohistochemical methods. Specificity of the endostatin antibody has been confirmed by Western blot analysis. To study RNA expression of collagen XVIII PCR has been performed.

Results: On PCR we found expression of collagen XVIII in all ocular tissues. In contrast for endostatin staining was restricted to the corneal endothelium, the unpigmented ciliary epithelium and to the lens cells. Extracellular endostatin was located at the anterior surface of the iris, the entire lens capsule and the inner limiting membrane.

Conclusions: Our results suggest that there might be a barrier of endostatin around the anterior chamber and the vitreous to protect the inner compartments of the eye from vascularization under normal conditions.

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Expression of monocarboxylate transporter subtypes 1 and 2 in the rat anterior uvea

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Purpose: Several monocarboxylate transporters (MCTs) are known to exist with one of their functions being to transport lactate and pyruvate across cell membranes. These transporters have been described to exist in the retina/RPE complex in rats. It is also known that lactate is produced in large quantities by anaerobic glycolysis in the lens yet the level of this compound remains steady in the anterior chamber. The aim of this study was therefore to determine the types and localisation of MCTs in the anterior uvea.

Methods: Whole Wistar rat eyes were fixed for 24 hours in Davidson's fixative and processed for the immunohistochemical localisation of MCTs 1-4, using commercially available antisera. Discrete tissues (lens, iris, ciliary body, cornea) were also isolated and examined for expression of both mRNA species encoding for MCTs by real time and reverse transcriptase-polymerase chain reaction (RT-PCR) and for translated MCT protein products by electrophoresis/Western blotting. All data was compared with known expression of MCTs 1-4 in the retina/RPE.

Results: Positive signals for proteins and mRNAs for MCTs 1-4 were initially confirmed to exist in the retina/RPE. MCT1 mRNA and protein were also present in the iris epithelium while MCT2 protein and mRNA were in the cornea, iris epithelium and ciliary epithelium. Moreover, protein and mRNA signals for MCTs 3 and 4 were not detected in the anterior uvea. The differential expression of MCTs 1 and 2 in tissues from the anterior uvea was largely confirmed by immunohistochemistry.

Conclusions: MCTs 1 and 2 are differentially expressed in the rat anterior uvea. This probably reflects their specific transport properties for defined monocarboxylates such as lactate or pyruvate.

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7-ketocholesterol modulates intercellular communication through gap-junction in lens epithelial cells

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Purpose: Connexin43 is one of a group of integral membrane proteins that form intercellular channels called gap junctions. The objective of this study is to evaluate if oxysterols affect gap-junction intercellular communication.

Methods: Primary cultures of lens epithelial cells were incubated with 7-keto, 25-OH or cholesterol. The levels of Cx43 present in membranes were assessed by its insolubility in Triton X-100 and determined by western blot. Subcellular distribution of Cx43 following treatment with 7-keto was evaluated by immunofluorescence confocal microscopy. Gap junctional intercellular communication was evaluated by transfer of the dye Lucifer yellow. The importance of the C-terminal in subcellular distribution of Cx43 was evaluated by transfecting cells 293T with cDNA encoding GFP-tagged wild type Cx43 or two C-terminal truncated forms of the protein. The effect of 7-keto on the endocytic pathway was determined by TRITC-dextran uptake.

Results: 7-keto induced a partition of Cx43 to Triton X-100 insoluble fraction, when compared to control cells or cells incubated with 25-OH or cholesterol. Accordingly, immunofluorescence analysis showed that 7-keto induces an accumulation of Cx43 in the plasma membrane and an increase in the intercellular communication through gap junctions, with no effects on the endocytic pathway. These effects are not dependent on the C-terminal of Cx43, since truncation of C-terminal did not affect distribution of the protein following treatment with 7-keto.

Conclusions: 7-keto induces an increase in the intercellular communication through gap junctions, that is probably due to an increased stability of protein in the plasma membrane.

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Lens epithelial repair after in vitro UVR exposure is dependent on the size and the region of the exposed anterior surface area

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Purpose: To further investigate how cataract develops after UVR exposure to different regions of the lens anterior surface.

Methods: Rat lenses in salt solution were exposed to UVR-B. The lenses were placed on buttons and washers with varying diameter, allowing for either central or peripheral blocking of the radiation. After exposure, the lenses were cultured in standard tissue culture environment for one week, and the degree of cataract was quantified daily by measurement of the amount of forward light scattering.

Results: The exposed epithelial region immediately exhibited a frosty appearance, which after one day was replaced by larger granules. Lenses with exposed equator developed cortical cataract within a few days. If the equator was spared from the radiation, the cataractogenesis was dependent on the size of the central concentric zone. With an exposed area of 50% or less, excluding the equator, both the size and the severity of the epithelial damage was macroscopically reduced during the week.

Conclusions: The peripheral epithelium is more sensitive to UVR than the central epithelium. Macroscopic repair of the lens epithelium occurs if the UVR-exposed area is sufficiently small and excludes the periphery.

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7-ketocholesterol induces differentiation of lens epithelial cells

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Purpose: To test the hypothesis that oxysterols induce differentiation of lens epithelial cells (LEC) into fiber cells.

Methods: Primary cultures of lens epithelial cells were incubated with 7-ketocholesterol (7-keto), 25-hydroxycholesterol (25-OH) or cholesterol at 10µg/ml for 10 days. Cells incubated with b-FGF 100 ng/ml were used as positive controls for differentiation. The expression of differentiation markers including crystallins, p57kip2 and PCNA following treatment with oxysterols or b-FGF was determined by western blot. Differentiation into fiber cells was further confirmed by immunofluorescence using antibodies against crystallins and the nuclear dye DAPI.

Results: LEC incubated with 7-keto presented higher levels of p57KIP2 and crystallins, compared to controls cells or cells incubated with 25-OH or cholesterol. The levels of markers for differentiation increased with time of incubation for cells incubated with 7-keto while there was a decrease in the proliferation markers. Differentiation was further accompanied by loss of intercellular organelles such as nuclei.

Conclusions: Results presented in this report show for the first time that 7-keto induces differentiation of lens epithelial cells and inhibits cell proliferation. The mechanisms whereby 7-keto induces LEC differentiation remains unknown. 7-keto may disrupt the highly regulated differentiation programme of the lens, thus compromising its transparency.

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Expression of CXCR4 Chemokine Receptor and SDF-1 in human corneal fibroblasts

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Purpose: The aim of the study was to investigate whether cultured human corneal fibroblasts express functional chemokine CXCR4 receptors on their cell surface and to determine the presence of its specific ligand, SDF-1a (CXCL12), in corneal fibroblasts.

Methods: Human corneal fibroblast cultures were obtained using human donor corneas. CXCR4 receptors were characterized using binding studies and autoradiography with [¹²⁵I] SDF-1a. Functionality of CXCR4 receptors was assessed by intracellular calcium measurement using a dynamic imaging microscopy system. CXCR4 and SDF-1a were detected by means of the reverse transcriptase polymerase chain reaction (RT-PCR) and Western-blot analysis.

Results: [¹²⁵I] SDF-1a specifically bound to cultured corneal fibroblasts. The presence of CXCR4 was confirmed by autoradiography of the radioligand on slices of corneal stroma. SDF-1a induced a rapid and transient intracellular calcium increase in cultured corneal fibroblasts that was blocked by the specific antagonist bicyclam. Moreover, a 48 kDa-protein was detected by Western-blot analysis of corneal fibroblast extracts, using a specific CXCR4 polyclonal antibody. Reverse transcriptase polymerase chain reaction showed the expressions of both CXCR4 and SDF-1a mRNAs in human corneal fibroblasts.

Conclusions: These results indicate for the first time that cultured human corneal fibroblasts express the chemokine receptors CXCR4 and its ligand SDF-1a.

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Calpain inhibitor III reduces retinal ganglion cell death in a model of intravitreal excitotoxicity

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Purpose: In this study we evaluated the effect of calpain inhibitor III on retinal ganglion cell (RGC) loss in a model of acute excitotoxic stimulation in rats. Calpain-mediated signalling pathways have been suggested in the pathogenesis of neuronal cell death. Calpains represent a family of calcium-activated neutral cysteine proteases. They require higher than normal levels of calcium which leads to an uncontrolled activation and results in structural and functional alterations which are cytotoxic to neurons.

Methods: For all experiments Wistar rats weighing 250-300 g were used. Excitotoxic lesions were induced by intraocular injection of 20 nmol NMDA, an endogenous glutamate agonist. A second group received in addition intraperitoneal injections of calpain inhibitor III (50mg/kg in DMSO) 0, 3 and 6 hours after the intraocular NMDA lesion. 7 days after the excitotoxic retinal damage, RGCs were labeled with FluoroGold[®]. After additional 2 days eyes were enucleated and retinal whole mounts counted for FluoroGold[®]-positive RGCs.

Results: RGC density in control retinas without lesion was 1855 ± 502 RGC/mm², whereas NMDA alone treated animals revealed a RGC survival of only 258 ± 121 RGC/mm². Animals treated with calpain inhibitor III resulted in a significant increase of the surviving RGCs to 530 ± 355 RGC/mm². Controls with calpain inhibitor alone did not show any effect.

Conclusions: Calpain-mediated proteolysis plays an important role in RGC death due to excitotoxicity. The inhibition of calpains may offer neuroprotective effects after an excitotoxic lesion. However, additional mechanisms by which an increased intracellular calcium concentration leads to retinal ganglion cell death exist.

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Biocompatibility of a new material in the anterior chamber of rabbits

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Purpose: To evaluate the biocompatibility of a new material with magnetic properties in the management of proliferative vitreoretinopathy. The new material is a ferrofluid in silicone oil. The present study evaluate the biological response in the anterior chamber of the rabbit eye in order to use it in the future as a vitreous substitute.

Methods: We inoculated 6 rabbit's right eyes with 0.1 cc of silicone oil ferrofluid. The silicone ferrofluid was introduced in the anterior chamber using a 30 G needle. They were followed and sacrificed at different times, two of them three days later, other two seven days later and the remaining two thirty days later. We designed a control group with the same number of animals. We inoculated them with oil silicone and sacrificed them with the same time scheme.

Results: We did not find any change in the control individuals. We did not find any remarkable change in lens vitreous or retina neither in light nor in electron microscopy. We found free ferrofluid particles in the extracellular space as well as inside siderofagous. We found large amounts of iron pigment deposits in our case individuals. In those who were sacrificed after a three days follow-up period we found acute inflammatory changes in their corneal endothelium, trabeculum and ciliar body. In the other case individuals we found chronic inflammatory changes.

Conclusions: These results suggests that silicone ferrofluids may induce an inflammatory reaction when inoculated in the anterior chamber of the rabbit, related to the presence of free iron particles because of the degradation of the silicone ferrofluid. Further studies are needed to test ferrofluids's safety when used in vitreous and under the influence of a magnetic field.

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VIP- NPY- and SP- in choridal human innervation

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Purpose: To study the presence of sympathetic, parasympathetic and sensory nerve innervation in the human choroid.

Methods: Seven human choroids were processed as whole mounts by indirect immunofluorescence. These were double labelled to demonstrate the co-localization of neurofilaments (anti-NF-200), and neuropeptides (anti-VIP, -NPY, -SP).

Results: Two basic patterns of nerve fibre staining were observed depending on the antibody used: 1) anti-VIP and anti-NPY marked thin fibres that stained discontinuously; 2) anti-SP showed thick fibres of irregular calibre but continuous staining. In the suprachoroid, the long and short ciliary nerves and their branches were stained by all three of the neuropeptides used. Additionally, in this layer we observed SP-positive long-tract axons that were not stained by VIP or NPY. In the vascular layer, the three antibodies used marked two types of axons in relation to the large and medium vessels: paravascular and perivascular axons. VIP-positive ganglion cells were observed in the suprachoroid. These formed a delicate network with processes extending through the choroid and were either isolated or in groups of 2-10 cells forming microganglia.

Conclusions: Innervation of the human choroid is of three kinds: parasympathetic, sympathetic and sensory. These reach the choroid through the long and short ciliary nerves, which branch to form a vascular plexus composed of paravascular and perivascular fibres. In addition, intrinsic VIP-positive choroidal ganglion cells are present in the suprachoroid.

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Tight junctions proteins composition in the blood-retinal barrier in experimental diabetes

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Purpose: To establish a correlation between inner Blood-Retinal Barrier (BRB) breakdown and the content of the tight junction transmembrane proteins, occludin, claudin-1 and claudin-5 in retinal vascular endothelial cells (RVEC) in an experimental diabetes model.

Methods: Experimental diabetes was induced in the Wistar rat (n=16) by intraperitoneal injection of streptozotocin. Blood glucose and hemoglobin A1c levels were determined to assess the diabetic state. Vitreous fluorophotometry was used to confirm BRB breakdown. Tight junction proteins expression was determined by immunofluorescence of cryosections of the retina and quantified in retinal lysates by immunoblotting using specific polyclonal antibodies. A control group of normal rats was used (n=8).

Results: A decrease in occludin immunoreactivity was observed in RVEC in experimental diabetes. The occludin content was significantly reduced in the diabetic animals in relation to controls. The expression of the other tight junctions proteins belong to the multigenic family of claudins and specifically expressed in vascular endothelial cells was altered in the diabetic animals.

Conclusions: Protein composition of the tight junctions in the BRB is being characterized to understand the paracellular barrier properties in normal and pathological states. The alteration of the protein composition of the tight junctions in the inner BRB in experimental diabetes can explain its permeability behaviour. Similar studies in an experimental ischemia/reperfusion model are being performed and could point to a common BRB breakdown mechanism in vascular retinopathies. Phosphorylation mechanisms of occludin is being to be characterized and seems to be also implicated in the alteration of permeability.

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Identification of retinal stem cells and differentiating neurons in the human, monkey and bovine eye

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Purpose: Retinal stem cells have been recently identified in dissociated cell cultures from the ciliary body epithelium of adult rodents. These cells express the neural stem cell marker nestin and can generate in vitro all the cell types present in the mammalian retina. The purpose of this work has been the immunological identification of these precursor cells in the adult mammalian eye and the in situ visualization of cells in the process of differentiation into mature retinal neurons and glia.

Methods: Cryostat sections of the ciliary body and adjacent peripheral retina from adult humans, macaques and cows were subjected to double immunostaining with specific antibodies against nestin and a set of phenotypic markers of the different retinal neurons and Müller glia. Cells were then viewed under confocal laser-scanning fluorescence microscopy.

Results: We have immunologically visualized nestin-expressing cells in the epithelium of the ciliary body pars plana, as well as in the peripheral retina area adjacent to the ora serrata. In this retinal area we have found, in the three species studied, a number of morphologically-undifferentiated cells which nonetheless express specific markers for the different mature neuronal cell types studied, i.e. photoreceptors, bipolar, horizontal, amacrine and ganglion cells, as well as Müller glia.

Conclusions: The far peripheral retina constitutes a region where retinal stem cells, present in the ciliary body of mammals, including humans, differentiate into the distinct neuronal and glial types of the retina. This process is likely to take place in adult animals with cell renewal purposes. Support: Generalitat Valenciana CTIDIB/2002/146 & Universidad de Alicante GR02-11.

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The effects of nicotine on retinal pigment epithelial cell function

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Purpose: This study investigates the effects of nicotine exposure on rpe cell functions such as vegf expression, phagocytosis and permeability.

Methods: Arpe-19 cells were plated on 24 well plates and grown to confluence. Cells were then exposed to nicotine @ conc of 10-6 to 10-8m in complete medium for 6, 12, and 24 hours. Supernatants were collected at these time points and vegf release quantified using elisa. Intracellular vegf protein was assessed by western blotting. Cell viability was assessed using an ldh cytotoxicity assay (roche diagnostics) and the aqueous one solution cell proliferation assay (promega). Bovine photoreceptor outer segments were isolated, labelled with fitc and fed to rpe cells that had been exposed to nicotine for 12 hours @ a conc of 2 x 10-7 particles /ml. After 12 hours these cells were trypsinised and phagocytosis quantified using flow cytometry (cell quest) to measure the mean channel fluorescence per sample. Rpe cells were grown on transwell inserts coated with laminin for the permeability experiments. The tear (transepithelial electrical resistance) was measured using endohm chambers (world precision instruments).

Results: Acute exposure to nicotine resulted in a decrease in vegf release and intracellular protein expression. Phagocytosis of photoreceptor outer segments was increased @ a conc of 10-7m nicotine. The permeability of the rpe monolayer was not altered at these concentrations of nicotine.

Conclusions: Decreased vegf levels may contribute to an atrophic rpe layer while increased phagocytosis may increase the level of oxidative stress on the rpe. The effects of acute and chronic nicotine exposure on the rpe may be quite different and therefore further studies are in progress.

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EGF induces neuron formation in retinal progenitor cultures

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Purpose: The generation of neurons from retinal progenitors (RPC) is a promising tool for the treatment of retinal degeneration. Here, we explore the influence of epigenetic factors on retinal progenitor differentiation and the generation of neurons. The present study aims to reveal the RPC fate under EGF stimulation.

Methods: We have isolated retinal progenitors from postnatal DBA mice. Progenitors were grown in EGF and FGF-2 and were passed 3 to 11 times before inducing differentiation. For the induction of differentiation, 50'000 single cells were transferred into a poly-ornithin substrate with or without laminin.

Results: Prior to differentiation most RPCs (60 to 90%) expressed nestin. Upon addition of EGF (20ng/ml), up to 7% of cells presented neuronal morphology and expressed beta-tubulin, an early neuronal marker. Then short-term (2-4 hours) or long-term (7-11 DIV) exposures to EGF were undertaken in order to identify the function of EGF: differentiation versus proliferation. Short-term stimulations are sufficient to induce neuronal differentiation: 11.5% of the cells were b-tubulin positive; whereas with long-term stimulations few b-tubulin positive cells were observed but with a complete mature morphology. Laminin also appears to be essential for neuronal differentiation. On the other hand, FBS alone or in addition to EGF completely abolishes neuronal formation.

Conclusions: It appears that EGF is not only a factor controlling glial fate as it was previously shown, but also a potent inducer of retinal neuron formation. EGF short pulse is sufficient for neuronal formation but its sustained action is necessary to obtain mature morphological neurons.

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Effects of intraocular injection of a low concentration of zinc on the rat retina

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Purpose: To investigate whether intraocular injection of low concentrations of zinc aids the survival of rat retinal ganglion cells after excitotoxic (NMDA) and ischemia/reperfusion injuries. Also to determine whether low amounts of zinc cause any detectable retinal toxicity.

Methods: Rats were injected intravitreally with zinc, NMDA, or NMDA plus zinc and killed after 8h or 7 days. Other rats were injected intravitreally with zinc, 1h or 3 days before receiving ischemia (IOP raised to 125mm Hg for 45 min) and killed after 7 days. Physiological evaluation of retinal injury was achieved by measurement of the ERG. Retinal toxicity was assessed by analysis of the levels of COX-2, iNOS, GFAP, FGF-2 and CNTF and of the ganglion cell-specific markers NF-L and Thy-1, using a combination of RT-PCR, Western blotting and immunohistochemistry.

Results: NMDA caused a substantial reduction in the mRNA levels of Thy-1 and NF-L. Co-injection of zinc neither diminished nor exacerbated the effect of NMDA. Ischemia/reperfusion caused a significant decrease in the levels of Thy-1 and NF-L mRNAs and a reduction in the b-wave amplitude of the ERG. These effects were not counteracted by zinc. Intraocular injection of NMDA caused a marked loss of the ganglion cell markers Thy-1 and NF-L and upregulations of COX-2, iNOS, GFAP, FGF-2 and CNTF. Interestingly, injection of zinc caused no changes in Thy-1 and NF-L, COX-2 and iNOS, yet elicited small upregulations in levels of FGF-2, GFAP and CNTF.

Conclusions: The results of this study suggest that a defined low concentration of zinc is neither toxic to the retina nor counteracts retinal injury caused by NMDA or ischemia/reperfusion. Changes seen in GFAP, FGF-2 and CNTF by zinc are thought to reflect mild activation of glial cells.

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Lead-induced microscopic changes in Retina

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Purpose: Detection of immediate and late histological changes in retina consequent on lead administration.

Methods: Two equal groups of adult (G1) and newborn (G2) albino rats were used. They received daily injections of lead oxide intraperitoneally (4 and 2%) for (2 and 8 weeks) respectively, to study immediate and late effects on retina. Specimens were examined by light and electron microscopy.

Results: Lead induced retinal changes were more obvious in growing than adults. It affected retinal pigment epithelium, photoreceptors and Ganglion cell layer as well as blood vessels.

Conclusions: Lead toxicity results in serious effects on retina leading to atrophy. Such changes are more profound on growing retina of newborn.

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Effect of VEGF on tight junctions in primary retinal endothelial cells

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Purpose: Elevated levels of Vascular Endothelial growth Factor (VEGF) is detected in the vitreous of eyes with diabetic macular oedema. VEGF is a growth factor with angiogenic and vasoactive potential and is thought to be central in the induction of diabetic macular oedema. Exposure of vascular endothelial cells in vitro to VEGF has been shown to increase permeability but little is known about the underlying mechanisms. Key components controlling permeability of endothelial cells are intercellular adherens and tight junctions. We were interested whether VEGF regulated endothelial permeability by modulating junctional integrity.

Methods: Primary microvascular endothelial cell cultures were treated with VEGF for various times and the subcellular localisation of a panel of junctional proteins determined by immunocytochemistry.

Results: VEGF treatment affected the subcellular distribution of many junctional proteins: It induced either their disappearance (for occludin and claudin 5) or their relocalisation from the junction to the cytoplasm (for catenins and ZO-1).

Conclusions: Our work suggests that elevated levels of VEGF might contribute to the pathogenesis of diabetic macular oedema by negatively regulating the intercellular junctions of retinal endothelium. Deciphering the underlying mechanisms may open novel therapeutic avenues.

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A binocular stereoscopic beamsplitter for slitlamp

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Purpose: Slitlamp biomicroscopy of the eye is an art; beginning ophthalmologists have to learn the artifices of this diagnostic tool. Some slitlamps nowadays are equipped with a video camera, however video images are less than ideal for teaching this technique. A binocular stereo co-observation tube is much preferable because it involves a more active participation by the observer.

Methods: We designed a novel binocular stereoscopic beamsplitter which fits on Zeiss slitlamps. The beamsplitting prism system is uncomplicated and results in a more compact device than previous models.

Results: Residents in training as well as staffmembers favourably appreciate this device. It is also useful when complex proceedings require the active participation of more than two hands.

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Improved technique of vapour-pressure osmometry for small tear volumes

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Purpose: We found the Wescor 5500 vapour-pressure osmometer gave irregular and anomalously high results with small tear samples (less than 2ul), and this seemed to depend on the lens-tissue discs supplied for the 2ul sample cup. We tested several disc materials for evenness and accuracy of performance.

Methods: 3mm discs were punched from lens tissue (LT), Whatman No. 1 paper (WP) and Millipore 0.2um filters (MP). The Wescor was calibrated using each disc type, and a 290 mOsm/kg standard was used throughout, for sample sizes between 2.0ul and 1.0ul. Broken pieces of Whatman Anodisc 0.2um filter (AP) of similar size were also tested.

Results: The mean reading for each paper type was 290 mOsm/kg but standard deviations for 2.0ul samples were: LT, ± 23.1 ; WP, ± 8.3 . On reducing sample size, SD for LT remained high at ± 19.5 for 1.5ul and ± 24.3 for 1.0ul, while WP showed a SD of ± 16.8 at 1.5ul rising to ± 19.4 at 1.0ul. MP showed an extremely small SD of ± 3.0 at 1.5ul rising to ± 16.3 at 1.0ul, while AP gave an SD of ± 29.0 at 1.0ul. If calibrated using WP, mean values using LT rose as sample size was reduced: 325, 385 and 376 mOsm/kg at 2.0, 1.5 and 1.0ul respectively.

Conclusions: The variation in errors with sample volume suggests that the Wescor Osmometer must be calibrated using the correct paper disc type. Use of LT tends to give higher results, but these may still be within the range of calibration readjustment of the instrument except at sample sizes below 1.0ul. The fibre distribution in individual LT discs suggests that LT has too variable a structure, and MP would be a better standard disc material. Anodisc is too friable for easy handling.

■ 123

Where does ARK-700A actually measure peripheral corneal curvature? A comparison with MEDMONT videokeratoscope

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Purpose: Modern auto-refractors are available in most eye-care clinics, some of them including the ability to obtain central and peripheral measurements of corneal curvature as well, auto-keratometers (AK). The aim of this study was to find a correlation between peripheral measurements taken with the AK and videoqueratoscope (VKS).

Methods: Central and peripheral corneal curvature and eccentricity were measured in orthogonal meridians with AK and VKS in 122 eyes from 61 university students (23 \pm 4 years or age). Peripheral curvature obtained with VKS at 2.0/2.25/2.5/2.75 and 3.0 mm were compared with peripheral measurements taken with AK to find the best correlation between both instruments. The narrower 95% confidence interval (95%CI) and the closer to zero mean difference (mean diff.) were used as criteria.

Results: VKS give higher values for corneal eccentricity (0.56 \pm 0.10) than AK (0.48 \pm 0.10) being significantly different from zero ($p < 0.001$). Point to point analysis display closer agreement at 3.0 mm superior (mean diff.=0.28; 95%CI = \pm 0.27), 3.0 mm inferior locations (mean diff.=0.19; 95%CI = \pm 0.22), 2.5 mm temporal (mean diff.= -0.04; 95%CI = \pm 0.22) and 2.75 mm nasal (mean diff.=0.15; 95%CI = \pm 0.35).

Conclusions: The application of peripheral curvature measurements with AK is limited and not comparable with VKS measurements. However, consistency of measurements makes AK a valuable tool to detect peripheral curvature variations in many clinical situations.

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P2 receptors increase tear lysozyme levels in New Zealand white rabbits

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Purpose: Analyse the effect of UTP, Ap4A and INS365 on lysozyme levels in order to know if these substances can increase the amount of this protein in the tear film.

Methods: Lysozyme amount was obtained by measuring the inhibitory halos around a Whatman n°1 disc of 5 mm soaked in rabbit tears along 6 hours. Discs were put on petri dishes with Agar medium where *Micrococcus Lysodeitikus* bacteria was grown.

Results: All the tested substances have revealed an augment of tear lysozyme in rabbits. The results show a percentage of increase over the levels of basal lysozyme of 88% for UTP, 135% for Ap4A and 12.5% for INS365. Antagonists studies suggest that this effect is mediated through P2 receptors. The P2 antagonist receptors, suramin and PPADS, partially antagonize the effect of UTP and Ap4A respectively.

Conclusions: These substances could enhance the bactericidal activity of tear film by means of increasing the tear lysozyme protein in New Zealand white rabbits.

■ 124

The development of tear sampling and bioassay techniques for the study of contact lens induced variations in tear protein profiles

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Purpose: This study has been particularly concerned with the development of techniques to enable the monitoring of specific tear proteins such as lipocalin as well as providing an overview of changes in more commonly monitored proteins. The effects of contact lens wear and diurnal variation are examined with the aim of providing an insight into causes of contact lens induced discomfort and particularly end of day effects.

Methods: Tear samples were collected by conventional microcapillaries, and two further techniques. The first used the lens in a novel way as a probe to sample a tear envelope (as distinct from tear products deposited on the lens). The second involved heating the lens in an extraction solution for 3 hours at 90°C to remove the protein bound or associated with the lens - both on and within the matrix. Samples were subjected to one dimensional SDS-PAGE. Gels stained in Coomassie blue or subjected to a modified Western Blotting technique.

Results: A great deal of information collected using electrophoretic techniques is of limited value because of the difficulty in processing, analysing rationalising and storing data.

Conclusions: We have been able to exploit improvements in software to produce processed data of a high standard, which has made it possible to follow changes in individual patients and assess changes in target proteins within large groups of patients very effectively. This poster describes the techniques involved and illustrates their use in patient monitoring.

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Protein uptake study on Focus Dailies and 1-Day Acuvue based on ex-vivo analysis of lenses from A 12hr clinical trial

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Purpose: To assess the protein deposition profile of high water, non-ionic Dailies and high water, ionic 1-Day Acuvue contact lenses worn for 12 hours.

Methods: Surface protein deposition was measured using fluorescence spectrophotofluorimetry and total protein was measured using ultra-violet spectroscopy. The saline solution used to store the worn lenses was also analysed to measure the protein and tear envelop from the lenses.

Results: Dailies lenses had a low level of deposited protein (average 30 microgrammes/lens) and 1-Day Acuvue had a higher level of deposited protein (average 457 microgramme/lens). Average protein from the Dailies storage solution was 89 microgrammes/ml and for the 1-Day Acuvue was 196 microgrammes/ml.

Conclusions: The non-ionic lens had less surface protein and released less protein into the saline solution and the ionic lens took up more protein on its surface and in its bulk. Contact lens wearers who have ocular sensitivity or allergic reactions driven by protein deposition may benefit from wearing a non-ionic material.

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Continuous and daily silicone hydrogel contact lens wear: biochemical markers

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Purpose: This study investigated the effects of silicone hydrogel lens wear on tear protein composition and deposition. Many immunoresponsive proteins are present in the tears and these and other constituents of the tear film may provide predictive diagnostic markers of the aetiology of contact lens complications and clinical contraindications to contact lens wear. Changes occurring on introduction of biomaterials to the human body for the first time and changes occurring with time were studied.

Methods: This study combined clinically observed parameters with those taken under laboratory conditions and provided a unique and comprehensive perspective on patient tolerance and the biocompatibility of contact lenses over an eighteen month wear history.

Results: Differences between silicone-hydrogel contact lenses were examined and differences between daily and extended wear were observed. Seven marker proteins were analysed: IgA, IgG, lactoferrin, albumin, IgE, kallikrein and kininogen, the latter two are members of the kinin family with potent pro-inflammatory function. The clinical parameters and results are presented in the accompanying poster results obtained up until six months wear for all patients (n=47). The results presented compare the deposition profiles observed after 1 and 6 months wear and demonstrate distinct changes in concurrence with the clinical parameters performed.

Conclusions: Although none of these proteins could be regarded as being specific to one particular disease or adverse response, our findings in this study would suggest that their assessment may prove useful in the quantification of distinct events in contact lens wear.

■ 126

Frictional behaviour of contact lenses.

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Purpose: Biotribology is the study of the lubrication of biological surfaces. It is an important aspect of the interaction between contact lenses and the anterior eye and also reflects the in vivo behaviour of ophthalmic solutions. Currently no established in vitro measurements exist that reflect the differences in behaviour found in the eye. We have extensively investigated a technique to measure the coefficient of friction of contact lenses, under different conditions, since frictional behaviour and lubrication are directly related.

Methods: Measurements are made on the lens by placing it onto a convex slider. With the contact lens in place, the slider is in turn placed onto a substrate surface which may be varied to represent the surface against which the lens might move. A few drops of an appropriate lubricating solution are usually added to the substrate before the testing begins. The resistance to sliding is measured as the convex slider is dragged along the surface of the substrate. The resistance to motion is expressed in terms of the coefficient of friction μ .

Results: The difference between the static or start-up friction and dynamic friction is of great importance. During the start-up the greatest susceptibility to lubrication breakdown and adhesion occurred. The values of dynamic coefficient of friction obtained are very similar with all commercial soft lenses when used in conjunction with saline or an ophthalmic solution. The similarity in the values is a reflection of the ability of these relatively hydrophilic surfaces to sustain this hydrodynamic boundary layer.

Conclusions: Our most recent work reported here concerns dehydration and deposition behaviour of lenses and their ability to affect the hydrodynamic behaviour.

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Gender- and age-related differences in corneal topography pattern: preliminary results

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Purpose: To investigate gender- and age-related differences in the corneal topography of a normal population.

Methods: Topographic examinations were collected from 103 patients ranging in age from 18 to 74 years (average, 33.99 \pm 18.31 years). Data were segregated by gender and further divided into younger (less than 50 years) and older (50 years or more) age groups. Corneal curvature was evaluated at the centre and thirty-two peripheral locations in the vertical and horizontal meridians of the cornea by videokeratoscopy (Eyesys, TX, USA). The astigmatism pattern was determined and compared with respect to gender and age.

Results: In the younger and older groups, no gender-related differences in corneal curvature were found (ANOVA, p=0.16; p=0.78 respectively). The vertical corneal meridian, but not the horizontal meridian, showed statistically significant gender-related changes with aging (ANOVA, p<0.01). Older men had a significantly higher potential for against-the-rule astigmatism or decrease with-the-rule rate than women (ANOVA, p<0.01). In the younger group, no gender-related differences in astigmatism pattern were found.

Conclusions: Aging involves changes in astigmatic patterns in a different way in men and women. Age-related changes in axial ocular dimensions, as well as the decrease in levels of sex hormones, may play a role in gender-related changes in corneal structure with age.

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Gender- and age-related differences in corneal thickness: preliminary results

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Purpose: To investigate gender- and age-related differences in the corneal thickness of a normal population.

Methods: Pachymetric examinations were collected from 103 patients ranging in age from 18 to 74 years old (average, 33.99+/-18.31 years). Data were segregated by gender and then divided into younger (less than 50 years) and older (50 years or more) age groups. Corneal thickness was evaluated at the center and thirty-two peripheral locations in the vertical and horizontal meridians of the cornea using an Orbscan II system.

Results: No significant differences were found in central corneal thickness with gender. Significant differences in the thicknesses of central, midperipheral and peripheral cornea were found with age using analysis of variance ($p < 0.05$), except for the female central corneal position ($p = 0.251$). The horizontal corneal meridian, but not the vertical meridian, showed statistically significant gender-related changes with aging (ANOVA, $p < 0.05$).

Conclusions: The effect of age on human corneal thickness is still subject of research. This study suggests a tendency to increase corneal thickness with age. The strong evidence of a decrease on corneal hydration control with age appears to be the main explanation of such results.

■ 131

Modification of properties of corneal stroma by molecular cross-linking

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Purpose: To describe mechanical, morphological, metabolic and chemical changes in corneal stroma subjected to molecular cross-linking through treatment with Riboflavin and subsequent irradiation with 365 nm UV-light.

Methods: Stroma from human and pig corneas was treated with 1/10 saturated Riboflavin in 50 mM TRIS buffer pH 7.6 in 150 mM NaCl, followed by 30-60 min irradiation. Changes in tissue properties were studied by a variety of techniques, appearing in the results section.

Results: Treated stroma was mechanically stiff. It showed a reduced swelling capacity and pressure. The longitudinal stress-strain curve was altered. 2-D gel proteomics analysis showed a multitude of changes. Microscopy of swollen tissue revealed a condensed appearance with water clefts between lamellae but no intralamellar swelling. Treated human corneas were kept for 5 days in organ culture, after which period the endothelium appeared normal.

Conclusions: These preliminary experiments show that it is possible to modify the mechanical properties of the corneal stroma by a procedure compatible with continued vitality of the tissue. Clinical perspectives include stabilization of keratoconus cornea, reduction of stromal oedema in endothelial decompensation, control of post-graft astigmatism and development of optically stable epilenses for correction of ametropia.

■ 130

Protective role of corneal epithelium against UVR damage

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Purpose: It is known that corneal epithelium has an unique capability to absorb the biggest part of the UVR spectrum. The aim of the present study was to show the protective role of corneal epithelium against UVR damage by comparing the biological effects of UVR exposure on whole corneas and on de-epithelialized corneas.

Methods: Twelve New Zealand albino rabbit corneas were exposed to 280 nm UVR in 10-nm full wavebands at a dose that causes biomicroscopically significant keratitis (0.12 J/cm²). A half of them underwent manual de-epithelialization prior the UVR exposure. The control group of 6 rabbits underwent only manual de-epithelialization. Animals were killed 76 h after treatment. Corneas were stained with hematoxylin and evaluated by light microscopy.

Results: Corneas that underwent only the exposure to UVR showed loss of epithelial cells in the treated area. No damage on the keratocytes or the stroma was detected. Corneas that underwent the manual de-epithelialization showed loss of epithelial cells, and also keratocytes in the anterior one fourth part of the corneal stroma. However, corneas that were exposed to UVR after manual de-epithelialization showed very deep stromal damage. The keratocytes disappeared through the entire thickness of the stroma in the UVR exposed area.

Conclusions: The UVR at 280 nm alone do not produce any deep damage to the corneal stroma and keratocytes. Manual de-epithelialization causes disappearance of anterior keratocytes. However, the stromal damage caused by UVR in the de-epithelialized corneas is very deep. The corneal epithelium protects the deeper lying corneal structures against the UVR damage, probably by absorbing the biggest part of the UVR energy applied to the eye.

■ 132

Relationship between concentration of TGF-beta1 in tears and haze formation after PRK and LASEK for myopia

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Purpose: To evaluate and compare the release of tear fluid transforming growth factor-beta1 (TGF-beta1), refractive and visual outcomes, and grade of corneal haze after PRK and LASEK for myopia.

Methods: A prospective, comparative study was performed in 184 eyes of 92 patients who underwent laser correction of myopia (range -1.75 D to -7.5 D). In each patient, LASEK was performed in 1 eye and PRK in the fellow eye. The first eye treated and the surgical method used in the first eye were randomized. Grade of corneal haze, visual results (UCVA, BSCVA), refractive outcome and tear concentration of TGF-beta1 were evaluated and compared in both groups of eyes.

Results: The mean corneal haze level at 6 months was lower in LASEK eyes at 0.21 compared to 0.43 in PRK eyes ($P < 0.05$). The median TGF-beta1 release values postoperatively at day 1 were: 312.5 pg/min (range 121.7 to 1026.3 pg/min) for PRK and 169.7 pg/min (range 58.6 to 436.1 pg/min) for LASEK ($P = 0.032$). At day 2: 412.8 pg/min (range 123.8 to 1085.4 pg/min) for PRK and 175.6 pg/min (range 67.7 to 623.5 pg/min) for LASEK ($P = 0.0081$). At day 7: 85.4 pg/min (range 0 to 196.5 pg/min) for PRK, and 72.8 pg/min (range 0 to 142.1 pg/min) for LASEK ($P = 0.183$).

Conclusions: LASEK for correction of low to moderate myopia, provided significantly quicker visual recovery, reduced the haze level, as compared to conventional photorefractive keratectomy. Significantly lower amount of tear fluid TGF-beta 1 was released in the first 2 days after LASEK than in PRK. It coincides with the lower grade of corneal haze after LASEK than in PRK. This study confirmed a role of TGF-beta 1 in corneal wound healing after a surface laser refractive surgery.

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Role of mitomycin C in PTK retreatment for corneal haze and regression after myopic PRK

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Purpose: To assess the efficacy and safety of phototherapeutic keratectomy (PTK) with use of mitomycin C for retreatment of eyes with corneal haze and regression following photorefractive keratectomy (PRK) for medium and high myopia.

Methods: This retrospective study analyzed the refractive and visual results of 41 eyes (37 patients) who were treated by PTK with intraoperative application of 0.02% mitomycin C for corneal haze grade 2 to 3 and myopic regression. Retreatment was performed after 9 to 12 months following the initial PRK procedure. Mitomycin C was applied immediately after PTK with soaked microsponge over the ablated stroma for 2 minutes and then the cornea was irrigated with the chilled BSS. Refraction, uncorrected visual acuity (UCVA), best spectacle corrected visual acuity (BSCVA) and corneal haze after retreatment were evaluated. All patients were completed 12 to 24 months (the mean: 19.3 months) follow-up after retreatment.

Results: The mean BSCVA and UCVA before retreatment was 0.53 and 0.24, respectively. At one year after PTK with mitomycin C, the mean BSCVA and UCVA improved to 0.75 and 0.58 resp. The mean grade of haze before PTK was 2.61 and decreased to 0.45 at 1 year after PTK with mitomycin C ($P < 0.05$). No mitomycin C-related adverse effects and complications were found. An improvement of visual acuity was observed in all eyes.

Conclusions: PTK with intraoperative application of diluted mitomycin C 0.02% solution significantly reduced corneal haze and improved visual acuity in patients undergone previous PRK for medium and high myopia complicated by severe haze and regression.

■ 135

Determinating endothelial cell density in human donor corneas: a comparison of automatic software programs with manual counting

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Purpose: To compare commonly used software programs, the Endothelial Analysis System V1.4 (Rhinetec, Duesseldorf, Germany) and the NAVIS Cell Count Version 3.4.1 (Nidek Technologies, Erlangen, Germany), with manual counts of cell density in human donor corneas.

Methods: A total of 50 human corneas were analysed during organ culture. Photography was performed on conventional film and digitally on all corneas at the same time. Manual counting was performed on the conventional photographs by two observers. For each cornea, at least two different photographs were counted three times. The digital pictures were analysed automatically with the software programs Endothelial Analysis System V1.4 and NAVIS Cell Count Version 3.4.1. Analysis was repeated three times. Repeatability of manual and automatic cell counting were determined.

Results: The mean endothelial cell density obtained by manual counting was 2680 +/- 361 cells per mm² (+/- SEM). Values obtained by the digital analysis with the Endothelial Analysis System V1.4 were mean 1.9% higher (2713 +/- 280 cells per mm²). The difference was not statistically significant ($p < 0.05$). Analysis of the digital pictures with the NAVIS Cell Count Version 3.4.1 showed very similar results. Repeatability of automatic cell counting was very high.

Conclusions: Automatic digital analysis of cell density provided nearly equal results as manual counting, which is the gold standard. Therefore computer assisted digital analysis is a quick and reliable method to determine the corneal cell density during organ culture and before surgery. Results are highly repeatable and do not depend on the level of experience of the observer.

■ 134

Normal endothelial cell counts in the Pakistani population

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Purpose: To assess normal corneal endothelial cell counts in the Pakistani population. To compare Pakistani corneal endothelial counts with published normative data of Indian, Japanese and American populations.

Methods: 450 eyes of 225 patients (118 men and 132 women) mean age 45.43 years (range 20-70 yrs) underwent Konan nonconrobo SP6000 specular microscopy. Quantitative analysis was done using fixed frame methods. Statistical analysis was done using SPSS 10 and paired sample T-test compared endothelial cell counts between different populations. Cases with known endothelial risk factors were excluded.

Results: Mean cell counts of 2654 +/- 341 per mm² were observed in the Pakistani population. Mean endothelial cell counts decreased as age increased.

Conclusions: Similar endothelial cell counts were observed between Pakistani, American and Indian groups. Pakistani endothelial counts were significantly less than reported Japanese cell counts $p < 0.05$

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Prospective, randomized clinical and endothelial evaluation of two storage times for corneal donor tissue in organ culture at 31 degrees centigrade. Two years results

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Purpose: To compare the endothelial and clinical outcome of penetrating keratoplasty (PKP) with corneas stored in organ culture for up to 12 days (5 to 12 days, group 1) or more than 21 days (21 to 24 days, group 2).

Methods: Controlled double-blind trial. Storage durations were randomly assigned to the paired corneas, and endothelial cell density (ECD) was measured at the start and end of organ culture. Patients with low rejection risk and normal preoperative ECD were randomly assigned to one of the groups and underwent an 8.25 mm PKP (n=25 pairs). Follow-up at day 5, month (M) 1, M6, M12, and M24 covered central ECD and morphometry, graft transparency, visual acuity (VA), pachymetry, and complications. The primary endpoint was the central ECD at M12.

Results: At the end of organ culture, ECD of the short-stored corneas was higher by 273 cell/mm² (95% CI 178, 368; $p < 0.001$). One year after PKP, group 1 ECD was still comparably higher by 227 cell/mm² (95% CI 43, 411; $p < 0.018$). Two years after PKP, the difference was maintained: +201 cell/mm² (95% CI 8, 394; $p < 0.042$). Graft transparency, VA, pachymetry, and complication rate did not differ at any time, except for VA, which in group 1 was better at M1.

Conclusions: Shorter organ culture allows delivery of corneas with higher ECD. Recipients with normal ECD and low rejection risk retain this initial benefit two years postoperatively. This higher endothelial cell capital may prevent or delay late endothelial failure, the leading cause of failure in these recipients. We think that it is legitimate to prefer short storage for such recipients.

■ 137

Endothelial thermal effects in nonmechanical corneal trephination in rabbits using Q-switched Erbium: YAG Laser for penetrating keratoplasty

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Purpose: To assess thermal effects of Q-switched Er:YAG laser (2.94 µm) ablation of the corneal endothelium using slit masks in an animal model.

Methods: Laser trephination was performed on 28 rabbit eyes with the most favourable technical parameters we evaluated before (repetition rate 5 Hz, pulse energy 65 mJ, spot size 0.7 mm). We used metal slit masks placed onto the corneal surface. Using light microscopy after PAS staining of 8 µm sections we assessed the extension of endothelial cell loss. Additionally, two penetrating keratoplasties were performed to observe the endothelial regeneration in a rabbit eye model.

Results: The trephination depth was 65.4% (34 - 100%) over all. The endothelial cell loss (84.3 ± 124.0 µm) correlated significantly with the trephination depth (r=0.69, p<0.001). Sixty days after penetrating keratoplasty complete closed endothelial layer was observed.

Conclusions: Endothelial cell loss is more pronounced in nonmechanical corneal trephination using the Q-switched Er:YAG laser compared to the excimer laser. Although a complete closure of the endothelial layer due to wound healing can be observed we look for alternative laser sources like ultra short pulsed lasers to minimise thermal tissue damage.

■ 139

An atypical case of Fusarium solani keratitis treated with amphotericin B and voriconazole

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Purpose: To describe a case of resistant fusarium keratitis leading to loss of the eye, despite a microbiological response to antifungal therapy.

Methods: Case report.

Results: A 60 year-old woman developed a left, contact lens-associated keratitis following a hose splash in rural surroundings. The atypical keratitis did not respond to antibiotic, antiviral, steroid and anti-parasitic therapy. Corneal biopsy was negative for infectious agents but a subsequent penetrating keratoplasty yielded a positive culture for *Fusarium solani*. The keratitis progressed despite treatment with topical and systemic amphotericin B and topical pimaricin. A corneo-scleral graft was performed. An intra-cameral recurrence was treated with intra-cameral amphotericin B and systemic voriconazole. Poor visual prognosis, together with ciliary body shut-down and repeated loss of the anterior chamber led to evisceration of the eye. Histopathological examination and cultures of the eviscerated specimen were negative for fungal hyphae. A final episode in this patient's clinical course was the development of sympathetic ophthalmia in her fellow eye, which has been effectively controlled with systemic cyclosporin A and local and systemic corticosteroids.

Conclusions: Fungal keratitis should be considered in cases of atypical infectious keratitis in temperate countries. Systemic voriconazole may be considered in the treatment of ocular mycosis.

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High efficiency gene electrotransfer to the endothelium of human organ cultured corneas

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Purpose: Gene transfer to the corneal endothelium is a hopeful strategy to modulate cell functions through newly synthesised proteins. Gene electrotransfer is deemed safer and triggers dramatically less inflammation than viral vectors and is also reputed to be more efficient than other non viral vectors. Our aim was to develop an ex vivo gene electrotransfer to endothelial cells (ECs) of organ cultured human corneas.

Methods: Non-contact electrodes were custom-designed. Forty five human organ cultured corneas were transfected using a reporter gene coding for the β-galactosidase (0.5 g DNA/corneas). Eight pulses of 100 ms and 100 mA were delivered by the GET 42 generator. Negative controls were performed omitting the current pulses. The expression of β-galactosidase was assessed at 1 (n=10), 3 (n=20), 5 (n=10), 14 (n=5) days after electroporation using an image analysis software. EC toxicity was assessed by histological examination and detection of apoptosis by in situ TUNEL technique.

Results: All electroporated corneas carried positive cells. Transfection efficiency varied from 0.5 to 42 % of transfected ECs, mostly depending on initial EC density. Transfection rates were comparable in paired corneas. β-galactosidase expression began at day 1, peaked at day 3 and slowly decreased until day 14. Cell toxicity remained under 5-8% and apoptotic ECs did not colocalize with transfected ones.

Conclusions: We demonstrated for the first time the possibility to obtain a high efficiency gene electrotransfer to ECs of organ cultured human corneas, by mean of newly designed electrodes. Long term expression of the transgene remains to be investigated as is the efficiency yield with other genes.

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Impression cytology in limbal cell deficiency associated with K.I.D. syndrome

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Purpose: To describe the use of impression cytology as diagnosis and prognosis tool when dealing with patients with limbal stem cell deficiency (LSCD) in KID (Keratitis-Ichthiosis-Deafness) syndrome.

Methods: We report two cases of patients with K.I.D. syndrome: a 30 year old woman presenting with massive corneal neovascularisation who underwent two unfruitful penetrating keratoplasties in her left eye and a 16 year old boy with minimal corneal neovascularization.

Results: In the light of these two cases of limbal cell deficiency, we will discuss the contribution of impression cytology (Severity of LSCD being correlated with the presence of goblet cells) as well as ophthalmologic manifestations of KID Syndrome, its clinical characteristics, its physiopathology and its mode of transmission.

Conclusions: KID syndrome is a rare and as yet poorly recognized entity responsible for LSCD. Correct understanding of the physiopathological mechanisms may prevent unnecessary surgeries. Impression cytology is a simple tool in order to appreciate the severity of the disease.

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The ultrastructure of hypertrophic dendriform epitheliopathy.

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Purpose: To report the ultrastructure of hypertrophic dendriform epitheliopathy (HDE). A 51 year old woman underwent multiple lid surgeries and PKs following penetrating trauma. She developed a grey, elevated, dendriform figure resembling HZO keratitis which recurred despite multiple debridements, PKs and a limbal stem cell graft. Risk factors included dry eye and imperfect blinking. Culture and PCR for herpes simplex and varicella-zoster were negative.

Methods: Two epithelial and two penetrating keratoplasty specimens from of this patient were examined by electron microscopy. Tissue was processed for light and TEM.

Results: Striking intra-epithelial aggregates were found which were composed of electron-dense, microfibrillar deposits. The degenerate epithelium contained a dense cytoplasm and numerous microfilament and glycogen granules. Basement membrane contained long-spacing collagen and Bowman's layer was replaced by a collagenous pannus. Keratocytes within the pannus were degenerate, while collagen fibres were sparse and intermixed with microfilaments and long-spacing collagen. Large quantities of proteoglycans were present. Some of the keratocytes were degenerate and the lamellae disorganised. Descemet's membrane was arranged in multiple lamellae containing basement membrane material, long-spacing collagen and microfilaments.

Conclusions: We concur with Mannis et al. that HDE is a distinct syndrome occurring post-keratoplasty in patients with associated chronic inflammation and tear dysfunction. We speculate that it represents an aberrant form of keratinisation with secondary reactive stromal changes. Posterior changes were probably secondary to uveitis.

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Formulating adhesive hydrogels for use as return electrodes during iontophoretic ocular drug delivery

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Purpose: In recent years there has been an increased interest in non-invasive methods, such as iontophoresis, for the delivery of drugs to specific sites. Advantages of ocular iontophoresis, in particular trans-scleral iontophoresis used to treat infections of the posterior segment is preferable to intra-vitreous injections which may cause retinal detachment, vitreous haemorrhage and endophthalmitis.

Methods: In iontophoretic ocular drug delivery, the drug is applied to the eye by an electrode carrying the same charge as the drug. The return (ground) electrode has the opposite charge and is placed elsewhere on the body to complete the circuit. The main drawback of this system is the "prickling" pain sensation that occurs at the location of the return electrode at low current levels. In order to minimize this effect, the return electrode should provide a low impedance and current density path for the return current. If this is not satisfied, the "excess" current travels through alternate routes of lower resistance ensuing equally high local current density, which may cause, for example, damage to viable hair follicles or in extreme cases tissue heating and burns arising from resistive heating.

Results: Because this application is relatively new and is as yet employed on a modest scale, the devices have used conventional medical electrodes to complete the circuit. These are hydrogels that have some features in common with soft contact lens materials.

Conclusions: This poster discusses the factors in the design of these gels that could minimize impedance of the return electrode used in ocular drug delivery and improve the usefulness of this potentially valuable technique.

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Cultured limbal epithelial cells using preserved limbal tissue

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Purpose: Autologous cultured epithelial cells can restore damaged cornea. The techniques implied, however, are still being developed. We decided to produce and evaluate cultured epithelial limbal cells using limbal rings obtained from banked corneal limbal rings preserved in INOSOL™ at 31°C.

Methods: Tissues were harvested from dead donor corneas and preserved at 31°C in INOSOL™ during 20±3 days. Then, epithelial cells were obtained from trypsinized limbal rings usually discarded after keratoplasty (n=30). Epithelial cells were cultured in plastic dishes on a 3T3 feeder layer according to Rheinwald and Green (Cell. 1975; 6: 317-30). We measured Colony Forming Efficiency and kinetics parameters of cultured epithelial cells and defined their phenotype by immunostaining with monoclonal antibodies to keratins and mucins. We then made histologic sections of cultured epithelium.

Results: The culture was successful for 83% of the trypsinised limbal rings (n=12).

Colonies appeared in cultures after 7±2 days (n=12). For 100 epithelial cells cultured, the number of colonies greater than 50 cells was 0,44% ± 0,57% (n=6) after 12 days. We are not able to find any difference in expression of Muc1 and cK19 before and after culture but noted that cK3 and Muc5AC increased. Histologic sections showed a pluristratified epithelial dysmaturation.

Conclusions: Epithelial cells obtained from limbal rings preserved in INOSOL™ at 31°C can be expanded. We are now able to produce and compare cultured limbal epithelial cells on either amniotic membrane or fibrin sealant and to evaluate culture without xeno-cells.

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Hydrogel release systems in ocular & dermal drug delivery

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Purpose: Comparative uptake and release of actives from hydrogel and factors controlling this are influential in the use of hydrogels for drug delivery. Optimisation of controlled release systems is an important challenge in current pharmaceutical technology, particularly in the areas of ocular and dermal drug delivery. This study evaluates the influence of hydrogel structure and characteristics of the active on uptake and release behaviour, and investigation of modification of hydrogel structure in optimisation of these systems.

Methods: A series of conventional hydrogels and biphasic silicone hydrogel materials were used with marker molecules for actives of varying molecular weight, acidity and water-octanol partition co-efficient. A vertical diffusion cell system was used to measure the in vitro transfer rate of marker molecules from the hydrogel carrier vehicle into a receptor medium.

Results: Varying release rates coupled with similar kinetic profiles for all materials confirm the mutual importance of solubility and diffusion in the transport of a range of marker molecules. The relative hydrophilicity and hydrophobicity of marker molecules and their acidity/basicity was shown to inter-act with the structural nature of the hydrogels, identifying and quantifying these characteristics as controlling factors in drug release from hydrogels.

Conclusions: Many factors contribute to release behaviour of marker molecules through hydrogels. Manipulation of hydrogel composition controls the release scope of hydrogels as delivery vehicles for use in ocular and dermal applications. Understanding of delivery of lidocaine (for use in cataract surgery) and dermal delivery of ibuprofen is illustrated.

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Development of a Biomimetic Keratoprosthesis: ongoing Developments

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Purpose: Developing new synthetic analogues for corneal surgery is a particular and challenging requirement for use in corneal replacements or keratoprostheses. Despite the success of corneal transplants, there are still cases of corneal disease, such as Stevens-Johnson's syndrome and extensive chemical burns, where transplants will never succeed. For these patients the implantation of a keratoprosthesis represents the only potential for visual improvement.

Methods: Polymers commonly used in biomaterials applications today share one common feature: they have been developed for other commercial uses. A technique currently growing in importance and known as biomimesis seeks to use nature as a template. We have used biomimetic principles in this interdisciplinary project. The ideal keratoprosthesis would consist of two components, an optically transparent centre with an anterior surface and a porous peripheral skirt into which stromal fibroblasts (keratocytes) can penetrate and synthesise connective tissue, anchoring the synthetic material to the host tissue.

Results: Using the natural cornea as a model, several potential polymeric materials and device configurations have been synthesised and characterised for potential use in keratoprostheses.

Conclusions: Our recent work has been increasing the anisotropy of the synthetic structure to more closely mimic natural corneal tissue. By developing materials for corneal replacement based on biological templates it will be possible to achieve a higher rate of clinical success than is achieved with the materials currently available.

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A comparison of 0.3% Tobramycin Ophthalmic Solution (TOBREX2x) vs TOBREX Ophthalmic Solution (TOBREX) for treatment of bacterial conjunctivitis

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Purpose: To evaluate the efficacy and safety of topical TOBREX2x, a new viscosized formulation containing tobramycin compared to the marketed TOBREX for the treatment of bacterial conjunctivitis.

Methods: 276 patients, older than 1 year, clinically diagnosed with bacterial conjunctivitis. Pre-therapy cultures determined the aetiological agent(s) of the bacterial conjunctivitis. Patients were randomized to one of the two medications (TOBREX2x or TOBREX-1:1). Patients instilled one drop of TOBREX2x twice a day for 7+/-1 days. The primary efficacy variable was the percent of patients with sustained cure/presumed bacterial eradication (SC/PE) based on final clinical judgement at test-of-cure (TOC) visit (Day 12+/-1). Modified-Intent-to-Treat (MITT) group has been considered for comparison.

Results: The results demonstrated that in the MITT group, 94% of the patients treated with TOBREX2x and 90% of the patients treated with TOBREX were categorised as microbiological success (p=0.2423). There were no statistically significant differences between the two treatments for the final clinical judgement at the TOC visit (SC/PE: TOBREX2x 98%, TOBREX 97%, p=1.000).

Conclusions: TOBREX2x is as effective as TOBREX in the treatment of bacterial conjunctivitis. TOBREX2x administered twice daily is safe and well-tolerated in patients treated for bacterial conjunctivitis.

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Cross-infection and contact ophthalmic devices: the design & development of a disposable ophthalmic barrier system

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Purpose: his work addresses the problems of cross-infection associated with contact ophthalmic devices and proposes the use of a unique, inexpensive, sterile barrier system as a solution. The concerns raised are of particular importance given the recent recommendations from the Ministry of Health with respect to the potential of CJD transmission via ophthalmic devices and the potential risk of cross-infection e.g. Herpes Simplex, Prions. Established clinical standards of sterilisation, most commonly wiping with an alcohol swab, are inadequate and ineffective. There are several examples of contact devices that are susceptible to these problems, including: Goldman tonometer head Gonioscope lenses A-Scan ultrasound probes Ultrasound pachometry. None of the currently available solutions are popular, efficient or cost effective and are only concerned with tonometry.

Methods: This project is to design and develop a sterile, universal, ophthalmic barrier system, which is inexpensive, effective, adaptable to all contact ophthalmic devices and quick and easy to use.

Results: This comprises a disposable, optically transparent, laminate barrier consisting of two thin layers: one a barrier film that contacts the eye and the other a hydrogel adhesive that enables the barrier film to be readily and reversibly attached to the tonometer head or ophthalmic devices.

Conclusions: This project has defined a number of potential films for use as ophthalmic barrier systems.

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Molecular diagnosis in fungal keratitis

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Purpose: To contribute toward assessing the effectiveness of PCR and DNA typing as a rapid and sensitive method in diagnosis of fungal keratitis.

Methods: Fifteen corneal scrapings from patients with suspicion of fungal keratitis were taken for microbiological study, including classic cultures and PCR. Internal transcribed spacer 1 (ITS1) and ITS2 and 5.8S rDNA were amplified by PCR and semi-nested PCR. Amplified DNA were sequenced and aligned against sequences in GenBank at National Institutes of Health to identify the pathogen.

Results: The results were PCR positive for fungal primers for eight corneal samples, one of them was negative by culture. All the positive samples by culture were positive by PCR. The PCR result was obtained in 5 hours after the sample was taken. Cultures needed an average of 2 to 7 days to grow. The identification of the pathogen by DNA-typing showed *Aspergillus fumigatus*, *Alternaria alternata* (two cases), *Alternaria infectoria*, *Fusarium oxysporum*, *Candida albicans*, *Candida parapsilosis*, *Aspergillus flavus*. The molecular identification was confirmed by classical microbiological techniques.

Conclusions: Amplification of ITS-5.8S rDNA and molecular typing shows potential as a rapid technique for identifying fungi in corneal scrapings.

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Lamellar femtosecond laser keratoplasty with conical incisions and positional pikes

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Purpose: To report on lamellar femtosecond laser keratoplasty with conical incisions and positional pikes.

Methods: Using a femtosecond laser, an intrastromal corneal incision running parallel to the corneal surface was created in five freshly enucleated porcine eyes. In a second step, a circular conical posterior-anterior incision was performed extending from the peripheral edge of the first incision with a diameter of 8 mm to the corneal surface with a diameter of 7 mm. Additionally, four positional pikes were formed in the graft with corresponding notches in the surrounding tissue.

Results: For all eyes included in the study, the lamellar corneal buttons with the four positional pikes could be prepared and transplanted into the recipient beds of other eyes without major difficulties. The preparation of the lamellar buttons took less than 5 minutes in all cases.

Conclusions: Femtosecond laser technology allows the preparation of lamellar corneal grafts with conical incisions and positional pikes which may decrease postoperative astigmatism and increase postoperative stability of the graft.

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In vitro MDA cross-linking of human crystallins is age related

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Purpose: The aim of this work is to relate the aging and the structural changes of lens crystallins with their ability to link a lipid peroxidation product (Malondialdehyde).

Methods: Non-pathological clear human lenses (30-75 years old), obtained from an Eye Bank, were divided into cortex and nucleus by a sham surgical extracapsular approach. Nuclei were homogenated in a 50 mM phosphate buffer, 0.1 M NaCl, pH 6.8, and centrifuged at 15000 rpm for 15 min to obtain the water soluble fraction. Aliquots of the water soluble fraction were incubated with increased concentration of MDA (Malondialdehyde) at various times. The MDA linked crystallins were detected by a fluorimetric method.

Results: To investigate how the modification of the crystallins structure is aging related we have identified the experimental conditions where MDA is unable to link the intact structure of proteins (molar ratio crystallins:MDA = 1:20 for 24 hours). MDA cross-linking results increased in lens belonging to over 50 years old.

Conclusions: We speculate that the lens proteins structural change occurred with aging increases the MDA cross-linking mechanism involving the cataract development.

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0377, a mutation in the bB2- crystallin encoding gene (Crybb2) in mice

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Purpose: Genetical and molecular characterization of a dominant cataract mutation recovered in the offspring of parentally irradiated mice.

Methods: Genome-wide mapping, Micro-satellite genotyping, RT-PCR, DNA sequencing and Western blotting are the techniques used for this study.

Results: The mutation has been mapped to chromosome 5, between the markers D5Mit239 and D5Mit138, which is the region containing the Crybb1, Crybb2 and Crybb3 gene cluster. Sequencing of cDNAs of all three genes revealed a 57bp insertion at the beginning of exon 6 in Crybb2. The remaining two genes were not affected. Analysis of genomic DNA indicated the mutation to be due to an A to T bp substitution at the first position of the intron 5 acceptor splice site leading to inclusion of 57 bp from intron 5 into the cDNA. Western blot analysis showed the mutated protein in lens is around 1.9KDa larger than the wild type protein (23KDa). Transcripts of this mutated gene (with 57bp insertion) have also been detected in all regions of the brain. However, repeated Western blot analysis of the protein from brains of different animals of 0377 mutant line did not reveal any difference in band size when compared to the wild type.

Conclusions: The predicted mutated protein in lens contains a 19 amino acid insertion in the 4th Greek-key motif of the bB2- crystallin protein. It probably affects the folding of the protein, thereby building aggregates which disturbs the lens structure. This mutant is expected to play a significant role in determining the function of bB2-crystallin in brain when compared with wild type. Results of Western blot analysis of the protein from brain indicates some specific changes in the Crybb20377 transcript immediately prior to translation or some immediate post-transcriptional changes leading to the apparent same molecular weight of the protein as in the wild type form in brain.

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Effect of alcohol intake on cataract, Reykjavik Eye Study

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Purpose: To investigate the effect of alcohol intake on cataract in the Icelandic population 55 years and older.

Methods: The 1045 subjects who participated in The Reykjavik Eye Study (RES) in 1996 were reexamined 5 years later in 2001. 846 or 88.2% of the survivors participated again in the RES in 2001 and were selected as subjects for the study. Classification of the three main types of opacity was done with photographed images using the WHO simplified cataract classification and grading system. Retrodots (RD) were determined by slitlamp examination. Cases with any of the three main types of lens opacity grade 1 and over and those with pseudophakia or aphakia were judged as cataract. The details of current alcohol consumption were assessed by questionnaire. Alcohol consumption was characterized into lifetime abstainers, former drinkers, drinkers of less than one drink a month and those that drank more than twice a month. The latter were defined as drinker. Statistical analysis was performed using logistic regression.

Results: The proportion of drinker was 38.6%. There was no significant difference of the risk for cataract in drinkers compared with non-drinkers. A decreased risk of cataract was found in wine drinkers (odds ratio=0.57, 95% confidence interval (CI)=0.34, 0.94, p=0.031) and red wine drinkers (odds ratio=0.55, 95% CI=0.32, 0.92, p=0.026). No significant association was found for beer and spirits drinkers. The tendency for a decreased risk of nuclear cataract was found in wine drinkers (odds ratio=0.49, 95% CI=0.20, 1.06, p=0.090). No significant associations were found for cortical, posterior subcapsular cataract and RD.

Conclusions: This study supported the hypothesis that wine intake decreases the risk of cataract.

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Human lens capsule thickness as function of age and location along the sagittal lens border

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Purpose: To verify the anterior peripheral thickening of the human lens capsule (Fincham 1937) and to investigate if there is a change with age.

Methods: Altogether 21 human donor lenses in 3 age groups (45-55, 65-75 and 85-100 years) were fixed in 3.6 % buffered formaldehyde and paraffin embedded. Sagittal sections were stained for collagen with periodic acid-Schiff (PAS) stain. Capsule thickness was measured at 5 locations along the sagittal lens border (anterior pole, anterior periphery, equator, posterior periphery and posterior pole) using a Zeiss Photomicroscope, a 25x objective and a digital camera.

Results: Capsular thickness at the posterior pole (2 µm), posterior periphery (4 µm) and equator (9 µm) was not changing significantly with age. There was an increase in the thickness at the anterior pole from the youngest to the oldest age group (9 to 12 µm). Anterior peripheral thickening of the lens capsule was seen in all age groups and was increasing with age (10 to 14 µm).

Conclusions: The human lens capsule shows an anterior peripheral thickening. This thickening is constantly increasing above the age of 45 years. There is no change in capsular thickness at posterior pole, posterior periphery and equator for the investigated age groups. The given values of the capsule thickness are for tissue after fixation, dehydration and paraffin processing, which induces a shrinkage of about 50%. Therefore they are not real absolute values, but allow relative comparison between different ages and locations along the lens border.

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UVR-B induced cataract development in C57 mice

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Purpose: To investigate the evolution of morphological appearance and intensity of light scattering in C57 mice lenses after UVR exposure.

Methods: A total of 80 six weeks old female C57 mice were divided into 4 groups (n=20). One eye in each animal was exposed in vivo with UVR-B (300 nm) to a dose of 5 kJ/m² for 15 minutes. The radiation output of the UVR- source had MAX at 302,6 nm with 4.5 nm [FWHM]. The animals were consecutively sacrificed 1, 2, 4 and 8 days after the exposure. Light scattering measurements of the exposed and non exposed lenses were taken and morphological lens changes were documented using grid- and dark field illumination photography.

Results: The light scattering of the exposed lenses was higher on all investigated days (day 1, 2, 4, 8) compared to the non-exposed lenses. No significant variation of the difference of light scattering between the exposed and non exposed lenses was found during the time interval studied. Three different morphological types of cataract (anterior subcapsular, cortical, nuclear) were identified at all investigated time points.

Conclusions: A dose of 5 kJ/m² UVR-B (300 nm) induces light scattering in C57 mice lenses. The increase of light scattering, appears to be more variable than the time dependant light scattering changes within the investigated interval.

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The impact of repeated exposure on maximum tolerable dose for UVR induced cataract

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Purpose: Purpose: To investigate the impact of repeated exposure with different time interval on maximum tolerable dose (MTD) for ultraviolet radiation induced cataract.

Methods: Methods: A total of 100 six weeks old female Sprague-Dawley rats were randomly divided into 5 groups with different time interval between repeated exposure. The time intervals between the repeated exposure were 6 hours, 1, 3, 9 and 30 days respectively, with 20 rats in each group. Each time interval group was divided into 5 dose-subgroups, with 4 rats in each subgroup. One eye of each rat was exposed to ultraviolet radiation (300nm). The doses incidence on the corneas were 0 ~ 10 kJ/m² and exposure time was 15 minutes. One week after the second exposure the rats were sacrificed and both lenses were extracted. The intensity of forward light scattering was measured and morphological changes were documented by photographs under microscope with dark illumination. MTD of each time-interval group was estimated based on the experimentally determined dose-response function.

Results: Results: MTD for ultraviolet radiation induced cataract for the groups with time interval of 6 hours, 1, 3, and 9 days was estimated to be 3.0 kJ/m². Most lenses from the time interval of 30 days were destroyed for the higher doses.

Conclusions: Conclusions: The biological responses with time interval of 6 hours, 1, 3 and 9 days between two repeated exposure can not be detected by MTD. Lower exposure doses should be used for 30 days interval group.

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Partial coherence laser interferometry (IOL-Master) in intraocular lens power calculation for silicone-oil filled eyes.

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Purpose: To assess the practical feasibility, the quality and variability of axial length measurements by partial coherence interferometry in silicone-oil filled eyes with retinal and other ocular pathology.

Methods: Design: Consecutive case series. Participants: 45 patients who had undergone pars plana vitrectomy with silicone oil tamponade. Intervention: At least 2 axial length measurements by the Zeiss IOL master with a signal-to-noise ratio (SNR) > 2 were undertaken in both eyes of the patients after performing complete ocular examination. Main outcome measures: Variability of the axial length measurements and the SNR in the silicone oil-filled eyes and the contralateral eyes were analyzed.

Results: Axial length measurements were feasible in all silicone-oil filled eyes except three patients with cataract, central retinal detachment or intraocular hemorrhage. The SNR of the first two measurements was significantly smaller (p=0.04) in silicone-filled eyes compared to the contralateral eyes without tamponade. Although the SNR increased in silicone-filled eyes by repeated measurements, values for the axial length did not become significantly different if biometry was performed twice or more.

Conclusions: Partial coherence laser interferometry shows a good clinical practicability, quality and reproducibility in silicone-oil-filled eyes with stable retinal situation and clear media. Further studies have to prove the reliability of the measurements concerning postoperative refraction after IOL implantation.

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Comparison of dysphotopsia in patients with monofocal and multifocal intraocular lenses after Nd:YAG capsulotomy

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Purpose: To compare photic symptoms in patients with monofocal and refractive multifocal (AMO Array[®]) intraocular lenses post Nd:YAG capsulotomy.

Methods: 49 eyes of 49 subjects, 20 with multifocal and 29 with monofocal IOLs had halo, glare, and flicker assessed in scotopic conditions with computer-generated psychophysical tests (Glare & Halo[®]). All subjects had uncomplicated clear corneal phacoemulsification cataract extraction by one surgeon and had full clinical examination and refraction prior to testing. Concurrent ocular pathology led to exclusion. Four quantitative questionnaires (Tester, Javitt and Winther-Nielson Questionnaires along with the Sedgewick Instrument) were used to assess subjective dysphotopic symptoms.

Results: All subjects had achieved LogMAR acuity of 0.3 or better (mean = -0.08) and 0.5 or better (mean = 0.39) for distance and near respectively and had a spherical equivalent of +/- 5 DSph after refraction with less than +/- 1 DCyl. No significant difference between the two groups was found in halo (p=0.3), glare (p=0.3) or flicker (p=0.7). Results from all the questionnaires showed no significant differences in the subjective appreciation of photic phenomena.

Conclusions: The presence of dysphotopic symptoms in patients with refractive multifocal IOLs may become comparable to monofocal IOL patients after Nd:YAG capsulotomy.

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Visual outcome after cataract surgery in uveitis patients

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Purpose: After cataract surgery in uveitis, aggravation of inflammation during postoperative period is a main concern for ophthalmologists. Our aim is to report the clinical course after phacoemulsification with intraocular lens implantation in uveitis.

Methods: Patients presenting an uveitis operated from cataract between June 2001 and June 2003 were involved in an opened prospective study. Pre and postoperative immunosuppressive treatment was established according to the severity of uveitis. In severe uveitis, patients were operated after a three day course of 1/2 mg / kg / day of prednisone associated with local prednisolone acetate 1% therapy. Each patient underwent complete ophthalmologic examination at day 1, 7, 15 and monthly until stabilization of uveitis. Visual acuity (VA) was assessed by Snellen chart.

Results: Thirty-nine eyes (23 patients) of uveitis patients were operated by phacoemulsification between June 2001 and June 2003. The mean age of patients was 56 ± 17 years (range 26 - 86 years). Major causes of uveitis were Fuchs heterochromic cyclitis (4), Sarcoidosis (3), HIV (3), Reiter's syndrome (2), Herpes (2), Lyme disease (1), Rubella (1), Crohn's disease (1), Behçet's syndrome (1), ARN (1), others (4). The mean initial best VA was of 0.3 (SD 0.3). Mean follow-up period was of 8 months. The mean final VA was 0.8 (SD 0.3). No per operative complications was observed. Cystoid macular edema was seen in 1 eye, 5 months after surgery. Two eyes had unchanged VA due to macular scars. None of the eye had a latest VA worse than preoperatively.

Conclusions: Phacoemulsification and IOL implantation appears as a safe procedure, when inflammation is treated by adequate anti-inflammatory therapy. Macular scars or retinal lesions are poor prognosis indicators.

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First clinical animal experimentation experiences and histological examinations with Intraocular lenses (IOL) consisting of Polyurethane

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Purpose: We aimed to give biological evidence of biocompatibility of a new polyurethane intraocular lens of high refractive index. We compared this material to the biocompatibility of acrylic lenses being in clinical use.

Methods: After in vitro proof of biocompatibility of the material we implanted intraocular lenses of polyurethane and acrylic in each 6 rabbit eyes following standard phacoemulsification in general anesthesia. The animals were examined after one day, one week, 1 and 3 months by means of slit lamp examination. Criteria were status of the cornea, anterior chamber depth, fibrin formation, centric IOL position and damage to the iris. The optical properties and the fixation by means of the haptic was examined.

Results: The implantation procedure of the yet not optimized haptic of the IOL was more difficult as in acrylic IOL. This was due to the large haptic. During the experiment we found a high biocompatibility compared to acrylic IOL. There was posterior capsule fibrosis in both groups somewhat more homogenous in the Polyurethane group. Lens epithelial regrowth was similar in both groups.

Conclusions: In this study we found first the perfect biocompatibility of Polyurethane IOL within the rabbits eye. The optimization of the implantation process is improved by means of and more flexible smaller haptic.

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Image filtering technique for assessing posterior capsule opacification

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Purpose: This paper presents a new image analysis approach for the assessment of Posterior Capsule Opacification (PCO) based on retroilluminated digital images of the eye's lens capsule.

Methods: A derivative filter is applied to the digital image. Textured areas are characterized by high response to derivative filters, contrary to smooth areas which produce a low response. Thus the response of the image to the derivative filter is able to indicate the amount of the texture in the image, reflecting therefore the amount of PCO in the capsule. The filter response can be evaluated by simply computing the energy of the filtered image. We also incorporated formulae to account for distance of PCO from central visual axis, which has not previously been described.

Results: Light does not appear to leave traces except some false boundaries that do not affect seriously the values of the PCO scores. The filtered images seem to reflect with a reasonable faithfulness the granularity and the texture of the capsule surface as it can be noticed for the rest of the images. Scores from example posterior capsules before and after capsulotomy show a high degree of discrimination between clinically significant and insignificant PCO.

Conclusions: This work presents a simple, objective and effective approach for assessing the amount of PCO in digital images. Preliminary results suggest good clinical validity.

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Expression of HOX11L1 in human adult lens epithelial cells

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Purpose: Posterior capsule opacification (PCO) is a common complication that affects an important percentage of patients who have undergone cataract surgery. PCO reflects a high proliferation capacity of lens epithelial cells (LECs), therefore new approaches to the discovery of genes expressed in the adult cells and that are implicated in the pathogenesis of PCO may be helpful to prevent this complication. HOX11L1, a homeobox gene with a role in eye development, was studied in order to know if it is expressed in the adult LECs or not.

Methods: RNA isolation from LECs attached to the anterior capsule obtained during the cataract surgery of capsulorhexis specimens from cataract patients (75 years), RT-PCR analysis was performed following standard protocols.

Results: Absence of expression of HOX11L1 was detected in the adult LECs during the study.

Conclusions: HOX11L1, a transcription factor involved in embryonic lens development, is silenced after birth. The absence of expression of HOX11L1 verified in this study suggests that it has no role in the proliferation of the epithelial cells. Further studies about genes expressed in the adult LECs which are implicated in the proliferation of these cells leading to PCO, must be done.

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Expression of vascular endothelial growth factor A and C in the uveal melanoma eye.

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Purpose: To investigate the expression of the VEGF-family in uveal melanoma an its relations with known prognostic factors.

Methods: In the aqueous humour of 74 and 46 eyes with uveal melanoma, the expression of VEGF-A and VEGF-C was determined with an ELISA. These expressions were compared with results in 30 control eyes. To determine the source of VEGF-A and VEGF-C, expression was determined in retinal and uveal melanoma tissue, using western blot analysis. The expression of these angiogenic growth factors was also determined in supernatants of uveal melanoma cell lines and melanocyte cultures.

Results: VEGF-A and VEGF-C were both significantly higher expressed in the aqueous humour of uveal melanoma eyes in comparison with control eyes. VEGF-A was expressed both in retinal and uveal melanoma tissue. VEGF-C was expressed especially by the uveal melanoma. All uveal melanoma cell lines expressed VEGF-A and VEGF-C, but none of the melanocyte cultures.

Conclusions: Both VEGF-A and VEGF-C are expressed by uveal melanoma cells and in the uveal melanoma eye. These cytokines may play a role in establishing an anti-angiogenic strategy in uveal melanoma.

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Localisation of Atrial Natriuretic Peptide (ANP) to cells of Epiretinal Membranes (ERMs) from patients with proliferative diabetic retinopathy (PDR)

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Purpose: Diabetic ERMs are the main pathological changes of PDR which may cause severe complications resulting in blindness. One possible cause for progression of ERMs is the production of growth factors by cells within ERMs that provide autocrine or paracrine stimulation. Vascular endothelial growth factor (VEGF) has been localised to cells of ERMs and may play such a role. Given that ANP has been recently described as an endogenous inhibitor of the synthesis and angiogenic action of VEGF, this study investigated the presence of ANP within ERM of eyes with PDR.

Methods: Ten ERMs were surgically excised from 10 diabetic patients with PDR during pars plan vitrectomy. The presence of ANP was determined by immunohistochemical staining using monoclonal antibodies against ANP and several cellular "marker" proteins.

Results: All ten ERMs from diabetic patients with PDR stained positive for ANP. This peptide appears to be located at vascular endothelial and vascular smooth muscle cells, glial and RPE cells, macrophages and fibroblast-like cells in ERMs, yet seems to be absent from the extracellular matrix.

Conclusions: This is the first demonstration that ANP occurs in most of the cells that make up the ERMs. Thus, ANP may have a role in regulating membrane growth in PDR. Further studies are needed to define its full pathophysiological role in the formation of ERMs.

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The invasive properties of uveal melanoma cell lines and their response to Interleukin-6 (IL-6) and Vascular Endothelial Growth Factor (VEGF)

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Purpose: Uveal melanoma cells have been shown to express cytokines, such as IL-6 and VEGF, which may affect their invasive ability. We investigated the invasive potential of five uveal melanoma cell lines, and their response to IL-6 and VEGF.

Methods: Five uveal melanoma cell lines (92.1, MKT-BR, OCM-1, SP6.5, UW-1) were used. An invasion assay was performed using a modified Boyden chamber system with a 8 µm pore membrane coated with Matrigel, an artificial basement membrane. 1x10⁵ cells were seeded on the top portion of the chamber, with 10% FBS as a chemo-attractant in the bottom portion of the chamber. The invasion assay was repeated using IL-6 and VEGF as chemo-attractants at concentrations of 10 and 20 ng/ml respectively. Invading cells were stained and counted in twenty high-power fields. Experiments were performed in triplicate.

Results: The average invasive ability of cells with only FBS varied from a high of 19.6 cells for OCM-1 to a low of 7 cells for UW-1. VEGF produced up to a six-fold increase in the invasion of all uveal melanoma cell lines. The addition of IL-6 produced a two-fold increase in invasion of 92.1, SP6.5 and MKT-BR, while inhibiting the invasion of OCM-1 and UW-1.

Conclusions: Different uveal melanoma cell lines have different invasive potentials. In this study VEGF has been proven to be a more potent stimulant than IL-6 to the invasion of uveal melanoma cells. IL-6 stimulated invasion in three of the uveal melanoma cell lines, inhibiting invasion in the other two. Different cytokines may have varying effects on the invasive properties of different tumor cell populations.

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Expressions of cyclin D1 are infrequent and different in uveal and skin malignant melanoma

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Purpose: Increased expression of the proto-oncogene Cyclin D1 protein has been observed in a number of different malignancies including cancer of the breast, esophagus, lung, bladder, and liver. In a limited study in melanomas increased cyclin D1 protein expression was seen in 33% of cases. We investigated the frequency of cyclin D1 amplification in malignant melanomas at various locations including uveal melanomas.

Methods: A tissue microarray of 382 melanoma samples was constructed. The array comprised 101 uveal melanomas, 154 skin melanomas (including 22 of acral lentiginous type), 10 mucosal melanomas, 66 nodal metastases, and 51 hematogenous metastases. Dual-color fluorescence in situ hybridisation (FISH) was performed with differentially labeled cyclin D1 and chromosome 12 centromeric (as a reference) probes.

Results: Overall, 326 melanomas could be analyzed by FISH. Cyclin D1 was amplified in 13 tumors (3/8 forearm, 5/28 head/neck, 1/5 mucosa and 4/17 acral lentiginous type) and in 8 lymph node metastases. No amplification could be observed in uveal melanoma.

Conclusions: Amplification of Cyclin D1 could be demonstrated in skin melanomas either from strongly sun exposed areas or from locations without sun exposure. No amplification of Cyclin D1 was found in intraocular uveal melanoma. Our results suggest that Cyclin D1 amplifications might play a role in the pathogenesis of certain skin melanomas depending on their location but not in ocular melanomas.

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Detection of spiked uveal melanoma cells in peripheral blood

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Purpose: The identification of circulating malignant cells (CMCs) in uveal melanoma (UM) patients is the most important indicator of patients at risk of developing metastatic disease. Detection of CMCs using RT-PCR has been investigated in skin melanomas using Tyrosinase and Melan A to identify these high-risk patients who may benefit from adjuvant therapy. The purpose of the study is to test the sensitivity, specificity and lower detection level of RT-PCR for the detection of UM cells spiked in peripheral blood (PB) of normal volunteers.

Methods: PB was collected from 18 healthy volunteers and divided in 6 groups of 3 volunteers each. PB from each volunteer (20 ml) was divided in 10 aliquots of 2ml each to obtain a final number of 30 samples per group. Group 1 (G1) was spiked with 10 cells, G2 with 100 cells, G3 with 1,000 cells, G4 with 10,000 and G5 with 100,000 cells. G6 was used as negative control. RT-PCR was performed using Tyrosinase and Melan A.

Results: All 30 samples were positive for both markers in each G5, G4, and G3. Nested RT-PCR detected spiked UM cells in G2 (18/30 samples with Tyrosinase and 15/30 with Melan A) and G1 (18/30 samples with Tyrosinase and 15/30 for Melan A). All samples in G6 were negative.

Conclusions: RT-PCR, using either tyrosinase or Melan A, is a sensitive and specific method for the detection of UM cells. Identification of CMCs in PB of UM patients by RT-PCR may be a useful non-invasive method to monitor high-risk patients. The detection of subclinical spread of disease may enable adjuvant therapies to be initiated at early stages of dissemination.

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Expression of sex hormone receptors and cell cycle proteins in conjunctival melanocytic lesions using Tissue Microarrays (TMAS)

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Purpose: To evaluate the expression of sex hormone receptors and cell cycle proteins in melanocytic lesions of the ocular conjunctiva.

Methods: Formalin-fixed, paraffin-embedded material from 76 tumors - 69 conjunctival nevi (junctional [n=18], compound [n=32], dermal [n=18], and blue [n=1], 5 specimens of primary acquired melanosis (PAM), and 2 conjunctival melanomas - were included in a tissue microarray (TMA) format. The TMA sections were analyzed by immunohistochemistry with antibodies for sex hormone receptors (progesterone, estrogen), and cell cycle related proteins (MIB1-Ki67, p53, p16).

Results: Progesterone receptors were highly (96%) and similarly expressed in all lesions, whereas estrogen receptor expression was completely absent. In addition, progesterone receptor expression showed a tendency to increase with age ($p=0.06$). p16 was expressed in 97%, p53 in 34% of the lesions. MIB1-Ki67 was expressed at low levels (13 ± 14 SD %) in 79% of the lesions. No differences of expression were found between the different lesions and nevi types. The mean age of the patients was highest in conjunctival melanoma (70 ± 22 SD y), followed by PAM (60 ± 19 SD y) and nevi (36 ± 18 SD y). The different types of nevi did also show a significant age-dependency (junctional [25 ± 17 SD y] < compound [34 ± 17 SD y] < dermal [49 ± 15 SD y]).

Conclusions: Our findings suggest a role for progesterone, cell cycle regulators (p16, p53), and the proliferation marker MIB1-Ki67 in the pathophysiology of melanocytic lesions of the ocular conjunctiva.

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Clinicopathological and genotype analysis of orbital lymphoproliferative lesions

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Purpose: To evaluate the validity of genotype analysis in addition to histopathological examination for diagnosis of lymphoproliferative lesions in orbit.

Methods: The subjects were 3 cases (49 year old woman (case 1), 66 year old man (Case 2), 59 year old woman (Case 3)) of the orbital lymphoproliferative lesions treated in our hospital. The surgical biopsy specimens were examined histopathologically and immunohistochemically. As for genotype analysis, Southern blot hybridization was used to detect immunoglobulin gene rearrangement.

Results: Case 1 had the mass lesion in the bilateral orbits and left ethmoid sinus, Case 2 had the unilateral orbital and ethmoidal mass lesion, and Case 3 had the unilateral orbital mass lesion. The histopathological and immunohistochemical examination suggested Cases 1 and 2 were the inflammatory pseudotumor, and Case 3 was the mucosa-associated lymphoid tissue (MALT) type malignant lymphoma. The genotype analysis revealed the immunoglobulin light chain gene rearrangement in Case 1, and no rearrangement in Cases 2 and 3. Taken together, Case 1 was diagnosed as malignant lymphoma and treated with chemotherapy and radiation. Cases 2 and 3 were diagnosed as inflammatory pseudotumor and treated with steroid therapy and radiation. The prognosis of each case was good after the follow-up of 10.6 months (average).

Conclusions: The analysis of rearrangement was confirmed to be useful to diagnose orbital lymphoproliferative lesions.

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Immunolocalisation of opticin in the human eye

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Purpose: To localise opticin, a novel extracellular matrix glycoprotein, in the adult human eye.

Methods: Two polyclonal rabbit antisera were raised against the opticin peptides called G989 and G869. These antisera were used to probe paraffin-embedded sections of two normal eyes. The opticin antibodies were then detected using the Vectastatin Elite "Universal" ABC kit and VIP staining agent.

Results: Opticin was found almost exclusively localised to the vitreous humour. Labelling was most intense at the vitreous base and the posterior cortical vitreous and appeared to follow the distribution of the collagen fibrils. In addition, specific staining was found along the surfaces of the basement membranes including the internal limiting lamina, the posterior and the anterior surface of the lens. Both antibodies showed similar patterns of staining although G989 was stronger.

Conclusions: The labelling of opticin being confined to the vitreous cavity and lens surface supports our in-situ hybridisation data on mouse and humans where opticin expression was found localised to the posterior non-pigmented ciliary epithelium. The surface staining of basement membranes, especially the internal limiting lamina suggests a role for opticin in vitreoretinal adhesion.

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Prevalences of eye diseases in Japanese population of high age-Sagae Study in Japan

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Purpose: We examined the prevalences of eye diseases to investigate the vision-related quality of life in the Japanese population with the age of 70 years and examined the correlation between ocular diseases and systemic states by population-base survey in Japanese.

Methods: The subjects were 70 years old living in Sagae City, Yamagata, Japan. Visual acuity (VA), intraocular pressure were measured. The ocular anterior segments were observation by slit-lamp and fundus photographs were taken to evaluate the prevalences of various pathological states of ocular fundus. The examination of systemic factors, including body height, weight, west/hip ratio, blood pressure, and blood examination were performed. All the study procedures were approved by the Ethical Committee of Yamagata University School of Medicine.

Results: Sixty eight persons (male 28, female 40) with 136 eyes participated in this study and corresponded to 60% of all the 70 year old population in this study area. Fifty four percent of the subject eyes had VA above 1.0 (=20/20). The prevalence of acquired blindness was 0%. In total, 50% of the eyes had eyes diseases. The findings of arteriolosclerosis were detected in 27% of the eyes. The findings of arteriolar narrowing (one of the arteriolosclerosis findings) was correlated significantly with the mean arterial blood pressure ($p=0.049$), the total cholesterol ($p=0.0003$), however, arterio-venous nicking or arteriolar reflex increase was not.

Conclusions: In this population-based study, 50% of the eyes had at least one pathological conditions, however, 96% retains useful visual acuity. The fundus evaluation suggests that the findings of retinal arteriolosclerosis have different clinical significances.

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Survey of ophthalmologist and optometrist attitudes to dry eye and preferred tests for its diagnosis

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Purpose: To survey ophthalmologist's and optometrist's attitudes towards diagnostic testing for dry eye.

Methods: A web-based anonymous questionnaire was sent to 35 ophthalmologists and 30 optometrists practicing in the UK and Australia. Questions related to reasons for preferred diagnostic tests, rank order of preference for tests, important characteristics to be considered in the development of future tests, overall satisfaction with diagnostic tests and therapeutic options in dry eye.

Results: Fifty-five practitioners completed the survey. Reasons for test choices related to cost (inexpensive) 78%, ease of administration (66%) and supported by good evidence (2%). History was the most frequent first choice for dry eye diagnosis (42%) followed by fluorescein break-up time (36%), fluorescein staining (16%), and Schirmer test (8%). However the Schirmer test was frequently recorded in the top four choices (52%). Important characteristics for new diagnostic tests were ranked in order of evidence for tests? validity, time taken to perform, patient comfort and cost. Overall practitioner satisfaction with available tests was 5.4 out of 10 (SD±1.9) and for available therapeutic options 4.1 (SD±2.1) with 10 signifying total satisfaction.

Conclusions: The importance of multiple tests is evident since practitioners are not satisfied with any single diagnostic test. There is a wide variation in preferred tests. A large discrepancy exists between the most highly valued characteristic of a test, that is, evidence for its validity and the current evidence for tests that is available. Overall satisfaction with diagnostic tests and therapy available for dry eye is low.

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Childhood myopia and parental smoking

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Purpose: To examine the relationship between exposure to passive parental smoke and myopia in Singapore Chinese children.

Methods: The initial cross-sectional results of 1,334 Chinese children from 3 schools in Singapore, all participants in the Singapore Cohort study Of the Risk factors for Myopia (SCORM), are presented. Information on whether the father or mother smoked, number of years smoked and the number of cigarettes smoked per day during the child's lifetime were derived. The children's cycloplegic autorefractometry, corneal curvature radius and biometry measures were compared with reported parental smoking history.

Results: There were 434 fathers (33.3%) and 23 mothers (1.7%) who smoked during the child's lifetime. After controlling for age, gender, school, mother's education and mother's myopia, the mean refraction (-1.31 D) of children whose fathers smoked was similar to that of children whose fathers did not smoke (-1.37) ($p=0.60$). There were no significant trends observed between the number of cigarettes the father smoked, duration of smoking in years and refractive error, axial length or any other biometry parameter. In similar multivariate analysis, mothers who had ever smoked during their lifetime had more "positive" refractions [adjusted mean = -0.28 (Diopter) D versus -1.38D] compared with children who did not ($p=0.012$).

Conclusions: Although experiments in chickens suggest that myopia may be inhibited by antagonists to nicotinic acetylcholine receptors, our study findings do not establish any firm link between passive smoking and myopia. "Positive" refractions in smoking mothers could possibly be attributed to other causes such as lower socioeconomic status or different behavioral lifestyle factor.

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Role of haptoglobin phenotypes and ace, in type 2 diabetic retinopathy-preliminary study

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Purpose: To study the angiotensin converting enzyme activity (ACE) and haptoglobin (Hp) phenotypes in type 2 diabetics, with and without retinopathy.

Methods: 44 type 2 diabetic subjects, 18 F and 26 M, with 66,58,5 years, were studied; 18 diabetics with and 25 without. The ACE activity (U/l) was determined by spectrophotometry, and Hp phenotypes by PAGE. 4 individuals were Hp 1.1, 15 Hp 2.2 and 25 Hp 2.1. Statistical methods were Student's t test, ANOVA, and X².

Results: We observed a predominance of Hp 2.2 in diabetics with retinopathy (50%) vs the other Hp phenotypes (2.1 Hp-33,3%; 1.1 Hp-16,7%) and conversely a predominance of Hp 2.1 in diabetics without retinopathy (72%) ($p=0.036$). The overall ACE activity was 2417.6. There was no differences vs controls (25,310,7). It was highest in diabetics with Hp 2.2 phenotype (34,3 13,1), lowest in diabetics with Hp 1.1 (12,717,2), and intermediate in Hp 2.1 (19,717,7) $p=0.02$. However it didn't show significant differences between the groups with and without retinopathy.

Conclusions: The Hp 2.2 with higher ACE activity, is associated with diabetic retinopathy. Hp phenotype seems to be a good marker of retinopathy specially if associated with higher ACE activity in type 2 diabetics.

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Ace genotypes and activities of ace and Epinephrine Oxidase (EPOX) in type 2 diabetic retinopathy

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Purpose: To study the relationship between angiotensin converting enzyme (ACE) activity and genotypes (I/I, I/D, D/D), with EpOx in type 2 diabetics, with and without retinopathy.

Methods: 44 type 2 diabetics, 18F and 26M, with 66,5±8,5 years, were studied; 18 with retinopathy and 25 without. Plasma ACE (U/l) and EpOx (mmol/ml/h) activities were determined by spectrophotometry, and ACE genotypes by PCR. 5 were ACE genotype II, 8 ID and 10 DD. Statistical methods Student t test, ANOVA, and X².

Results: EpOx activity was lower in controls (28,4±9,9) compared with diabetics (43,7±11,8) p<0,001. There was no differences of ACE activity in controls vs diabetics. We observed a trend for higher ACE genotype II in diabetics with retinopathy (50%) compared to the others (ID-25%; DD-25%), conversely a predominance of ACE DD in diabetics without retinopathy (53,3%) vs II (6,7%) (p=0,055). The ACE II genotype corresponds to the lowest EpOx activity compared with the others (p=0,059). The overall ACE activity (24±17,6) didn't show differences between its genotypes neither between the groups with and without retinopathy, the same for the EpOx activities. We observe a strong correlation between the ACE and EpOx activities only in the retinopathy group (p=0,003; r=0,690).

Conclusions: ACE II was predominant in the retinopathy type 2 diabetics showing lowest EpOx activity; ACE DD was predominant in group without retinopathy with the highest EpOx activity. ACE and EpOx activities were strongly correlated within the retinopathy group. This study suggests a relation of these enzymes in diabetic retinopathy, supporting an inflammatory mechanism.

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Macular corneal dystrophy: discovery of twelve novel mutations in the CHST6 gene

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Purpose: To identify mutations in the CHST6 gene in fifteen patients from eleven unrelated French families affected with recessive macular corneal dystrophy.

Methods: Genomic DNA was extracted from peripheral blood leukocytes of the affected patients and their healthy family members, and the mutational status of the CHST6 gene was determined for each patient by a PCR-sequencing approach. Serum concentrations of antigenic keratan sulfate for each proband were determined by ELISA.

Results: ELISA showed that all affected patients, but one, were of MCD type I or IA. Fourteen distinct mutations were identified within the CHST6 coding region: 2 nonsense, 2 frameshift, and 10 missense mutations. Of these, 12 were novel and a nonsense mutation in the homozygous state was reported for the first time.

Conclusions: These molecular results combined with those reported from previous studies indicated CHST6 mutational heterogeneity. The characterization, here, of nonsense mutations is in keeping with the fact that MCD results from loss of function of the CHST6 protein product.

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Expression of the CCT subunit and ALPHA-SMA during corneal wound healing

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Purpose: To demonstrate the expression of the eta subunit of the hetero-oligomeric particle CCT (the chaperonin containing T-complex polypeptide 1) and its substrate, alpha-smooth muscle actin (alpha-SMA), in normal corneas and corneas treated with ultraviolet radiation (UVR). The CCT chaperonin in the wound may promote the formation of myofibroblasts by the folding of sufficient alpha-SMA to form the stress fibers characteristic of these cells. Therefore, this investigation could be a helpful and interesting step in solving the problem of corneal scarring and loss of transparency.

Methods: New Zealand albino rabbit corneas were exposed to UVR at 310 nm at the dose producing biomicroscopically significant keratitis. Expression of CCT chaperonin and alpha-SMA genes in the corneas was investigated by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR).

Results: There was slight CCT chaperonin gene expression in the control group. One day after UVR exposure, the expression level of CCT chaperonin was slightly increased (p<0.05). Two days after exposure, the gene expression was highly increased (p<0.01), and decreased again to the 1 day level at 5 days after exposure (p<0.05). No alpha-SMA expression was observed in the control group. One day after UVR exposure, alpha-SMA gene was strongly expressed. The expression of alpha-SMA was decreased but still observed over days 2 and 5, however this decrease is not statistically significant (p<0.1).

Conclusions: The UVR exposure leads to a strong expression of alpha-SMA gene, followed by upregulation of CCT chaperonin expression. These data indicate that CCT chaperonin as alpha-SMA folding complex is involved in the corneal repair process after UVR exposure.

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Differential expression of ABC-transport proteins in vasospastic persons with increased endothelin-1 plasma levels

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Purpose: To quantify the gene expression levels of the ABC-proteins MDR1 (P-glycoprotein) and MRP (multidrug resistance-associated protein) isoforms in isolated mononuclear cells of vasospastic persons with increased Endothelin-1 plasma levels.

Methods: Expression levels of the MDR1 (P-glycoprotein) gene and MRP1 to MRP5 genes in mononuclear cells derived from vasospastic subjects compared to healthy controls was determined by quantitative real-time RT-PCR.

Results: Mononuclear cells of vasospastic subjects showed a significant decrease in the expression of MDR1 (P-glycoprotein) gene (p=0.029), MRP2 gene (p=0.003) and MRP5 gene (p=0.013) when compared to healthy controls.

Conclusions: Self-reported observations from vasospastic subjects about enhanced drug sensitivity might have their explanation in a reduced expression of several multidrug resistance (MDR) proteins.

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Management of slow flow vasculopathy of the anterior segment

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Purpose: To describe the role of the ophthalmologist in the management of the rare but clinically important condition of slow flow vasculopathy of the anterior segment, by description of its clinical features, diagnosis and treatment.

Methods: Description of a plethoric patient with persistent bilateral hyperaemia of the conjunctiva and sclera unresponsive to treatment with topical antibiotics or mast cell stabilising agents. Fundal examination was unremarkable. Viral serology and scrape for chlamydia were negative. Further investigations performed included full blood count and standard screen for scleritis.

Results: Haemoglobin was in excess of 18 g/dL, leading to diagnosis of very severe, life-threatening polycythaemia. Non-haematological parameters were unremarkable. Staged treatment with venesection in collaboration with haematologists was implemented to haemodilute the patient. Both conjunctival and scleral hyperaemia subsided in parallel to haemodilution, with full reversal of ocular and systemic hyperaemia.

Conclusions: Severe polycythaemia is a rare but important cause of venous stasis in the anterior segment, and one in which ophthalmologists have a crucial role in diagnosis. This life-threatening condition is a rare but extremely important cause of a red eye that thus merits being highlighted. Prompt diagnosis is required for effective management to reduce plasma viscosity. Ocular changes are fully reversible with systemic plasma haemodilution, which is also life-saving.

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Intravitreal triamcinolone acetonide as treatment for extensive exudative retinal detachment?

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Purpose: To describe the effect of intravitreal triamcinolone acetonide on eyes with extensive exudative retinal detachment.

Methods: Two patients suffering from long-standing extensive exudative retinal detachment received an intravitreal injection of 25 mg triamcinolone acetonide.

Results: In both patients, anterior chamber flare and flare in the vitreous cavity markedly diminished. The extension of exudative retinal detachment increased with formation of new subretinal strands.

Conclusions: Despite its anti-phlogistic, anti-edematous and anti-angiogenic effect, intravitreal triamcinolone acetonide may be associated with a paradoxical increase of subretinal proliferations leading to an enlargement of exudative retinal detachment. Possible reason may be the enhancement of retinal pigment epithelium proliferations by low concentrations of triamcinolone acetonide.

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Biomechanical evaluation of admissible ballistic and constructive parameters of shooting toys bullets for excluding dangerous child's eye contusion

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Purpose: The results of contusion injuries caused by shooting toys bullets of 34 children of age from 3 to 14 are examined. It was necessary to develop an adequate criteria of estimating the danger contusion level of a shooting toy for the child's eye and to introduce them as an obligatory condition for an issuing the shooting toy's safety certificate.

Methods: The clinical description of contusion character was set up with the help of vision and refraction analysis, ophthalmoscopy and biomicroscopy of the cornea and the lens. With the help of specially developed methods the ballistic characteristics were measured on the pendulum test bench where the sphere, which was made of visco-elastic material and was hung on unstretchable rope, was used as a load. The bullet stuck in this hung load then the angle of deflection was measured. Knowing this angle one could evaluate the energetic characteristic of the impulse. The velocity of the bullet was estimated by the results of video record interpretation. The danger of geometry of the bullets head-part was evaluated by the comparative value of its penetrating into the plasticine provided that initial energy.

Results: The round bullets have seemed to be the most traumatic. The cylindrical bullets with spherical head-part were the least traumatic. The bullets which had the diameter not exceeding 2 mm and the mass not exceeding 5 grams were equally dangerous for all 6 types of shooting toys. The energy evaluated as 0.07 J and the velocity of flying evaluated as 5 m/sec form the upper admissible level. The cylindrical bullets have better ability to get on ricochet that's why the level of contusion was less.

Conclusions: The estimated energy and velocity levels are considered to be the main criteria for defining the dangerous toys. These criteria can be taken as a basis for developing the special safety standard for shooting toys.

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Duration of the effect of intravitreal triamcinolone acetonide as treatment of exudative age-related macular degeneration

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Purpose: To evaluate the duration of the effect of intravitreal triamcinolone acetonide on visual acuity in patients with exudative age-related macular degeneration.

Methods: The prospective clinical interventional case-series study included 42 patients (44 eyes) with exudative age-related macular degeneration, who had shown an increase in visual acuity by at least 2 Snellen lines after an intravitreal injection of 25 mg triamcinolone acetonide. Mean follow-up time was 10.4 ± 7.0 months (3.1 months - 31.7 months).

Results: Within the first week after the injection, visual acuity started to increase significantly (p=0.008) to reach a plateau-like maximum at 1 - 6 months after the injection. Visual acuity returned to baseline values 8 to 9 months after the injection. In a parallel manner, intraocular pressure started to significantly increase about 2 weeks to 1 month after the injection, reached a plateau-like maximum 1 to 6 months after the injection, and returned to preoperative values about 8 to 9 months after injection. Occurrence and amount of intraocular pressure elevation was statistically (p>0.15) independent of the amount of increase in visual acuity.

Conclusions: In patients with an increase in visual acuity after intravitreal injection of 25 mg triamcinolone acetonide as treatment of exudative age-related macular degeneration, visual acuity and intraocular pressure start to increase with the first 4 weeks after the injection, reach a plateau-like maximum 1 - 6 months after injection, and return to baseline values about 8 to 9 months after the injection. It may suggest that triamcinolone may be re-injected about 6 - 9 months after a primary successful injection. Intraocular pressure has to be checked for several months after the injection. The finding that visual acuity increased and decreased in a chronological parallel manner with the steroid induced change in intraocular pressure suggests that the increase in visual acuity was connected with the intraocular presence of triamcinolone acetonide.

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Optical coherence tomography prior to and after intravitreal triamcinolone for diffuse diabetic macular edema

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Purpose: To describe morphologic and functional changes after an intravitreal injection of triamcinolone acetate as treatment of diffuse diabetic macular edema.

Methods: Six patients suffering from diffuse diabetic macular edema received an intravitreal injection of 25 mg of triamcinolone acetate. Optical coherence tomography, fluorescein angiography, and visual acuity measurements were performed for follow-up of the patient.

Results: Mean retinal thickness (circle of 500 µm radius around the center of the macula) decreased significantly ($p=0.032$) from preoperatively 575 ± 158 µm (mean \pm SD; range, 349 µm to 793 µm) to 242 ± 71 µm (160 µm to 330 µm). Fluorescein angiography showed a reduction of fluorescein leakage. Visual acuity increased from 0.19 ± 0.07 to 0.30 ± 0.11 .

Conclusions: As shown by optical coherence tomography and fluorescein angiography, intravitreal triamcinolone can lead to pronounced morphologic restitution and marked functional improvement lasting for at least 5 months in patients with diffuse diabetic macular edema.

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Edema index in laser scanning tomography of the macula

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Purpose: To evaluate the significance of the edema index of the Heidelberg Retina Tomograph II (HRT) in assessment of eyes with and without macular edema.

Methods: 156 eyes of 110 consecutive patients underwent examination with the HRT using the newly developed software MEM. 58 eyes (37,2%) of the 156 eyes were diagnosed with macular edema due to various reasons (AMD, diabetes, venous occlusion and others) using ophthalmoscopy and fluorescein angiography. Edema index was obtained for circles of 1,0 mm (e1), 2,22 mm (e2) and 3,45 mm (e3) diameter. All circles were concentric with the middle in the assumed or detected center of the macula.

Results: In eyes without macular edema e1 was $1,28 \pm 0,47$ (mean \pm SD; range, 0,62 - 2,68), e2 was $1,30 \pm 0,39$ (0,77 - 2,29), e3 was $1,33 \pm 0,35$ (0,81 - 2,20). In eyes with macular edema e1 was $2,48 \pm 0,79$ (mean \pm SD; range, 1,09 - 4,11), e2 was $2,27 \pm 0,62$ (1,03 - 3,46), e3 was $2,15 \pm 0,54$ (1,03 - 3,02). The difference of the both groups was highly significant for all circles ($p<0,001$; t-test for unpaired matches).

Conclusions: In all groups signal width and edema index was significantly higher in eyes with macular edema compared to eyes without macular edema. Further studies of larger populations are required to define a cut-off point for diagnosing macular edema.

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Comparison of optical coherence tomography and laser scanning tomography for macular assessment

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Purpose: To compare two recently commercially available imaging systems (Optical Coherence Tomographer Model 3000 (OCT) and Heidelberg Retina Tomograph II (HRT)) for assessment of the macula.

Methods: 156 eyes of 110 consecutive patients underwent examination with the OCT and the HRT. Of the 156 eyes 58 eyes were diagnosed with macular edema due to various reasons (AMD, diabetes, venous occlusion and others). In OCT six line scans in a radial pattern were performed for macular mapping, in HRT the macular program MEM was applied. For OCT averaged retinal thickness (RT) was calculated and for HRT signal width (w) and edema index (e) were obtained for circles of 1,0 mm, 2,22 mm and 3,45 mm diameter. All circles were concentric with the middle in the assumed or detected center of the macula. Bivariate correlation (Pearson) of the three different averaged macular thickness values from OCT to signal width and edema index in HRT was calculated.

Results: For the whole study population, correlation of RT within the 1 mm circle to w and e was 0,670 and 0,776 resp. ($p<0,001$); correlation of RT within the 2,22 mm circle to w and e was 0,657 and 0,763 resp. ($p<0,001$); correlation of RT within the 3,45 mm circle to w and e was 0,620 and 0,694 resp. ($p<0,001$). Sex, refraction, intraocular pressure, and visual acuity did not show significant influence on the results.

Conclusions: Results of OCT and HRT in macular assessment show a good correlation. In HRT, edema index is more strongly correlated to retinal thickness than signal width. In contrast to OCT, which measures retinal thickness in µm, HRT produces dimensionless values. Further studies for evaluation of the edema index are necessary.

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Intravitreal triamcinolone acetate for treatment of central retinal vein occlusion

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Purpose: To evaluate the effect of intravitreal triamcinolone acetate on visual acuity and intraocular pressure in patients with central retinal vein occlusion.

Methods: The prospective comparative non-randomized clinical interventional study included 16 patients (17 eyes) with central retinal vein occlusion. The study group consisting of 10 patients (11 eyes) received an intravitreal injection of 25 mg of triamcinolone acetate. The control group including 6 patients did not receive any treatment. Mean follow-up was $6,9 \pm 3,4$ months in the study group and $4,4 \pm 3,2$ months in the control group.

Results: In the study group, mean visual acuity increased significantly ($p=0,038$) from $0,12 \pm 0,12$ preoperatively to a best visual acuity during follow-up of $0,21 \pm 0,19$. Visual acuity measurements determined 1 and 3 months after the injection were significantly ($p=0,043$ and $p=0,046$, respectively) higher than the baseline values. Visual acuity measurements returned to the baseline level about 5 months after the injection. Gain in visual acuity measured in Snellen lines did not vary significantly between eyes with ischemic versus non-ischemic central retinal vein occlusion. In the control group, baseline visual acuity and best visual acuity during the follow-up did not vary significantly ($p=0,89$), and visual acuity at the end of follow-up was significantly ($p=0,043$) worse than at start of the study. Comparing study group and control group with each other, gain in visual acuity was significantly ($p=0,003$) higher in the study group.

Conclusions: A single intravitreal injection of triamcinolone acetate temporarily increases visual acuity in central retinal vein occlusion.

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Predictive factors influencing visual acuity change after intravitreal triamcinolone injection for diffuse diabetic macular edema

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Purpose: To evaluate which factors influence change in visual acuity after intravitreal injection of triamcinolone acetonide as treatment for diffuse diabetic macular edema.

Methods: The prospective clinical interventional study included 38 eyes with diffuse diabetic macular edema who received an intravitreal injection of 25 mg of triamcinolone acetonide. Mean follow-up was 6.9 ± 3.4 months (1.0 to 13.6 months).

Results: In a multiple linear regression analysis, improvement in visual acuity after the intravitreal injection of triamcinolone acetonide was significantly and negatively correlated with the degree of macular ischemia ($p < 0.001$), preoperative visual acuity ($p = 0.002$), and grid laser treatment of the macula prior to inclusion into the study ($p = 0.009$). Improvement in visual acuity was significantly and positively correlated with an increase in intraocular pressure ($p = 0.046$). Change in visual acuity after the intravitreal triamcinolone injection was statistically independent ($p > 0.20$) of age, gender, and pseudophakia.

Conclusions: A steroid induced increase in intraocular pressure may be associated with an increase in visual outcome after intravitreal injection of 25 mg triamcinolone acetonide as treatment of diffuse diabetic macular edema. Marked macular ischemia, high preoperative visual acuity and pre-injection grid laser treatment of the macula may have a negative impact on an increase in visual acuity after intravitreal triamcinolone injection.

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Intravitreal triamcinolone acetonide for neovascular or edematous macular diseases: follow-up of intraocular pressure

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Purpose: Analysis of intraocular pressure after intravitreal triamcinolone acetonide (TA) injected for neovascular/edematous macular diseases.

Methods: 71 patients (75 eyes) with progressive age-related macular degeneration ($n = 64$) or diffuse diabetic macular edema ($n = 11$) and treated with 25 mg TA are included in prospective consecutive non-comparative interventional case-series; mean follow-up 6.86 ± 2.52 months.

Results: Intraocular pressure (IOP) increased significantly ($P < 0.001$) from 15.43 ± 3.26 mm Hg preoperatively to mean maximum of 23.38 ± 8.37 mm Hg postoperatively. Increase of IOP > 21 mm Hg in 39 (52%) eyes, developing 2 months after injection. Preoperative predictive factor for the rise in IOP: younger age. It was statistically independent of refractive error, presence of diabetes mellitus, indication for injection. In all but 1 eye, IOP was controlled by topical antiglaucomatous medication without glaucomatous optic nerve head changes. In the eyes with increased IOP it was normalized about 6 months after injection. Increase in IOP after re-injection developed only after an increase after 1st injection.

Conclusions: After 25 mg of intravitreal TA, increase in IOP develops in 50%, starting 1 to 2 months after injection. Except for 1%, IOP was normalized by topical antiglaucomatous medication and without further medication after 6 months.

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Ocular blood flow and scleral buckling. A color doppler study

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Purpose: This study describes the blood flow changes using Color Doppler imaging in patients who underwent scleral buckling procedures for retinal detachment.

Methods: The study was carried out in 14 patients with monolateral rhegmatogenous retinal detachment without cardiovascular or blood disorder. Color Doppler imaging was used to measure blood flow velocities in the ophthalmic artery, central retinal artery and posterior ciliary arteries before surgery and three months after the operation.

Results: The authors observed a reduction of blood flow velocity in the central retinal artery (12.770 ± 0.306 vs 11.920 ± 1.105 cm/sec ($p < 0.045$)) with increase of the resistance index in the central retinal artery (0.633 ± 0.025 vs 0.672 ± 0.027 ($p < 0.001$)) and in posterior ciliary arteries (0.652 ± 0.054 vs 0.734 ± 0.040 ($p < 0.003$)). In some patients with poor visual recovery they found a marked reduction in the peak systolic velocity in the posterior ciliary arteries: 13.600 ± 1.794 vs 9.800 ± 2.066 cm/sec ($p < 0.047$), with an increased resistance index: 0.607 ± 0.085 vs 0.767 ± 0.049 ($p < 0.020$).

Conclusions: These results may be related to choroidal compression by the encircling band and indirectly by the release of local vasospastic substances. The authors suggest to reduce the surgical trauma as much as possible and avoid the release of vasospastic substances.

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The timing of silicone oil removal following retinal reattachment surgery and its effect on intraocular pressure

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Purpose: To investigate whether the timing of silicone oil removal following retinal reattachment surgery has a long term effect on the intraocular pressure (IOP).

Methods: A longitudinal retrospective study was undertaken of the medical records of patients who underwent vitrectomy and silicone oil tamponade for rhegmatogenous retinal detachment surgery. The main outcome measures were IOP at the last follow up appointment and duration between injection and removal of silicone oil.

Results: A total of 31 eyes of 31 patients (19 males and 12 females) were identified and included in the study. The mean age of the subjects was 53.4 ± 24.5 years and the mean interval between injection and removal of silicone oil was 6.1 ± 2.7 months. Linear regression analysis revealed that longer duration of intraocular silicone oil before its removal was significantly associated with higher levels of final IOP over a mean follow up of 15 ± 7.1 months ($r = 0.699$, $p < 0.001$). When the silicone oil was removed within 5 months from its injection into the vitreous, the mean final IOP was 13.1 ± 2.5 mmHg as opposed to 18.2 ± 1.0 in case silicone oil was retained for more than 5 months ($p < 0.001$).

Conclusions: Extended maintenance of intraocular silicone oil following vitreoretinal procedures may result in long-term ocular hypertension. Early removal of silicone oil could minimize the risk of irreversible damage to the trabeculum and subsequent development of secondary glaucoma.

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5-year incidence of age-related maculopathy. Reykjavik eye study

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Purpose: To examine age and sex specific 5-year incidence of drusen, retinal pigmentary abnormalities and age related macular degeneration in Icelanders 50 years and older at baseline.

Methods: 1045 randomly sampled persons were examined in 1996 and 5 years later in a follow up study 846 persons were re-examined. We did simultaneous colour stereo fundus photography centred on the fovea. Typing and grading of the macula photographs was done at the Reading Centre of Moorfields Eye Hospital in London using the international classification and grading system for age-related maculopathy (Surv. Ophthalmol. 1995).

Results: For right eyes, those 50-59 years at baseline the 5-year incidence for soft intermediate drusen 63-125mm sexes combined was 2.6%, for soft indistinct drusen > 125mm it was 0.7% and for hyperpigmentation >63mm it was 0.4% and for early ARM 9.3% and the same figures for those 80 years and older at baseline are 6.7%, 6.3%, 5.9% and 15.4%, respectively. Altogether 4 men and 3 women had progressed to geographic atrophy during the 5 years all 70-79 years at baseline and 1 man had progressed to neovascular AMD. Out of 11 persons with geographic atrophy in 1 eye at baseline 3 had progressed to bilaterality.

Conclusions: The 5-year incidence of geographic atrophy is 0.9% and the same for neovascular AMD is 0.1%. This high incidence of geographic atrophy is in agreement with our prevalence studies and higher than in other studies.

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A novel scalar measurement for assessing the degree of visualisation of the posterior pole

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Purpose: To describe a novel scalar measurement of posterior polar visualisation, and to assess its repeatability and internal consistency.

Methods: Patients had their posterior pole visualisation graded according to the appearance of the optic disc and the macula. Both scores were graded from 0 to 2 and combined to produce an overall posterior pole visualisation score from 0 to 4. The examinations were repeated 4 weeks later, and the repeatability and internal consistency of the scalar measurements evaluated. The components of the grading scores were as follows: Optic disc Grade 0 - Clear view of disc margin, blood vessels and adjacent nerve fiber layer. Grade 1 - Blurred blood vessels and/or nerve fiber layer, but clear disc margin. Grade 2 - Blurred disc margin, blood vessels and nerve fiber layer. Macula Grade 0 - Clear view of foveal reflex, peri-foveal blood vessels and nerve fiber layer. Grade 1 - Blurred foveal reflex, clear blood vessels and nerve fiber layer. Grade 2 - Blurred foveal reflex, blood vessels and nerve fiber layer.

Results: 14 cases had their posterior pole assessed on two separate occasions. Complete agreement of the posterior pole visualisation score for the two occasions occurred in 12 of the 14 cases. Cohen's kappa coefficient = 0.754, showing excellent agreement. Internal consistency was also very high, with Cronbach's alpha coefficient = 0.796.

Conclusions: This newly described scalar measurement for visualisation of the posterior pole is both repeatable and internally consistent. It may be a clinically useful tool for assessing patients with medial opacities, and their improvement following treatment.

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Macular pigment in diabetic patients

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Purpose: To compare macular pigment optical density (MPOD) in diabetic patients & controls.

Methods: A previously described method of heterochromatic flicker photometry with LED's (Mellerio et al, Current Eye Res, 25:37, 2002) was used to measure MPOD in one eye of 44 males & 46 females with diabetes & 54 male & 44 female healthy eyes in Sydney, Australia.

Results: The mean MPOD in the diabetes set of 0.14 ± 0.07 (mean \pm sd) was significantly lower ($p < 0.01$) than in controls (0.29 ± 0.07). There was a negative correlation between duration of the disease & MPOD ($r = 0.67$). There was a significant difference between the male & female normal patients (0.34 ± 0.07 & 0.25 ± 0.04) though this difference disappeared in the diabetic group, but the diseased eyes had significantly less MPOD than the same gender normal eyes. 12 patients had no retinopathy, 47 had non-proliferative retinopathy (NPR) and 39 had proliferative retinopathy (PR). For patients with (PR) the MPOD was 0.09 ± 0.04 which was significantly lower than in those with no retinopathy (0.26 ± 0.04) & for those with NPR, MPOD was 0.14 ± 0.06 which was significantly lower than for those with no retinopathy. Patients with panretinal photocoagulation (PRP) (0.13 ± 0.05) or macular laser treatment (0.10 ± 0.04) had significantly lower MPOD than those with no laser treatment. Patients who had PRP & macular laser had significantly lower MPOD than those who had PRP alone.

Conclusions: Macular pigment density is reduced by diabetes. It is also reduced by laser treatment of the disease, a reduction that may reflect loss of tissue volume due to the disease process and exacerbated further by cellular loss resulting from laser irradiation.

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Health related quality of life in 260 patients with ARMD compared to visual function and lesion size

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Purpose: The National Eye Institute Visual Function Questionnaire (NEI-VFQ) was used to assess self-reported visual quality of life (QOL) in correlation to lesions size, type of lesion and visual function in patients with ARMD.

Methods: A cross sectional study included 260 patients with all stages of ARMD. Examinations included ETDRS visual acuity, ophthalmoscopy, fundus photography and National Eye Institute Visual Function Questionnaire (NEI-VFQ). The NEI-VFQ is a 51-items questionnaire with 12 subscales based on a 10 to 100 scale. Higher scores are more favourable. Lesion size and type was assessed on digital fundus fotos according to the Age Related Eye Disease Study (AREDS) Classification.

Results: 260 patients were included. 199 patients were grouped into AREDS categories: 27 eyes in cat.1, 70 eyes in cat.2 and 102 eyes in cat.3. 206 eyes presented an active CNV (mean: 15,74 mm²), 84 eyes with a central scar (mean: 21,17 mm²), 26 eyes showed a geographic atrophy (mean: 10,04 mm²). Mean lesion size was 23 mm². Visual acuity (VA) ranged from hand movements to 20/12,5 (mean VA 0,55 + 0,39). Overall NEI-VFQ score was 73,5 compared to 88,4 in control group (n=360). The scores in the most relevant scales (near activity, distance activity, driving) were lower with increasing lesion size. Kruskal-Wallis test showed a significant positive correlation between visual acuity, lesion size and QOL in all AREDS categories analysed.

Conclusions: Health related quality of life in patients with ARMD is also significantly related to the size of the lesion. and not only to the measured ETDRS vision. QOL measurement should be part of a comprehensive assessment of visual function in ARMD in future treatment trials.

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Health care resource utilisation in 200 patients with ARMD in a one year longitudinal study

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Purpose: Routine diagnostics and treatment are not the only services for patients with Age related macular degeneration (ARMD). Magnifying devices, disease related in-and outpatient care, private nursing and other resources are frequently needed. Here we investigated the Health care resource utilisation of patients with ARMD in a longitudinal study.

Methods: Two hundred patients with all stages of ARMD were recruited to study their health care resource utilisation. They were presented with a questionnaire recording time and cost of medical treatments, hospitalisations, glasses, magnifying devices, nursing and other medical aids at baseline, 3, 6 and 12 month. Examinations also included ETDRS visual acuity, ophthalmoscopy, fundus photography and National Eye Institute Visual Function Questionnaire (NEI-VFQ) for each patient.

Results: While hospital visits, regular outpatient care and the use of emergency units remained stable over time, the need of additional home nursing, paid help with everyday tasks like shopping, cooking and transportation increased and was significantly related to decrease of visual acuity and measured quality of life. As an example help with meal preparation was needed from 12% at baseline compared to 31% after one year. The use of magnifying devices increased from 47% to 62%.

Conclusions: With progression of their disease patients with ARMD use more health care resources. A significant part of the generated cost are covered privately by the patient in order to cope with the disease. This extra burden of ARMD patients should not be underestimated in the economical dimension of the disease.

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Central serous chorioretinopathy associated with systemic corticosteroid

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Purpose: To report two patients who developed chronic central serous chorioretinopathy after using systemic corticosteroid.

Methods: (Case Report) We present two patients with decreased visual acuity that associated with systemic corticosteroid.

Results: First case, a 56-year-old male with thyroid ophthalmopathy, received IV solumedrol 500mg for 3 days just after orbital decompression and then tapered with oral prednisolone. On FAG, ICG and OCT, bilaterally multifocal RPE changes and recurred central serous chorioretinopathy on his right eye were noted. Second case, a 44-year-old female who had received renal allograft before 13 years, had long-term oral prednisolone for prevention of allograft rejection. Bilaterally multifocal RPE changes and chronic central serous chorioretinopathy on her right eye were noted on FAG.

Conclusions: These two cases demonstrate that systemic corticosteroid can be associated with central serous chorioretinopathy. Before, during and after high-dose or long-term use of systemic corticosteroid, regularly periodical fundus examination and careful medical history are necessary to prevent serious complications.

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Macular thickness in patients with choroidal neovascularization determined by RTA and OCT 3. Comparative results

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Purpose: The authors conducted a study to compare retinal thickness in patients with choroidal neovascularization (CNV) using optical coherence tomography (OCT) and retinal thickness analyzer (RTA).

Methods: Eleven eyes from 11 patients with subfoveal choroidal neovascularization were examined with OCT and RTA.

Results: Three patients could not be explored by RTA due to lack of fixation and high myopia. Mean foveal thickness was $289.9 \pm 92.1 \mu\text{m}$ with OCT and $207.7 \pm 60.8 \mu\text{m}$ with RTA. Mean perifoveal thickness was $293.8 \pm 46.3 \mu\text{m}$ and $200.8 \pm 61.3 \mu\text{m}$ respectively. Maximal perifoveal thickness measured by OCT was $335 \pm 104.0 \mu\text{m}$ and $316 \pm 86.8 \mu\text{m}$ by RTA.

Conclusions: OCT and RTA are able to detect increases in retinal thickness due to the presence of CNV and of fluid extravasation. They can be used in measuring retinal thickness in patients with CNV though measures are not comparable between both systems.

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Cardiovascular disease and development of macular edema in patients with uveitis

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Purpose: The development of macular edema (ME) has a decisive effect on the visual outcome of many ocular disorders including uveitis, diabetic retinopathy and retinal vein occlusions. The exact pathogenesis of inflammatory ME is not known, nor the factors which determine either visual recovery or irreversible loss of vision. Systemic vascular diseases were reported in patients with ME due to diabetic retinopathy and vein occlusions. In this study we evaluate the impact of generalised cardiovascular diseases (CVD) on the development of ME and visual acuity in uveitis patients.

Methods: 97 consecutive uveitis patients and 49 non-uveitis control patients were given an extensive questionnaire for the presence of CVD, risk factors and family history of CVD, smoking and drinking habits, diet and stress. Clinical data were obtained from the medical records (diagnosis, presence and duration of ME, the impact of ME on the visual acuity, the presence of vasculitis, retinal occlusions) and were compared to the outcome of the questionnaire.

Results: The prevalence of CVD among patients with uveitis was 13.4% (13/97) and 20.8% (10/48) among the controls ($p=0.25$). No differences were found for the prevalence of CVD in ME versus non-ME uveitis patients ($p=0.18$). Logistic regression showed that the simultaneous presence of 4 or more risk factors correlated positively (0.011) with the presence of ME. Correcting for age, the presence of multiple CVD risk factors did not correlate significantly with the presence of ME.

Conclusions: This study reports on age being a major risk factor for the development of ME in uveitis. The contribution of the presence of CVD or multiple risk factors for CVD to the development of ME is little, compared to the influence of age.

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Effect of macular pigment quantity on the directional properties of foveal cones according to age

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Purpose: Directional properties of foveal photoreceptors depend on their structure, likely to be deteriorated with age. We recognize now a protect role of macular pigment from oxydative damage in the central retina, which increase during ageing. Therefore, we have measured directionality factor and ratio of directional to diffuse flux of foveal cones according to optical density of macular pigment in 30 healthy subjects (aged 20 - 70 years).

Methods: 30 healthy volunteers are included in the study: 3 groups of different age (young adults, middle age adults and senior adults). We have determined directional characteristics of foveal photoreceptors by measuring the distribution in the pupil of light reflected by a retinal area (diameter: 2 degrees) centred on the foveola. The macular pigment optical density was also assessed by reflectometry (sample field: 2 degrees).

Results: The ratio of directional to diffuse flux decreases significantly with age. No age correlation in the directionality factor was found, nevertheless we have noted that the standard deviation is higher in the senior group than in the young group. Furthermore, the optical density of macular pigment tends to decrease with age.

Conclusions: The ratio of directional to diffuse flux is directly linked to the photoreceptor outer segment - retinal pigment epithelium interface. Consequently, its significant age-related decrease seems to be due to an alteration of this interface in aged subjects and might be associated with a decrease of macular pigment density. Moreover, an important dispersion of the directionality factor in senior group reveals a modification of the structure of their foveal cones.

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Fixation behaviour in occult and classic choroidal neovascular membranes before and three months after verteporfin photodynamic therapy

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Purpose: The prospective study was undertaken to analyze the fixation behaviour in relation to visual acuity and optical coherence tomography (OCT) findings before and 3 months after the first Verteporfin Photodynamic Therapy (PDT) in occult and classic choroidal neovascular membranes (CNV) secondary to Age Related Macular Degeneration (ARMD).

Methods: Eleven patients with classic subfoveal CNVM and 13 patients with occult subfoveal CNVM treated by PDT for the first time were included. The location and stability of the fixation point was determined by scanning laser ophthalmoscope (SLO), OCT findings of macula, and visual acuity were recorded for each patient before and 3 months after PDT.

Results: Stable and central fixation was present in the classic CNV group before and after PDT. Percentage of eccentric fixation in the group with occult CNV was 23% (3/13) before PDT, it increased to 30% (4/13) after PDT. Mean visual acuity increased in both groups and there was no statistically significant difference in mean visual acuity improvement (visual acuity expressed in logMAR) between the occult and classic group (paired t-test, p=0.77, p>0.01) but in the occult group, mean visual acuity only increased in patients who fixated centrally before PDT. Retreatment with PDT was higher in classic group (63%) than occult group (38%). Improvement in OCT findings were more important in the group with occult CNV.

Conclusions: Fixation is a complex behaviour that is unpredictable by visual acuity and OCT findings alone. In occult CNV, PDT may be more effective as regards increase of visual acuity after 3 months in patients with central fixation before PDT.

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Volume changes of the macula measured by OCT after phacoemulsification and PCL implantation

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Purpose: The purpose was to measure the change in the volume of the macula after phacoemulsification and foldable PCL implantation (phaco +PCL) by OCT (Optical Coherent Tomograph) and to compare two groups: Patients in Group A received steroids in the postoperative period, patients in group B did not receive steroids but only non-steroidal anti inflammatory eyedrops.

Methods: Twenty consecutive cataract patients were enrolled. They had no other ophthalmic disease. They did not undergo previous ophthalmic surgery. The phacoemulsification (Accurus equipment) and the implantation of foldable PCL were carried out without any complications. The average phaco time was 1.44 min (0.12-5.2). The average visual acuity was 0.6 on the first postoperative day, and 0.8 at the end of the first week. OCT examinations were performed before surgery (if possible) and at the end of the first and of the sixth week. We compared macular volume in both groups within the 3.5 and 6 mm diameter ranges.

Results: The average macular volume in group A before surgery was 2.49/6.93, at the end of first week was 2.51/7.06 and at the sixth weeks 2.54/6.98 within the 3.5/ 6 mm diameter ranges. At the same time in group B the macular volume was respectively 2.54/7.03, 2.54/7.09 and 2.6/7.09. New posterior vitreous detachment was observed in 6 cases.

Conclusions: No significant differences were detected in macular volume before or after surgery at any time or in any group. The change in the posterior vitreous surface might be a result of the pressure changes in the vitreous caused by phaco.

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UBM and OCT studies in cases of rhegmatogenous retinal detachment and in post-surgery follow-up

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Purpose: To study the vitreoretinal junction at the ora serrata (vitreous base) and at the macular region with UBM and OCT respectively in cases of rhegmatogenous retinal detachment and in the healing period after ab externo surgery.

Methods: 47 patients were included in these studies (17 male and 30 female, mean age 52,8 y). All patients were affected by rhegmatogenous retinal detachment, without macular involvement. UBM and OCT were performed before as well as 1 and 3 months after the surgical intervention. The following parameters were recorded: (i) modification of inter-space between retina and vitreous measured by UBM at the vitreous base and expressed in millimeter (ii) variation of thickness (micron) and volume (mm³) of macular area as detected by OCT.

Results: About 35 % of patients showed at the 1 and 3 months follow-up a remarkable changes of the inter-space between retina and vitreous base (ora serrata) as well as changes of both thickness and volume of the macular area: (i) mean decrease of inter-space between retina and vitreous base was 52 % (ii) mean decrease of the macular thickness was 35 % (iii) mean decrease of the macular volume was 35 %.

Conclusions: These findings suggested that scleral cerclage may affect not only the vitreous base but also the macular region resulting in increase of both thickness and volume of the underlying retina. Pathophysiologic role of mechanical forces played in the development of rhegmatogenous retinal detachment will also be discussed.

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Short-wavelength automated perimetry and retrobulbar hemodynamics in age-related maculopathy (ARM)

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Purpose: To investigate the correlation between visual function assessed by short-wavelength automated perimetry and retrobulbar hemodynamics assessed by Color Doppler Imaging in patients with age-related maculopathy (ARM).

Methods: In a prospective study 56 patients with ARM were included. Short-wavelength automated perimetry was performed in the 10 degree field with a conventional perimeter (Humphrey field analyzer, prg. 10-2). In the ophthalmic artery (OA), the nasal and temporal short ciliary arteries (PCA), and the central retinal artery (CRA) flow velocities (peak systolic and end diastolic velocities) were measured with Color Doppler Imaging (Siemens Sonoline Sienna) and resistive indices calculated.

Results: The end diastolic velocity in the CRA was significantly correlated with mean short-wavelength sensitivity ($p=0.007$, $r=0.37$). All other parameters lacked significant correlation.

Conclusions: Decreased sensitivity in short-wavelength automated perimetry is known to be a functional risk factor for exudative age-related macular degeneration (AMD). In this study we found a correlation of downstream end diastolic velocity in the CRA and visual function in ARM. These results suggest the possible impact of disturbed microcirculation in the pathogenesis of AMD.

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Computer-assisted microaneurysms counting on digitized retinographies

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Purpose: In order to improve human grader reliability in the identification and counting of microaneurysms (MA) on color fundus images (retinographies), software was developed for eye movement compensation, color correction and identification of each MA by its coordinates.

Methods: Existing color slides were digitized using a Minolta SC-100 slide scan to produce non-compressed TIFF files (RGB), of 4032 by 2688 pixels. The images contain two major objects over a black fundus: the patient identification (ID) and; the region of interest (ROI). The fundus image was segmented and enhanced without considering the background or the patient ID. The segmentation of the ROI is achieved by a Hough transform for the detection of the circle and the two parallel lines that enclose it. Due to the lower quality of the images available to be displayed on the computer screen, two new digital versions were created: one with color correction considering the whole color space (option A), and the other with color correction considering each of the RGB channels separately (option B). For the data processing of the MAs counting several parameters were computed such as the lifetime and the quality factor.

Results: The human grader is able to choose among the three different versions of the same fundus image. The two graders involved elected option B as the best one.

Conclusions: This work showed the possibility to improve the performance of the human graders in detecting MAs over color fundus images. Simultaneously, the earmarked MAs will provide detailed information for a better description of MAs as seen on regular films, such as the color and shape information that will be useful for an automatic detection system.

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Macular hole : a rare complication of Purtscher's retinopathy as revealed by OCT

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Purpose: Purtscher's retinopathy is a severe acute vascular retinopathy due to sudden impairment of choroidal and retinal blood supply. It may have traumatic, inflammatory or hematologic origin, and usually resolved in few weeks. The aim of the present study was to describe an unusual case and outcome of a Purtscher's retinopathy.

Methods: Case report of a Purtscher's retinopathy occurred after vomiting in a male affected by high myopia. The disease was followed, the first time, by OCT.

Results: A male patient with -14.0 D myopia on both eye was examined due to sudden visual loss of the right eye after an episode of severe vomiting. Visual acuity was finger-counting at 30 cm. Clinical examination and OCT revealed retinal edema at the papillo-macular area with subretinal haemorrhages. Fluorescein angiography showed occlusion of the cilio-retinal vessels. After resolution of retinal edema and reperfusion of cilio-retinal vessels, OCT verified typical full thickness macular hole.

Conclusions: Although, the Purtscher's retinopathy is potentially reversible disease, in patients affected by high myopia it may be complicated with macular hole. The authors suppose that abnormal anatomical situation of the posterior pole of highly myopic eye might play a role in the development of an unusual occurrence and outcome of Purtscher's retinopathy.

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Fundus autofluorescence of the retinopathy associated with the mitochondrial A3243G point mutation

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Purpose: To characterise the clinical features and fundus autofluorescence in patients with the A3243G mutation associated with maternally inherited diabetes and deafness (MIDD).

Methods: Patients presenting with visual symptoms, or signs suggestive of MIDD-related maculopathy, and whom tested positive for the A3243G mutation were investigated clinically, including retinal autofluorescent imaging.

Results: Twelve probands with macular dystrophy and the A3243G mutation were identified. Of these, 5 were visually asymptomatic, 4 were diabetic, 10 had neurosensory deafness, 3 had systemic neurological abnormalities, one had cardiac disease and one had renal disease. A family history for known A3243G clinical features was present in only 5 pedigrees. Fundus findings consisted of two primary phenotypes: discontinuous perifoveal atrophy orientated circumferentially (9/12) or an appearance consistent with pattern macular dystrophy (3/12). In both phenotypes, pale subretinal deposits and subretinal pigment clumping were seen.

Conclusions: The A3243G mutation is associated with abnormalities of multiple organ systems including the retina, which shows a variable clinical presentation. Fundus autofluorescence of the maculopathy associated with MIDD reveals a recognizable phenotype that has distinctive features from juvenile macular dystrophies and geographic atrophy in AMD. Determination of the A3243G mitochondrial genotype is important in the assessment of an atrophic maculopathy.

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How can combinations of F6H8 and silicone oil be used in treatment of retinal detachment?

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Purpose: To explore whether combined use of the semi-fluorinated alkane perfluorohexyloctane (F6H8) and silicone oil can act as an inferior tamponade: a) solutions of F6H8 in silicone oil, and b) double filling with F6H8 and silicone oil.

Methods: Surface contact for different combinations was photographed in transparent model eye chambers made of surface modified PMMA that mimics the retina: a1) solutions of F6H8 in silicone oil with densities of 1.01, 1.03 and 1.06. a2) for the 1.06 solution the surface contact was plotted against volume and compared with F6H8 and n-octane. b) different proportions of F6H8 inferiorly and silicone oil superiorly was injected and merged to a single bubble. The model was agitated to study dispersion.

Results: a) Solutions of F6H8 in silicone oil do not have the good contact properties of pure F6H8. The higher the density of the solution, the better was the contact. b) Double filling with F6H8 and silicone oil does not provide good simultaneous superior and inferior tamponade. As the proportion of silicone oil increased the area of lost contact increased and progressed inferiorly. Double fill reduces dispersion.

Conclusions: The solutions behave like silicone oil in that they are hydrophobic. Nonetheless, the mere availability of a silicone oil-like agent that sinks is sufficiently attractive to warrant clinical trial. Solutions of F6H8 with silicone oil of other proportions or with other semi-fluorinated alkanes may be of interest. The model eye chamber is an efficient way of screening for candidate tamponade agents.

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Anti-retinal auto-antibodies are present in patients with early and late stage age-related macular degeneration

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Purpose: There is increasing evidence that the immune system is involved in the pathogenesis Age-related macular degeneration. In this study, we assess whether these auto-antibodies are the result of the healing response of the development of choroidal neovascularisation (CNV) or whether they are present before the end-staged event

Methods: Patients with bilateral drusen (n= 13) and with CNV (n=19) were recruited from the Macular Clinic whilst age-matched normal controls (n=5) were recruited from other Clinics. The serum of these subjects were analysed for Anti-retinal autoantibodies with murine (balb/c) retinal sections using indirect immunocytochemistry.

Results: Three staining patterns were identified, namely nuclear layers, outer plexiform layer and inner segments of photoreceptors. Chi-Square Test confirmed that all three patterns were significantly different from normal in the CNV group (p<0.05) whilst the staining pattern in the outer plexiform layer staining (p<0.05) were significantly different from normal in the drusen group.

Conclusions: Anti-retinal autoantibodies are present in patient with bilateral drusen and exudative AMD. This implies that these autoantibodies are not simply a reactive response to the CNV but might play a significant role in the pathogenesis of AMD. The role of these autoantibodies in pathogenesis and as a prognostic factor for exudative AMD warrants further investigation

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Results of macular hole surgery with ILM peeling

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Purpose: To report the anatomical and functional outcome of macular hole surgery with internal limiting membrane (ILM) peeling. To analyse if hole form factor (HFF) may be used as a predicting factor for anatomical results of macular hole surgery.

Methods: We examined prospectively 48 eyes of 48 patients with idiopathic full-thickness macular hole (MF) undergoing vitrectomy with ILM-peeling and compared the best-corrected visual acuity with postoperative features of optical tomography (OCT) and scanning laser ophthalmoscopy (SLO) images. The HFF was definite as a relation between the sum of the left and right arm length of macular hole on OCT image to the base diameter of the hole.

Results: The treatment was anatomically successful in 38 of 48 eyes (79.1%), after secondary intervention in 85.8%. 70.83% showed an improvement of at least two Snellen lines. Mean retinal thickness was 410nm preoperatively. Postoperatively it decreased to 325nm (P<0.01, Student t-test). The foveal thickness ranged from 80 to 300nm (mean 170nm). Preoperative SLO of 13 patients disclosed retinal folds in 7 cases. In 5 eyes these correlated with cystoid macular edema, elevated edges and subretinal cuff on OCT images, which disappeared after surgery. Fixation-point was central in 66.6% of successful cases. A hole form factor (HFF) greater than 0.9 showed anatomical success in 100% and less than 0.5 only in 50% of cases.

Conclusions: Biomicroscopic and OCT-documented results correlated with the functional improvement of visual acuity and fixation. The hole form factor can predict the postsurgical anatomical outcome.

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Topographical behaviour of choroidal neovascularisation after PDT

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Purpose: Aim of the study was to examine the change of size and topography of choroidal neovascularisation (CNV) in age-related macular degeneration (AMD) treated by means of photodynamic therapy (PDT).

Methods: 50 eyes of 49 patients (32 female, 17 male, mean age 78,8 yrs, range 50-97yrs), who had photodynamic therapy of classic CNV in AMD, were followed up by means of fluorescein angiography. Indication of PDT was selected according to TAP guidelines. Additionally carefully examinations including measurement of best corrected visual acuity (VA) and fundus biomicroscopy were performed 3 months after each PDT session. Images of the early phase of fluorescein angiography were selected for digital measurements of lesion size, diameter and topographical changes.

Results: CNV tends to enlarge most likely after the first treatment (mean plus 64% of lesion size), and slowly stabilizes after retreatment (plus 25% in size after second PDT, plus 24% after third treatment). There is a significant tendency (p=0,03) for the CNV not to enlarge at the temporal border, but towards other directions (inferiorly, nasally and superiorly). Compared to the initial lesion size small CNV enlarge relatively the most (p <0,001).

Conclusions: Our results indicate that progression of lesion size can be expected most likely after the first PDT, suggesting not to falsely consider the first PDT as a failure and thus to cease therapy. We cannot confirm that PDT is more effective in cases with smaller CNV. The latter tends to enlarge relatively more than already extended CNV. Further improvements of PDT are needed in order to obtain an early stabilization of the process especially in cases with small lesion diameter and good visual acuity. We have no explanation why CNV prefers not to enlarge temporally.

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Peripapillary idiopathic choroidal neovascularization in young patients

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Purpose: Introduction: Peripapillary idiopathic choroidal neovascularization is a rare disorder when occurring in young patients. The purposes of this study were to describe clinical and angiographic features, clinical course, risk factors, and to compare with other peripapillary choroidal neovascularizations related to a definite cause.

Methods: Patients and methods : Four young patients (three males and one female; age range, 9-33 years) files were retrospectively analysed. All these patients presented choroidal neovessels located to less than one papillary diameter distance from the margin of the optic disc. Visual acuity measurement, fundus examination, fluorescein and ICG angiographies, optical coherence tomography were performed in all the patients. Only two eyes were assessed with B-mode echography.

Results: Results: Pseudotumoral biomicroscopic and angiographic findings, rapid and pejorative clinical course, hypermetropia and changes of the peripapillary pigment epithelium were constant in these four young patients.

Conclusions: Conclusion: When comparing with data from previous studies, the data of this study were quiet different and this lead to the hypothesis that these four cases could belong to a singular entity.

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Photodynamic therapy with Verteporfin for choroidal neovascularization in patients with age-related macula degeneration

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Purpose: To evaluate the safety and short-term visual effects of photodynamic therapy (PDT) with verteporfin, in patients with choroidal neovascularization (CNV) caused by age-related macula degeneration (AMD).

Methods: A retrospective review of 29 eyes with AMD treated with verteporfin. Lesions' greatest linear dimension was 5400 micrometers. Best-corrected visual acuity ranged from 1/10 to 5/10. At follow-up examinations every 3 months, retreatment was applied if angiography showed fluorescein leakage.

Results: The mean patients' age was 73.85 years. A mean of 3.31 treatments per eye was performed. The mean follow-up was 13.62 months. Most patients (55.17%) stabilized or improved their visual acuity over one-year period. The effect was greatest among eyes with predominantly classic CNV.

Conclusions: Although the follow-up time and the number of patients in this study were limited, the use of PDT seems to reduce the risk of vision loss in CNV caused by AMD.

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Validation of a semi-automatic drusen quantification software

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Purpose: 1) An inter-institutional comparative validation of computer-aided drusen quantification 2) Assessment of the practical application of the software

Methods: We are developing computer-aided drusen quantification software based on Adobe Photoshop[®]. Ten images with significant numbers of drusen were selected. Graders from the Columbia University and from King's College Hospital (one expert and one non-expert from each institution) quantified the percentage of drusen in the middle Wisconsin subfield. The differences between the mean software grading from each institution and each grader were computed. Secondly, images from 100 consecutive images of patients with AMD were analysed to assess the practical limitations of the software.

Results: Inter-institutional comparison: the mean difference over all images was 6.0 +/- 8.5%. Only three images showed greater than a 10% difference. After the resolution of disagreements in threshold for segmentation in two images, the mean difference was 3.3 +/- 6.7. Poor image quality and lack of stereo, caused a tendency to include RPE atrophy as drusen. There were no significant differences between expert and non-expert graders. Practical application: 79 were found to be suitable for analysis by the software. 13 had extensive mixed RPE changes limiting drusen identification, 5 had reticular drusen, which are poorly identified by the software, and 3 had multiple small areas of RPE atrophy which are difficult to distinguish from drusen.

Conclusions: Comparable measurements were achieved by the two institutions and by the expert and non expert graders, demonstrating portability and feasibility. This semi-automatic software has the potential to assess the change of drusen in a drusen reduction trial.

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Role of trypan blue in epiretinal membrane surgery

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Purpose: Idiopathic epiretinal membrane results from detachment of the posterior hyaloid and is believed to be related to naturally occurring defects in the internal limiting membrane of the retina; vitrectomy and peeling are treatment of choice. Recently it was shown that Trypan blue (TB) stains epiretinal membrane and internal limiting membrane. It allows selective and complete removal, facilitating surgery, with less retinal damage. An ultrastructural study was performed showing on one hand ultrastructural features of idiopathic epiretinal membranes (ERM) and on the other hand the presence of internal limiting membrane respecting the retinal side, demonstrating the value of a selective stain during vitrectomy.

Methods: 15 cases of ERMs underwent surgery successfully. After pars plana vitrectomy and induction of posterior vitreous detachment, 2 ml TB 0.15% is injected over the ERM in an air filled eye. The stained tissue is peeled with intraocular forceps. Specimens are at once collected in 4% glutaraldehyde for electron microscopic study.

Results: Results show that TB allows a complete ERM and ILM peeling. The major cellular contingent is represented by glial cells and participates actively in neocollagen synthesis. The presence of photoreceptor fragments supports the hypothesis of a migratory movement of the retinal cells towards the vitreoretinal side.

Conclusions: Trypan blue does not seem to present toxic effects and might be very helpful in making more easy epiretinal membrane surgery.

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Radial optic neurotomy for central retinal vein occlusion

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Purpose: No efficient treatment is actually available for severe forms of central vein retinal occlusion (CRVO). Recently, radial optic neurotomy (RON) was proposed by Opremack et al. with good results.

Methods: Prospective analysis of 12 eyes of 12 patients with severe CRVO with visual acuity of 20/200 or less. They underwent fluorescein angiography, visual field testing with Goldman perimetry before and after the RON.

Results: The average age was 68 years. One vitreous and one sub-retinal transient haemorrhages were noted after the procedure. Ten patients had clinical improvement as determined by fluorescein angiography. Venous anastomosis was observed in 6 cases. In all cases, an improvement of the central scotoma was noted. A temporal fascicular scotoma corresponding to the site of neurotomy appeared in 9 cases. By 4 months, a mean visual acuity increase of 16 letters on ETDRS was noted. Vision improved by 3 lines or more in 8 eyes.

Conclusions: Our results are encouraging. A randomized study should be conducted to assess the efficiency of radial optic neurotomy.

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Transpupillary Thermotherapy (TTT) for the treatment of new onset subfoveal CNV in AMD patients ineligible for PDT.

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Purpose: To assess the efficacy of TTT for the treatment of new onset subfoveal CNV in AMD patients ineligible for PDT.

Methods: A retrospective noncomparative study of 48 eyes of 48 patients who underwent TTT for subfoveal CNV secondary to AMD. Snellen visual acuity and fluorescein angiographic characteristics were assessed. Patients deemed treatable by the TAP protocol were excluded. TTT was delivered using a diode laser at 810nm via a Mainster Wide Field contact lens. A retinal laser spot size of 1.8-4.5 mm was used with a power setting 320-1000mW. Outcome was determined by Snellen visual acuity, clinical examination and fluorescein angiography.

Results: 83% (n=40) of patients had predominantly occult (54% pure occult, 29% minimally classic); 17% (n=8) had predominantly classic subfoveal CNV but with visual acuity <6/60. Haemorrhage was present in 79% of lesions at presentation. Mean follow-up time was 23 weeks (range 16-52 weeks). 45% of patients avoided moderate visual loss (= 2 lines) in the predominantly occult group (42% pure occult, 50% minimally classic). Visual acuity increased (mean 1.7 lines) in 47.5% of predominantly occult group (46% pure occult, 50% minimally classic). All patients with predominantly classic lesions had stable or increased visual acuity. 91.7% (n=44) eyes demonstrated a decrease in leakage on fluorescein angiography and clinical examination. All eyes demonstrated late staining of subretinal fibrosis. There was progression of haemorrhage in one eye. No other complications were noted.

Conclusions: TTT has a potential benefit in reducing exudation associated with subfoveal CNV secondary to AMD and maintaining or improving visual acuity.

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Surgical management of an angiomatous retinal lesion in Cowden Disease

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Purpose: To show the surgical management of an atypical ocular angiomatous localization of Cowden Disease.

Methods: A 50-year-old woman with a multiple tumor history (breast, thyroid, ovary and mucocutaneous hamartomas) was diagnosed with Cowden Disease that was confirmed by the discovery of a mutation in the PTEN gene. She had developed visual loss in her left eye two years previously but had not consulted her ophthalmologist. Visual acuity was 20/20 OD and 20/200 OS. Right eye fundus examination showed an epiretinal membrane associated with a peripheral and temporal inferior angiomatous lesion.

Results: Treatment consisted in cryoapplication and surgical removal of the epiretinal membrane after cautious central vitrectomy. Although the anatomical result was satisfactory, visual acuity remained unchanged.

Conclusions: Some ocular lesions of hamartomatous origin have been described in Cowden's disease. However, to our knowledge, this is one of the first documented cases of retinal angiomatous lesions in a patient with PTEN gene mutation.

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F6H8 in treatment of retinal detachment. Anatomic result better than functional?

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Purpose: To report our clinical experience using the semi-fluorinated alkane perfluorohexyloctane (F6H8) as tamponade in complicated retinal detachment compared to silicone oil.

Methods: 15 patients with "hard to manage" retinal detachments were enrolled in a study of 3-4 weeks tamponade with F6H8. 15 controls were matched for similar pathology. We monitored complications and results (attached, partially detached and completely detached) as well as pre- and postop BCVA, IOP, F6H8 droplet formation, cataract progression and causes for reoperations. The mean follow-up time in the F6H8 group was 13 months and in the silicone oil group 9 months.

Results: Temporary elevated IOP in 2 patients and droplet formation in the AC in 7 patients was registered as complications of F6H8. In the F6H8 group 12 patients had an attached retina, 1 had a detachment temporarily and 2 had a complete detachment. 6 patients had silicone oil in situ at follow-up. In the control group 12 patients had an attached retina, 2 had a detachment inferiorly and 1 patient had a complete detachment. 2 patients had silicone oil in situ. In the F6H8 group BCVA was improved in 3, remained the same in 4 and deteriorated in 8 patients. In the control group BCVA was improved in 11, remained the same in 3 and deteriorated in 1 patient.

Conclusions: In this pilot study the most desperate cases with were selected for F6H8 tamponade. The anatomic result of F6H8 as a 3-week tamponade seem similar to silicone oil. F6H8 may increase the chance of reattachment inferiorly. The poor visual function in the F6H8 group may depend on case selection. The higher frequency of reoperations indicate that patients in the F6H8 group had a more complex pathology. Whether F6H8 may induce retinal damage is discussed.

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ICG angiography in MEWDS

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Purpose: To assess the value of indocyanine green (ICG) angiography for the diagnosis and the follow-up of Multiple Evanescent White Dots Syndrome (MEWDS).

Methods: Observational Case Report. A 29-year-old woman complained of a quick decrease of visual acuity in both eyes with central scotoma.

Results: Fundus examination, fluorescein and ICG angiographies showed lesions in accordance with the diagnosis of MEWDS. The progressive disappearance of the dark spots on ICG contrasted with the persistence of a granular aspect of the macula.

Conclusions: ICG angiography demonstrate typical lesions for the diagnosis and the follow-up of MEWDS.

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Use of prisms in congenital nystagmus without strabismus

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Purpose: Congenital nystagmus is a difficult to treat oculomotor dysfunction, responsible for often severe bilateral amblyopia. The goal of prism treatment is to decrease or stop the nystagmus, and thus, to stop accompanying head movements, and to fight bilateral amblyopia.

Methods: 15 patient files were retrospectively analyzed over several years. Children were assessed at birth and treatment was initiated at 3 months of age. Only isolated nystagmus (non-eventful ophthalmologic examination: no strabismus, no other abnormalities) was taken into account. The prisms used were conventional prisms (4 to 5 dioptres) rapidly inserted in the eyeglasses.

Results: They were assessed taking into account: (a) The decrease of nystagmic movements (b) The absence of abnormal head movements (c) Behavior: awakening state, change of attitude, interest for pour the environment in the days following prism insertion. Assessing the development of visual acuity remains a difficult task to perform early and is often done in a later stage. Comments: The technique is non-invasive, easy to implement, and well accepted. The precocity of treatment is a success factor.

Conclusions: The authors recommend an early management, a prolonged monthly follow-up, pleoptics rehabilitation as early as three years of age.

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Unilateral blepharospasm induced by diplopia - failure of alleviation by monocular occlusion

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Purpose: To describe a unique case of unilateral blepharospasm induced by diplopia (interventional case report).

Methods: We followed up a patient with an intracavernous meningioma who had diplopia from 3rd, 4th and 6th nerve palsies in the right eye. Right involuntary lid closure also developed. We attempted to treat her symptoms with botulinum toxin injections, lid props and occlusive contact lenses.

Results: Botulinum toxin injections to the medial rectus failed to alleviate the diplopia. We felt that the right involuntary lid closure was due to diplopia and attempted monocular occlusion with a right occlusive contact lens. However, the right eye closure persisted but on left monocular occlusion, the patient was able to keep her right eye open for short periods. However, this caused vertigo and the left contact lens had to be removed. A lid prop in the right eye was also unsuccessful. She finally required both botulinum toxin injections for her blepharospasm and an occlusive contact lens in the right eye for the diplopia.

Conclusions: Certain brainstem mechanisms controlling unilateral eye closure may be stimulated by diplopia and result in a unilateral blepharospasm.

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Effect of monovision correction on binocular vision: measurement of the horopter

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Purpose: The aim of this work is to study the effects of monovision correction on binocular vision by measuring the Horopter. Determining this function, information about fusion and stereopsis will be achieved. These results will be compared with the results obtained with bifocal contact lenses.

Methods: Three presbyopic patients participated in the study. Two methods for presbyopic correction were used: MV correction and bifocal contact lenses. The horopter was measured with the apparent frontoparallel plane method. The device consists on tracks equally spaced in angle over which rods can be moved. The patient adjusts the distances of the rods until they all appear to be at the same distance as the middle rod in the plane parallel to the subject's face. In our experience, the fixation rod was one meter from the patient. The position of each rod was obtained from ten measurements. For the Monovision correction, subjects were fitted with a contact lens for distance correction in one eye and with the near correction on the other. The adaptation was done to minimize the resulting anisometropia. The bifocal contact lenses used were Acuvue bifocal in all cases.

Results: For an emmetropic observer, all rods are placed at the same distance as the middle rod located at 100 cm. For patients wearing monovision correction, the horopter is rotated around the fixation point towards the magnified eye. The horopter's rotation was increased with the addition. With binocular contact lenses, the horopter is not biased.

Conclusions: With monovision correction, binocular vision is disturbed due to the differences in image size between the two eyes. Patients wearing bifocal contact lenses show the best behaviour near to the theoretical value.

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Longitudinal observation of a congenital pulsating proptosis in a child

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Purpose: To report ten years observation of a case of congenital pulsating proptosis (exophthalmos) in a 10-year-old boy, caused by congenital anomaly of the right orbit.

Methods: 1. Ophthalmological investigation; 2. Computed Axial Tomography (CAT) and a Magnetizing Resonance Imaging (MRI) studies; 3. Consultations with pediatrician, neurologist, neurosurgeon were provided.

Results: A 10-year-old boy is presented with facial dysmorphism - the right orbit is 2 mm lower than the left one, and with ocular pulsation, simultaneous with cardiac activity and 4 mm amplitude. Besides that, epiblepharon and trichiasis were determined too. VOD = 0,8 with +1,0 d sph and + 1,0 d cyl 110 0; VOS = 1,0. The child is in a good condition, no operated. General damages are not established.

Conclusions: Although pulsating proptosis usually is found in adults after trauma and injury of a carotis interna they should be found as a congenital manifestation too and must be considered in the differential diagnosis of structural orbital lesion in children.

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Report of 5 cases of bilateral nonarteritic anterior ischemic optic neuropathy associated with sleep apnea syndrom.

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Purpose: Recently, reports by Mojon suggest an association between sleep apnea syndrom (SAS) and glaucomatous neuropathy, and also between SAS and nonarteritic anterior ischemic optic neuropathy (NAION). We report five cases of bilateral NAION associated with SAS.

Methods: We recruited 5 patients with bilateral NAION; 4 men and 1 woman. The age ranged from 48 to 73 years. We eliminated by clinical, biological and laboratory exams, the diagnosis of giant cell arteritis. Then, we performed overnight polysomnography and determine the respiratory disturbance index during night sleep, a value used to diagnose and grade SAS.

Results: There were more men than women. The SAS grading determinate all patients in severe form of SAS.

Conclusions: Our study is very similar to the Mojon's study regarding to the age, sex, and clinical signs of the patients. In all cases, the common therapeutics were not efficient. With our findings, we think that it's interesting to performed overnight polysomnography for every NAION. If the SAS is confirmed, we can purpose a treatment by continuous positive airway pressure (CPAP). The efficiency of the CPAP to treat the NAION and to prevent on the involvement of the second eye has to be studied.

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The influence of anisometropia on the monovision success

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Purpose: The effect of the anisometropia, due to mono-vision (MV), on the success ratio of the correction technique for presbyopia is analyzed.

Methods: Twenty presbyopic subjects having different ages and additions are considered in this study. All had 6/6 Snell visual acuity in both far and near vision after correction and normal binocular vision. The dominant eye was fitted with the distance correction and the fellow eye with the near correction. This is the ordinary procedure when MV technique is applied. After a training period a group of ten subject had rejected the MV contact lenses (unsuccess group) while the other ten subjects were successful adapted to MV lens (success group). In several papers, the mean age and add power had not statistically significative difference on the success ratio. Nevertheless, both parameters (age and addition) are related with the amount of anisometropia induced by MV to the subjects.

Results: The t-test shows statistically significant differences between the mean values of the anisometropia of the success and unsuccess group with $p = 0.017$. Therefore we can say, with 1.7% of error, that the mean value of the anisometropia in the MV success group is lower than in the MV unsuccess group. The t-test provides also a significant difference between mean values of age with $p = 0.016$.

Conclusions: 1) The amount of anisometropia influences the probability of achieving success with MV. This probability is about 80% with anisometropia equal or lower than 2.25 diopters. 2) The age of the subject is also important, the percentage of success dramatically decreases out of the 44-50 years interval. 3) It is best to adapt the addition to that eye (dominant or fellow is indifferent) that will cause the lowest possible anisometropia.

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Pupillograms in school-children with accomodative lesions

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Purpose: Pupillograms can be helpful in neurophysiology with the purpose of diagnosis the autonomic nervous system activity.

Methods: The Filatov Institute of Eye Diseases and Tissue Therapy, Odessa, Ukraine and Institute of TV jointly developed a device and corresponding software for pupillographic method. The pupillography was performed in 30 school-children aged 6-7. 25 school-children (83%) had astenopic complaints resulting from infringement of visual functions. Methods of research included direct, associate, accomodative-convergentive reaction of pupils to flash; the dynamics of size change in time during accommodation; visual acuity testing for near and far distances, refractometry.

Results: We obtained computer images of pupils and Pupillograms for direct and consensual reactions of pupils to light flashes and recorded accommodation-convergence dynamics. We have discovered 7 temporal periods describing pupils state. There were correlations of amplitude, speed, fluctuation frequencies of pupil area with visual acuity, accommodation, refraction in the periods of direct flash, consensual and near in accommodative-convergentive reaction. In the 1st period visual acuity correlates with speed of pupil area changes ($r=0.61$); accommodation correlates with speed of pupil area changes ($r=0.77$) and anisocoria factor ($r=0.72$). In the 2nd period of latency there was marked a correlation between visual acuity, mezoopic vision and the duration of this period ($r=0.59$). In the 5th period of pupil redilation accommodation correlates with fluctuations frequencies of pupil area ($r=0.72$).

Conclusions: The analysis of Pupillograms may be used as objective test in the diagnosis of lesions along the visual of pupillomotor pathway.

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Treatment of the toxic optic neuropathy with high dose corticosteroids

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Purpose: Toxic and toxic-allergic optic nerve neuropathy were always an actual problem in ophthalmology because lead to atrophy of the optic nerve. Widening of the arsenal of the medicines, using them without control, application a lot of neurotoxic chemicals, toxicomania, abuse of alcohol, tobacco- are the main reasons of this pathology. The treatment of toxic opticopathy present some difficulties because the pathologic changes of optic nerve are sometimes irreversible. In this connection search after new effective remedies are still the issue of the day in ophthalmology. To investigate the clinical manifestations, evaluate efficacy of high dose corticosteroid therapy on toxic optic neuropathy.

Methods: Retrospective analysis was done on 7 patients with toxic optic nerve damage. All patients were treated by IV methylprednisolone 30 mg/kg every 6 hours 3 days. It is important to note that all of our patients before had been treated in the hospitals by the corticosteroids in standard dosage without visual improvement.

Results: The present data suggest the efficacy of pulse steroids therapy in patients with toxic damage of optic nerve in spite of beginning of treatment after some time after poisoning. Explanation of this is probably part of the optic nerve fibers is on the parabolic stage and after steroids therapy they begin to functionate.

Conclusions: The treatment toxic optic nerve damage by high dose corticosteroids is soundly and has effect even distant in- time of poisoning.

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Functional and morphologic alterations in Stargardt's macular dystrophy

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Purpose: To assess relation between functional changes (visual acuity and electric activity) and morphologic parameters (foveal thickness and macular volumen) of the central retina in patients with Stargardt's maculopathy.

Methods: 20 eyes of 10 patients (aged 16-35) and 10 healthy age-matched controls were examined. Multifocal electroretinography (mfERG, FOK) was obtained binocularly, using Retiscan (Roland Consult) equipment. The central 30° (radius) of the retina was stimulated by 61 hexagons. An ERG jet was used as a different electrode. Response density of the b wave in the central 5 rings was measured. Optical coherence tomography (OCT, Humphrey-Zeiss Instruments, USA): six, 6 mm long, radial scans manually centered on the fovea were obtained in each eye. Foveolar thickness (FT) and total macular volume (TMV) were measured automatically, using the mapping software of OCT in the 9 areas (3500µm of diameter) recommended by the Early Treatment Diabetic Retinopathy Study. Correlation was examined between visual acuity and foveal thickness (FT), visual acuity and response density (RD) in the central 0-2° (ring 1), and between FT and RD.

Results: Visual acuity and foveal thickness showed the highest correlation ($r = 0,58$). Electric activity correlated weaker to both foveal thickness ($r = 0,29$) and visual acuity ($r = 0,2$).

Conclusions: Both methods seem to be suitable to detect and follow pathological changes of the posterior pole in SMD. Visual acuity seems to show a stronger correlation to morphologic changes, than electric activity.

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30 Hz flicker multifocal ERGs in retinitis pigmentosa

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Purpose: This is a preliminary study for testing the technical application and practical usefulness of the 30 Hz Flicker stimulation mode in mfERG to detect residual central cone-photoreceptor function in retinitis pigmentosa.

Methods: 10 patients (aged 20-30) with retinitis pigmentosa and 10 healthy age-matched controls were examined. The basic criteria for selecting the patients were: visual acuity of 0.5 or better and visual field constricted to 20° (or less) concentrically. Visual fields were tested on Octopus 101 (G2 program). The 30 Hz Flicker mfERGs were obtained binocularly using Retiscan equipment (Roland Consult). The central 30° (radius) of the retina was stimulated by 61 hexagons. An ERG jet was used as the different electrode. For each patient and control, the retinogram field (calculated on the Fourier Analysis) was recorded and the response density (DFT [Digital Fourier Transformation] BW [nV/deg]) in the 5 concentric rings were measured. (In each case, the second of the three peaks was used as the basis of measurement.)

Results: The above method enabled good quality retinograms to be obtained over the whole retinogram field in the case of the controls. In the case of the patients with fields of between 5° and 20° useful retinograms were recorded, indicating the patients' conditions. For patients with visual fields of 5° or less, no useful retinograms were detectable.

Conclusions: This method proved to be appropriate for measuring central cone-photoreceptor function in healthy individuals and patients with moderate to expressed visual field loss. It is not, however, sensitive enough to measure central cone function in patients with extremely restricted fields.

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Multifocal ERG in the monitoring of chloroquine retinopathy

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Purpose: To illustrate the use of multifocal ERG in the monitoring of disease progression, after cessation of treatment, in a patient with chloroquine retinopathy.

Methods: Illustrative case report. A 37 yr old woman with rheumatoid Arthritis was referred with difficulty with night vision. She had been taking chloroquine for the last 16 years.

Results: Examination revealed UCVA 6/9 BE, Ishihara reduced to 14/17 in BE. Fundoscopy revealed bilateral central foveolar hyperpigmentation surrounded by depigmented zone encircled by a ring of hyperpigmentation. Treatment was stopped. Electrodiagnostic showed reduced pattern and multifocal ERGs (L>R). 9 months later UCVA was 6/9 BE. However, repeat electrodiagnostics showed undetectable multifocal and pattern ERG bilaterally.

Conclusions: Patients that remain on longterm chloroquine therapy may develop progressive retinopathy despite cessation of treatment. The multifocal-ERG aids the clinician in assessing the longterm prognosis in such patients.

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Chromatic deficits in Best's macular dystrophy: insights for the development of new clinical tools

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Purpose: To analyze chromatic dysfunction in Best Disease, and to relate identified parameters with anatomical, clinical and electrophysiological markers of disease progression.

Methods: Color contrast discrimination was measured using two different strategies, based on the Cambridge Colour Test. The first measured three main confusion lines, and the second evaluated discrimination ellipses that were further fit using an alternative custom made algorithm. The study was run in age-matched control (n=41) and patient (n=34) eyes.

Results: Substantial chromatic dysfunction occurred even with relatively preserved visual acuity (VA). Still, strong significant negative correlations between all test parameters and VA could be found. Highly significant loss of performance was seen in all color confusion axes, but specially along the protan and deutan ones. Partial correlation analysis showed that protan/deutan loss is related to VA independently of tritan loss. The observed dysfunction increased monotonically with clinical Fishman staging as also confirmed by ellipsis length and axis ratio measurements. Significant positive correlations were also found between lesion size and chromatic dysfunction. EOG was useful for early diagnosis but did not correlate with other markers of disease progression.

Conclusions: Chromatic discrimination is often impaired in Best disease, even when other functional parameters, such as visual acuity are still spared. The relative degree of protan/deutan vs. tritan loss seems to be an indicator of the degree of macular dysfunction and its progression. Promising cut-off values offer a new quantitative clinical approach to study early functional involvement and disease progression in macular disorders.

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Improved mobility and independence of night-blind people by the use of night vision goggles

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Purpose: We studied whether the use of night vision goggles (NVG) by night-blind people improves their mobility and sense of independence.

Methods: 20 night-blind subjects with Retinitis Pigmentosa walked routes at night-time with and without use of NVG (Multi-Vision, Trivisio, Switzerland). We assessed the number of unintended contacts with obstacles (Hits) and Percentage of Preferred Walking Speed (PPWS) during routes at 3 circumstances: (1) darkened indoor corridor (2) moderate-lighted outdoor residential area (3) well-lighted outdoor shopping area. Assessments were carried out before and after a 5-week training period, during which subjects have practised the use of NVG in their own environment and registered their experiences in a diary and questionnaires.

Results: In the darkened corridor, mean Hits declined from 8 to 2 Hits with use of NVG. Mean PPWS (34%) did not improve. In the residential area, the mean of 8 Hits declined to practically no Hits. Mean PPWS increased from 60% to 72% and after training to 78%. In the shopping area, subjects walked at 93% PPWS without any Hits, which did not improve with use of NVG. Subjective scores from the diaries reveal a good sense of orientation, feelings of safety and relaxedness during mobility with use of NVG (mean scores > 9 on a scale from 1 to 10). In the questionnaires, all subjects indicated to travel independently much more often since using NVG.

Conclusions: Using night vision goggles improves night-time mobility in a safe way by decreasing unintended contacts with obstacles and increasing walking speed. Additionally, use of night vision goggles increases independence and is generally positively evaluated by night-blind people for use in everyday life.

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Electroretinography study of thyroid-associated eye disease

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Purpose: To define the early signs of sensory visual system involvement in Thyroid-Associated Eye Disease (TAED) in order to determine the prognosis and patient management strategy.

Methods: There were examined 23 patients (46 eyes) with TAED. The age ranged from 14 to 75 years. Visual acuity varied from 0,01 to 1,0. Maximal electroretinogram (ERG), local ERG (18°) to red, green and blue stimuli, oscillatory potentials (OP), 30-Hz flicker ERG, ERG to long-flash stimulus (200ms) and pattern ERG were recorded to evaluate electroretinography symptoms of TAED.

Results: ERG signs were depended on the degree of the clinical symptoms and TAED stage. The fundus examinations revealed mild optic head edema, smoothed macular reflex, dilated and tortuous veins. Patients with TAED demonstrated electroretinographical changes in the optic nerve and retina. Optic nerve changes performed pathological pattern ERG: diminished amplitude and prolonged implicit times of P50 and N95 components. Pathological retinal changes were localized in the central retina from photoreceptors to the ganglion cells and included pathological OP, diminished amplitude and prolonged latency b-wave of local ERG to red, green and blue stimulus, decreased on- and off-components of long-flash ERG. It's possible, the electroretinography symptoms reflect the early stages of ganglion cells apoptosis and early metabolic disturbances due to retinal ischemic syndrome occurring in this disease.

Conclusions: The degree of retinal and optic nerve involvement evaluated by ERG allows to assess the state of the sensory retina in order to predict the visual functions and to determine patient management strategy with TAED.

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Computerised calculation scheme for ocular magnification with the Zeiss telecentric fundus camera

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Purpose: Littmann related the size of a retinal feature to its measured image size on a telecentric fundus camera film. It requires only ametropia and keratometry. The purpose of this study is to describe a numerical calculation scheme of determining the magnification q of the eye using paraxial raytracing.

Methods: Our calculation scheme based on ametropia, keratometry, axial length, anterior chamber depth and thickness of the crystalline lens is described step-by-step in order 1) to determine the refractive power of both surfaces of the crystalline lens, which is not direct measurable in-vivo, 2) to derive the retinal image conjugate to a circular object using paraxial raytracing, 3) to fit an ellipse to the retinal image, 4) to determine the secondary principal points (Gaussian length) separately for both cardinal meridians and 5) to calculate the ocular magnification q. The power of the crystalline lens is estimated to compensate for the spherocylindrical refraction at spectacle plane and the corneal refraction with an astigmatic component thus creating a sharp image focused at the retinal plane.

Results: The capabilities of this computing scheme are demonstrated with five clinical examples. Our results are related to the respective values of the classical Littmann formula as well as to enhanced methods described by Bennett and Garway-Heath.

Conclusions: We present a novel strategy for calculation of 'ocular magnification q' for determination of the total magnification of a telecentric fundus camera and an individual eye using paraxial raytracing. The q value is determined separately for both cardinal meridians of the eye characterised by a spherocylindrical refraction and astigmatic cornea and biometric measurements.

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Localizing and Stabilizing the PRL with a Modified Slit Lamp

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Purpose: Patients with a central scotoma frequently develop an eccentric area of fixation commonly referred to as a preferred retinal locus (PRL). Localizing the PRL can be done precisely with Scanning Laser Ophthalmoscope (SLO). Other PRL localizing methods usually use indirect psychophysical methods, e.g. mapping the location of the blind spot. This paper presents a new method for localization of PRL with a modified slit lamp.

Methods: The rotating aperture disc controlling the height of the illumination slit of Haag-Streit slit lamp was disassembled. Five extra cone shaped holes were drilled to the disc. Several fixation targets were printed on a transparency sheet and mounted to the rotating disc. During standard clinical fundus examination with a modified slit lamp and +90 D lens the fixation targets were projected onto the macular area. The fixation preference of ten AMD patients was investigated with this method. The examination was also recorded on videotape for later analysis and was compared to immediate clinical results for the location of the PRL.

Results: The fixation preference (central, unstable, eccentric PRL, several different PRLs) could be identified for every patient. The fixation results were similar both in the clinical examination and in the video analysis afterwards. For one patient the rather unstable PRL was trained during the examination by instructing him to move his eyes to correct direction. This improved significantly his ability to read the words that were projected with the slit lamp onto the fundus. In a second examination after two months the PRL was more stable and he read more fluent than in the first examination.

Conclusions: Modified slit lamp can be used for localizing and training the PRL with AMD-patients.

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Statistical validation of diffractive eye model

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Purpose: In previous works, the authors have presented a diffractive method for studying optical quality of human corneas. In this communication, the method is extended to the whole eye, including the crystalline lens and real biometric measures of the anterior chamber and axial length.

Methods: The lens has been modelled following indications given by Kasprzak and Popiolek. An age correction proposed by Brown and Korteiz has been also introduced in the model. The final result consists of a complete "diffractive eye model" that has been validated on 20 healthy young eyes aging 21 ± 1 years. Experimental data consist of corneal topographies and biometric measures for each patient. With these data and the modelled lens, we are capable of obtaining the MTF and the Strehl ratio at the retinal plane and any other plane of relevant interest.

Results: Obtained statistics on MTFs and Strehl ratios show that our method is similar to those based on double pass techniques. With our algorithm we can calculate image formation at any distance inside the eye. Additionally, we can obtain the exact light distribution before and after passing the crystalline lens, thus permitting the study of this single element.

Conclusions: The advantages of our method in front of these techniques are less exploration time and less technology requirements. We would like to recall that the model can be calculated from data obtained with a corneal topograph and a non-contact biometer, which are widely available. An additional advantage that can be obtained is that substitution of lens model by an IOL model allows "a priori" exploration of lens implementation and even personal adaptation of these lenses.

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Measurement of forward light scatter in the eye: limitations of current clinical techniques

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Purpose: Intraocular light scatter affects retinal image quality, with forward scatter particularly relevant for visual performance. Current techniques to measure scatter assume its angular distribution is invariant, estimating its amount using a single fixed-angle ring source. Here we evaluate the variation in the angular scatter distribution in the eye and estimate the errors involved for a single annular source.

Methods: Five extended annuli measure scatter using flicker cancellation over a range of eccentricities. The empirical function $L_s = kE^{-(n)}$ is fitted to the data and parameters k , n and the new integrated straylight parameter k' , a measure of the total scatter in the eye, estimated. A second test uses only one scatter annulus with k and k' computed on the assumption that $n=2$. Normals completed both tests, allowing a comparison of the two methods.

Results: Values of n varied from 1.58-2.66 (mean 2.14). Subjects with $n \sim 2$ produced nearly identical results for both tests. Other subjects revealed a significant disparity: k showed large variability, whilst k' remained relatively unchanged. No correlation was found between changes in n with age or k' . A correlation was found between changes in n and k .

Conclusions: If the scatter index n is assumed to be 2 the resulting straylight parameter k will be inaccurate, suggesting that clinical methods using a single annulus to give k will be accurate only for subjects with $n \sim 2$. The integrated straylight parameter k' varies less, but provides no information on the angular scatter distribution. This highlights the need for a clinical, objective technique to measure the amount and angular distribution of scattered light in the eye as both are important for vision.

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Evolution, classification and execution of the primary and secondary accommodation mechanisms in human eye

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Purpose: We have examined the evolution of accommodation mechanisms, taking into account our own considerations as well as of other researchers, concerning the biomechanism of primary and secondary accommodation mechanisms, which humans inherited from their ancestors.

Methods: Using methods of morphology, physiology, ophthalmology, optics, mechanics and regulation theory of biosystems.

Results: In human eye there are several extraocular and intraocular accommodation mechanisms, but the basic one is deformation of the lens and change of its refraction power (the only mechanism acknowledged by Helmholtz). We consider it a key mechanism in one general mechanism which consists of a number of intraocular and extraocular ones. The middle tonical state of the Muscle ciliaris is not only provides the smallest time of readjustment, but also is the most efficient for outflow mechanisms, which depend on the state of accommodation. Relaxation or no accommodation correspond to the look at the distance. The primary mechanism seems to be used only after the secondary ones, such as change of refraction power of the cornea and narrowing of the pupil for increasing the focal depth. Developed principles of interconnected activity of primary and secondary mechanisms helped us to explain some clinical observations, which were not explained earlier from incomplete, but basically correct Helmholtz point of view as well as to classify all accommodation executive mechanism in one scheme.

Conclusions: In the eye there are several accommodation mechanisms. Primary is the change of refraction power of the lens regulated by the tension of zonular fibers (by Helmholtz), all others are considered secondary. But when primary mechanism is distorted, secondary mechanisms just don't have enough capacity to compensate even though they try. So the total refraction of the eye must be changed by means of IOL or change of cornea refraction.

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Foveal spatio-temporal detection surfaces for gratings and gabor stimuli

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Purpose: To compare the contrast sensitivities obtained in the fovea with spatio-temporal gratings and Gabor stimuli.

Methods: Achromatic spatial gratings with frequencies 0, 0.5, 2, 4 and 8 cycles per degree (cpd), with temporal sinusoidal modulation of frequencies 0, 2, 6, 12 and 24 cycles per second (Hz) were spatially modulated with either a rectangle subtending 5°x5° or a bidimensional Gaussian function with its standard deviation subtending (5/6)°. These stimuli were generated by a CRS-VSG 2/5 graphics card and presented in a Mitsubishi CRT display. Two observers carried out the measurements.

Results: The detection surfaces for both the gratings and Gabor stimuli have band-pass characteristics with spatial and temporal frequency, showing a maximum at 2 cpd and 6 Hz. The grating/Gabor sensitivity ratio reaches a maximum value of approximately 8 when both spatial and temporal frequency are zero, and decreases when either spatial or temporal frequency increase. A two-way analysis of variance of the results was applied.

Conclusions: When all the frequency combinations are taken into account, the grating/Gabor sensitivity ratio varies significantly only with spatial frequency. If 0 cpd is excluded, there is no significant difference for this ratio between frequency combinations, its average being approximately 1.7. If 0 Hz is excluded, a significant variation with spatial frequency appears again. If both 0 Hz and 2 Hz are excluded, the variations are significant when varying both spatial and temporal frequency.

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Clinical performance of Nidek ARK 700A autorefractor

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Purpose: The aim of this study was to estimate the agreement between an autorefractor (Nidek ARK 700A), and retinoscopy and subjective refraction.

Methods: Subjective refraction and retinoscopy were performed on 192 right eyes from 192 healthy young adults (mean age 24,5 ± 2,5), and compared with the measurements obtained with the ARK 700A. These measurements were performed without cycloplegia. The limits of agreement were used to determine the accuracy between the three methods.

Results: The refractive error of the sample, as represented by subjective refraction, ranged from -9,00 to +2,25 D mean spherical equivalent -0,29 ± 1,39D (Mean ± SD). The maximum amount of astigmatism was -2,50 D. The mean value of the refractive error obtained with each technique was: Retinoscopy = -0,19 -0,26 x 170 Autorefractor = -0,63 -0,22 x 168 Subjective refraction = -0,23 -0,12 x 165. By considering the conditions, sphere and cylinder component within ±0,25D and cylinder axis within ± 10 degrees, we may verify that in only 42 eyes (21,9%) the autorefractor agree with the subjective refraction compared with 96 eyes (50,0%) of the results of the retinoscopy.

Conclusions: The measurements obtained with the ARK 700A were slightly less positive or more negative than retinoscopy and subjective refraction, being these bias statistically significant. Retinoscopy continues to be the best method for obtaining an objective refraction value. The autorefractor can be used as a start point prior to subjective refraction, but never as its substitute.

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A comparison of glare and halo in contact lenses, emmetropes and spectacle wearers before and after lens cleaning

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Purpose: To compare glare and halo in a cohort of emmetropic subjects and ametropic subjects with various methods of refractive correction.

Methods: 98 eyes of healthy pre-presbyopes (emmetropes n=28, spectacles n=40, CL n=30) had glare and halo analyzed using computer-generated stimuli (Glare&Halo®). The emmetropic and contact lens groups were tested once. The spectacle group subjects were tested twice, once with the lenses in their natural, end-of-day, dirty state, then tested again after cleaning with an alcohol based propriety lens cleaning solution, and a micro-fibre cloth.

Results: No significant difference in glare was found between any of the groups (p>0.05). Statistically significant differences in halo size were noted between emmetropes (no refractive correction) and both contact lens (p<0.001) and uncleaned spectacle groups (p<0.05) but not with cleaned spectacles. The reduction in halo size, post lens cleaning was significant. (p<0.0187).

Conclusions: Contact lens and unclean spectacle-lens wearers both experience significantly larger halos than emmetropes and cleaned spectacles lens wearers. Uncleaned-spectacles and contact lenses will affect the accuracy of results in psychophysical and clinical testing.

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Absolute and incremental spectral sensitivities predicted by a new colour vision model

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Purpose: To predict absolute and incremental spectral sensitivities with a new colour vision model.

Methods: We have developed a neural colour vision model, ATTD, consisting of 1) a cone stage, with a Naka-Rushton-type non-linearity and a multiplicative adaptation mechanism; 2) a first opponent stage at LGN level, computed from the cones, yielding an achromatic channel (A), two red-green channels with opposite polarities (TLM, TML), and a blue-yellow channel (D), all with Naka-Rushton non-linearities, and multiplicative and subtractive adaptation mechanisms; 3) a second opponent stage at cortical level, with a subtractive adaptation mechanism and 4) a third opponent stage, yielding the perceptual channels. The parameters of the model were adjusted to fit a large set of psychophysical and physiological data. Thresholds of a normal observer were obtained as the envelope of the action spectra of the excitatory components of the four LGN mechanisms.

Results: The predicted absolute spectral sensitivity, with a sensitivity peak around 540 nm, basically agrees with the experimental data except at low wavelengths, where our model overestimates the sensitivity of the blue-yellow mechanism. The predicted incremental sensitivity function shows the typical three-peak shape, with maxima around 440, 520 and 610 nm and the Sloan notch around 570 nm.

Conclusions: The model predicts that the blue-yellow and the red-green cells with L-M polarity always detect at, respectively, the short and long-wavelength regions. Detection in the middle-wavelength range is mediated by achromatic and M-L cells in the absolute and incremental paradigms, respectively, although achromatic cells always detect around 570 nm. These results agree with the literature.

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Postoperative retinal thickness changes after vitrectomy for macular pucker

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Purpose: To determine postoperative changes in retinal thickness and visual acuity after vitrectomy for macular pucker.

Methods: Studied prospectively, pars plana vitrectomy was performed on 45 consecutive eyes of 45 patients with macular pucker. The follow-up interval ranged from 3 to 16 months. Peroperative and postoperative visual acuity (ETDRS and Snellen), and examinations by color fundus photography, angiography and optical coherence tomography (OCT) of the macula, were performed on all patients. Epimacular membranes, removed during surgery, were examined histopathologically (HES, GFAP, Vimentin, keratin, and specific markers for myofibroblasts: alpha-actine smooth muscle and desmine).

Results: We observed two histopathological types of epimacular membranes: reactive gliosis (fibro-glia membranes) with few myofibroblasts, and fibrosis with much myofibroblasts. Preoperative mean best-corrected visual acuity was significantly lower in patient with macular edema and high foveal retinal thickness. The postoperative mean best-corrected visual acuity was significantly better than the preoperative mean best-corrected visual acuity. The final visual acuity improved by 2 or more lines in 30 of 45 eyes (66%). The postoperative foveal retinal thickness at the last visit was significantly thinner than the preoperative foveal retinal thickness. High rates of postoperative visual acuity occurred in eyes with better preoperative visual acuity, thinner postoperative foveal retinal thickness and fibro-glia epimacular membranes.

Conclusions: Vitrectomy for epimacular membranes is an effective procedure for reducing macula edema and improving visual acuity. Postoperative improvement of visual acuity was correlated to postoperative reduction in retinal thickness.

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Low vision rehabilitation for people with AMD: a randomised controlled trial

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Purpose: To compare the effectiveness of enhanced low vision rehabilitation (ELVR), home-visits without enhanced low vision rehabilitation (CELVR, control for additional contact time with ELVR), and conventional low vision rehabilitation (CLVR; no home visits) for people with AMD referred for low vision rehabilitation (LVR).

Methods: The present trial was a single-centre parallel group RCT. Subjects included people newly referred for LVR with a primary diagnosis of AMD and VA worse than 6/18 in both eyes and equal to or better than 1/60 in the better eye. Main outcome measures included: Vision specific quality of life, QoL, (primary outcome) and generic health-related QoL (SF-36); psychological adjustment to vision loss; task performance; restriction in activities; use of low vision aids (LVAs).

Results: 226 participants were recruited (median age 82 years); 194 completed the trial (86%). Except for SF-36 physical and mental summary scores, arms did not differ significantly for any of the outcomes after adjusting for baseline scores. Differences on the SF36 favoured CLVR compared to ELVR (ELVR vs. CLVR: physical=-6.05, 95% CI -10.2 to -1.91, p=0.004; mental=-4.04, 95% CI -7.44 to -0.65, p=0.02). Differences in vision-specific QoL were: ELVR vs CLVR, 0.06 (95% CI -0.17 to 0.30, p=0.60); ELVR vs CELVR, 0.12 (95% CI -0.11 to 0.34, p=0.31); CELVR vs CLVR, -0.05 (95% CI -0.29 to 0.18, p=0.64). At 12 months, 94% of participants reported using at least one LVA.

Conclusions: ELVR was no more effective than CLVR. Researchers should be wary of proposing new LVR interventions without preliminary evidence of effectiveness, given the lack of effectiveness of the model of enhanced LVR evaluated in this trial.

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Consecutive development of optic neuropathy, cilioretinal artery and central retinal vein occlusion in a patient with chronic renal failure

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Purpose: To report a patient with chronic renal failure who developed anterior ischemic optic neuropathy, cilioretinal artery occlusion and central retinal vein occlusion consecutively.

Methods: Case report. Thirty six-year-old male patient was admitted to our clinic with blurry vision in the left eye. He has been on hemodialysis 3 times a week because of chronic renal failure for 12 years and had no other major systemic disease.

Results: His visual acuity was 20/20 in the right and 20/25 in the left eye.

Biomicroscopic examination revealed no pathologic findings in the anterior segment and the IOP was 15mm Hg in both eyes. Fundus examination showed sectoral optic disc edema and hemorrhage in the left eye while the right fundus was normal. Hematologic findings including ESR and CRP were within normal limits. The patient was diagnosed as non-arteritic anterior ischemic optic neuropathy. The next day after the diagnosis was made, his visual acuity decreased to hand motions in the left eye and fundus examination revealed cilioretinal artery occlusion and fluorescein angiography confirmed the diagnosis. Two days after, intraretinal hemorrhages developed throughout fundus of the left eye and the patient had no light perception. The patient was diagnosed to have central retinal vein occlusion.

Conclusions: Optic neuropathy, cilioretinal artery and central retinal vein occlusion may develop one after the other even in young patients with chronic renal failure. This clinical picture may follow an aggressive course and may end up with permanent amaurosis.

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Ultrasound biomicroscopy (UBM) follow up of rinostomy size after external dacryocystorhinostomy.

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Purpose: To evaluate the rinostomy size after external dacryocystorhinostomy, using Ultrasound Biomicroscopy (UBM), as postoperative successful index.

Methods: 20 patients (12 women and 8 men, mean age, 61 years) undergoing external dacryocystorhinostomy for low lacrimal tract obstruction were recruited. The vertical and anteroposterior size of the rinostomy were measured at 1 week, at 3 and 6 months after surgery using Ultrasound biomicroscopy (UBM, 20 Hz-13), referred to subjective symptoms (epifora).

Results: About 55% of mean reduction of rinostomy size and about 30% of epifora in patients were showed at 6 months postoperative follow-up.

Conclusions: The objective of dacryocystorhinostomy is to create a new drainage pathway bypassing lacrimal obstruction. In external dacryocystorhinostomy, a large bony window is opened in lateral nasal wall, and a muconasal anastomosis is created between the lacrimal sac and nasal cavity. The success of surgery depend on the remaining postsurgical anastomosis dimensions.

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Complex ocular anomalies in a patient with frontonasal dysplasia

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Purpose: New association of complex ocular anomalies in a patient with frontonasal dysplasia previously not reported in literature.

Methods: An eight month old girl presented with epiphora. Her birth was at term. Weight, head circumference and caryotype were normal. She had hypertelorism with telecanthus, broad nasal bridge, partial bifid nose and normal systemic examination. There was no positive family history or any history of co-sanguinity. Frontonasal dysplasia was diagnosed based on her appearance. Ocular examination showed objection at occlusion of right eye, bilateral horizontal coarse nystagmus, small limbal dermoids, left corneal leukoma, right morning glory syndrome and left normal optic disc. At 3-year old visual acuities were 3/36 in the right eye and 2/60 in the left.

Results: As no right nasolacrimal duct was found at probing, subsequent magnetic resonance imaging performed showed an intraorbital mucocele and intracranial lipoma over corpus callosum. Epiphora had improved after endoscopic dacryocystorhinostomy with tubes.

Conclusions: Frontonasal dysplasia is a developmental field defect of heterogeneous aetiology characterised by hypertelorism, S-shaped palpebral fissures, broad nasal root, bifid nose, and cleft of lip. Systemic associations have been described. In conditions associated with hypertelorism and facial clefting the visual function is usually good. Association of midline facial clefts with optic disc anomalies of dysplastic type like morning glory is uncommon and have substantial diagnostic implications. There are previous reports of patients with pulsatile nasal tumours patients complicated by recurrent rhinorrhoea after hazardous surgery. The corneal opacity present in our patient was most likely coincidental.

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Comparing 3 methods for determining scotopic pupil size: Colvard, Orbscan estimation and slit lamp biomicroscopy-based pupillometry

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Purpose: To compare two methods for measuring scotopic pupil diameter, Orbscan (OP) and slit lamp-based pupillometry (SLP), with a gold standard, infrared Colvard pupillometry (CP).

Methods: One single measurement of scotopic pupil size was performed by the same examiner in 34 eyes using the Orbscan II system, a slit lamp biomicroscope and the Colvard pupillometer. Statistical analysis of the agreement between the three techniques was performed with the method described by Bland and Altman.

Results: The mean pupil diameter was 4.27 ± 0.70 mm (range 3.10 to 6.00) with OP, 6.26 ± 0.79 mm (range 4.70 to 7.70) with SLP and 6.28 ± 0.80 mm (range 4.50 to 8.00) with CP. Mean differences between techniques was -1.99 ± 0.48 mm for OP and SLP ($p < 0.001$), 2.01 ± 0.54 mm for OP and CP ($p < 0.001$), and 0.024 ± 0.37 mm for OP and SLP ($p = 0.713$). Limits of agreement ranged from around 2 for the comparison groups OP-SLP and OP-CP to 1.44 for the comparison between CP and SLP.

Conclusions: Results indicate that the Orbscan method underestimated scotopic pupil size approximately 2 mm because of the luminance of the Placido rings. Therefore, the measures with this device and those obtained with the other two methods cannot be used interchangeably. However, OP and SLP provide readings more comparable.

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Vigabatrin reduces human Retinal Pigment Epithelial (hRPE) cell viability and proliferation in culture

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Purpose: Vigabatrin, an antiepileptic drug that has been implicated in visual field constriction, effects inner retinal electrophysiology at the muller cell level. Since very little is know about its effect on hRPE cells, we assessed the effect of vigabatrin on hRPE cells in culture and its effect on c-fos protein synthesis, a nuclear protein that is implicated in cell death.

Methods: Primary hRPE cell cultures were established from human eyes. Cells were allowed to grow to confluence and treated with vigabatrin (0-10 µg/ml). Cell viability was determined by the trypan blue exclusion method. Cell proliferation was monitored by 3H-thymidine (3H-thy) incorporation. 14C-methionine labeled-intracellular c-fos synthesis was determined by immunoprecipitation using c-fos specific antibodies. Data were analyzed by Student 't' test.

Results: FBS increased hRPE cell proliferation in a dose dependent manner. Increasing concentrations of vigabatrin in the presence of 10% FBS decreased 3H-thy incorporation in hRPE cells in a dose dependent manner. Vigabatrin in the presence of 10% FBS also decreased hRPE cell viability in a dose dependent manner with significantly higher number of trypan blue damaged cells (1000 ± 400 vs. 0 ± 0 , cell number \pm SEM, P value < 0.046). Light microscopy showed that addition of vigabatrin resulted in the loss of in epitheloid type morphology. Vigabatrin (10µg/ml) also stimulated immunoprecipitated 14C-c-fos (440 ± 43 vs. 343 ± 58 , CPM \pm SEM, P value < 0.05).

Conclusions: Vigabatrin reduces hRPE cell viability and proliferation. It also increases c-fos synthesis. The data suggest that hRPE cells may play a role in vigabatrin associated visual field defects.

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Pseudo-duplication of the optic disc by peripapillary coloboma

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Purpose: To describe four lesions simulating optic disc pseudo-duplication. Pseudo-duplication of the optic nerve head is a stunning clinical entity, where a circular lesion resembling an optic disc appears adjacent to the true optic disc. True doubling of the optic disc, with a dual system of retinal blood vessels, is rare.

Methods: Retrospective, non-comparative review of case notes. Four lesions simulating duplication of the optic disc are described.

Results: All four cases represent chorioretinal coloboma. Closure of the fetal fissure is not always constant, such that chorioretinal coloboma can lie superior to the optic disc. A double blind spot can be demonstrated by automated visual field testing. Fundus fluorescein angiography can differentiate between true doubling and pseudodoubling of the optic disc.

Conclusions: These anomalies are asymptomatic and require no treatment. Clinical examination of the retinal vessels can usually avoid unnecessary invasive and non-invasive investigations.

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Rapid recovery of night blindness due to obesity surgery after vitamin A repletion therapy

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Purpose: To describe a case of subacute night blindness due to vitamin A deficiency after abdominal surgery for morbid obesity, and rapid recovery after repletion.

Methods: A 39-year old male presented with a 6-month history of night blindness, progressing more rapidly in the last 2 weeks. Three years before he had undergone a partial gastrectomy and biliopancreatic derivation for morbid obesity (Scopinaro procedure). A full clinical, psychophysical and electrophysiological work-up was performed. Follow-up evaluations were performed on day 0, and subsequently on days 3, 5, 9, 22, 36 and 72 after instigation of repletion therapy. Serum retinol concentration (SRC) was measured regularly.

Results: At presentation, best corrected visual acuity was 6/5 in BE. Slit lamp examination and funduscopy were unremarkable in BE. Concentric narrowing in BE was seen on VF analysis. DA showed a considerable decrease in sensitivity. The EOG was subnormal (RE: 166%; LE: 146%). ISCEV-standard ERG showed only residual scotopic responses in BE. The SRC of vitamin A and E were subnormal. Our patient was repleted with 60,000 IU retinol/day and vitamin E 140 mg/day. Ten days after initiation of treatment, all ERG and EOG parameters returned to normal, although ERG amplitudes increased up to day 22. Complete normalisation of DA was also seen by day 10. From day 36 Goldmann visual fields were considered to be normal.

Conclusions: Malabsorption caused by biliopancreatic derivation surgery of the Scopinaro type can induce vitamin A deficiency with progressive rod-cone dysfunction. In the early stages of vitamin A deficiency, recovery of visual function rapidly follows after oral repletion therapy, and can be nearly complete one week after initiation of such therapy.

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A study of perceived image quality using a low cost home-made lensless ophthalmoscope versus a standard direct ophthalmoscope

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Purpose: To compare the image quality of a novel low cost home-made lensless ophthalmoscope with a standard direct ophthalmoscope as perceived by a cohort of student doctors.

Methods: Twelve fifth year Oxford University Medical Students participated in a prospective comparative study. Each performed fundus examinations under standardized conditions with both ophthalmoscopes, and completed questionnaires relating to each examination (n=56). The perceived image quality of several fundus features was scored, and the overall ease of use and instrument preference was recorded. Chi-squared or Fisher's exact test analyses were performed, and the effect of uncorrected refractive error greater than -2D was also determined.

Results: There was no statistically significant difference in perceived image quality of the red reflex (p=0.49), optic disc clarity (p=0.1) and macula view (p=0.09), but the optic disc cup image (p=0.026) and the overall ease of use (p=0.003) were better with the standard direct ophthalmoscope. When uncorrected refractive errors greater than -2D were eliminated, the differences between the perceived image quality of all parameters were mitigated but the overall ease of use still favored the standard direct ophthalmoscope (p=0.03). However, 36% of participants rated the overall perceived image quality of the lensless ophthalmoscope as at least as good as the standard direct ophthalmoscope.

Conclusions: The lensless ophthalmoscope offers means of performing good quality fundus examinations at a very low cost, and compares well to a standard direct ophthalmoscope.

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Three different tests of foveal function

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Purpose: To evaluate two new, computerized tests of foveal vision, the TRIVA test, which measures visual acuity (VA) at different contrast levels, the Rarebit Fovea Test (RFT), which probes the integrity of the neuro-retinal matrix within the central 3° visual field, in comparison with conventional VA testing.

Methods: Sixty-six healthy volunteers, aged 22-87, with subjectively normal vision participated in the study. The right eye from each subject was tested with decimal VA, the TRIVA and RFT. In addition biomicroscopy and fundus examination was performed.

Results: All subjects had normal fundus in the examined eye, and eight had minor lens opacities. The median decimal VA was 1.22 (range 0.65-1.6). There was a significant decline in decimal VA with increasing age. There was no significant age effect when measuring acuity with the TRIVA test at 90 or 50% contrast level. At the 25, 10 and 5% contrast level a significant age effect was observed, most pronounced at age > 65 years. Median mean hit rate in RFT was 100% (range 97-100) in the age group below 65 years and 87.5% (range 34-98) in subjects older than 65 years. The results from the subjects with lens opacities did not differ significantly from those with clear lenses. The reliability of the test results in all ages was good. In RFT there was no difference in the number of errors made between the two age groups, but test and reaction time was significantly higher in the age above 65 years.

Conclusions: In healthy volunteers a decline in foveal function with age, reflecting the loss of nerve fibres with increasing age, was demonstrated using three different tests. Both the RFT and the TRIVA test at contrast levels below 50% were more sensitive to the effect of age than conventional VA testing.

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Glucose increases Fibroblast Growth Factor 2 (FGF2) stimulated STAT1 and c-MYC synthesis in human Retinal Pigment Epithelial cells (hRPE)

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Purpose: FGF2 has been implicated in proliferative eye diseases. The purpose of this study was to assess the effect of high glucose on the FGF2 stimulated intracellular signaling molecules, STAT1 and c-Myc proteins.

Methods: Primary hRPE cell cultures were established from human eyes. Cells were treated with FGF2 and glucose (5-30 mM). Cell proliferation was monitored by 3H-thymidine (3H-thy) incorporation. 14C-methionine labeled-intracellular STAT1 and c-Myc synthesis were determined by immunoprecipitating using STAT1 and c-Myc specific antibodies. Data were analyzed by Student 't' test. c-Myc antibodies were used for c-Myc immunohistochemistry.

Results: FGF2 increased cell number and 3H-thy incorporation in a dose dependent manner in hRPE cells. The addition of glucose to FGF2 further increased 3H-thy incorporation in a dose dependent manner. FGF2 also stimulated immunoprecipitated 14C-STAT1 and 14C-c-Myc synthesis in dose dependent manner. Pre-treatment of cells with anti-STAT1 antibody blocked FGF2 stimulated 3H-thy incorporation. Addition of glucose (20 mM) further enhanced FGF2 stimulated 14C-STAT1 (3783±1242 vs. 2984±1002, CPM±SEM, p<0.05) and 14C-c-Myc (1071±224 vs. 534±103, mean CPM±SEM, p<0.05) synthesis. Immunohistochemical studies also confirmed an increase in c-Myc synthesis.

Conclusions: Glucose enhances FGF2 stimulated hRPE cell proliferation and STAT1 and c-Myc synthesis. Signaling pathways involving STAT1 and c-Myc synthesis may play a role in FGF2 induced hRPE cell proliferation. Modulation of this pathway by elevated glucose may play a role in diabetes related ocular complications. Supported by the Skillman Foundation.

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A1 adenosine receptors of human schlemm's canal cells as a potential target of cross-talk between inflow and outflow of aqueous humor

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Purpose: A1 adenosine receptor (AR) agonists lower IOP in rabbits, mice and monkeys by increasing outflow facility. ATP is released both from ciliary epithelial and trabecular meshwork cells and can be metabolized to adenosine by ectoenzymes. The main target site and underlying mechanism are unknown. We developed a technique to isolate Schlemm's canal (SC) inner-wall cells from human donor corneas to evaluate the effect of A1 AR agonists on ion transport by these cells.

Methods: Cells were isolated enzymatically. The resulting cells were cultured in medium F99 plus FBS. Before reaching confluence, cells were harvested and retained with CD31 monoclonal-antibody coated cell culture dishes; non-adherent cells were subsequently rinsed off. Whole-cell currents were recorded at passage 1 - 3.

Results: Inner-wall SC cells could be isolated within hours, as opposed to previous approaches requiring weeks of explant incubation. Employing CD31 as an SC-cell marker in the outflow region ensured the homogeneity of the harvested cells. The dominant conductance was an outwardly-rectifying TEA-inhibitable K⁺ channel. The A1AR agonist CPA activated 18 / 19 cells. At 50nM CPA produced a consistent activation in 9/11 applications to 6 cells. At 15nM CPA activated currents in 2/4 applications to 3 cells. At 100nM (S)-ENBA increased currents in 18/22 applications to 9/10 cells by an even higher magnitude than observed with CPA.

Conclusions: Inner-wall SC cells can now be cultured more selectively and more quickly than previously possible from tissue explant cultures. These SC cells display functional A1 ARs supporting the idea that inner-wall SC cells may be a potential site of IOP modulation.

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Vasomotion in small and large retinal arterioles

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Purpose: Recently focus has been put on rhythmic contractions of resistance vessels, vasomotion (VM), as a central factor in the regulation of the retinal microcirculation and fluid haemostasis. We have hypothesized that disturbances in VM in retinal arterioles of different calibre may participate in the pathogenesis of retinal vascular diseases, such as e.g. diabetic maculopathy. Therefore, experimental setups were established in order to study and differentiate vasomotion in larger and smaller retinal arterioles.

Methods: Double-barrelled pipette technique: Small porcine retinal arterioles (inner diameter 55 microns) were dissected and mounted between two double-barrelled pipette systems. Changes in the diameter of the arterioles were registered by video recording. Wire myograph: Large porcine retinal arterioles (inner diameter 140 microns) were dissected and mounted in a small-vessel myograph for isometric force measurements. Periods of VM were analysed by fast Fourier transformation and the frequencies determined.

Results: There was significant difference ($p=0.026$) between the frequency observed in the smaller retinal arterioles: 0.041 ± 0.011 Hz (SEM), $n=13$, as compared with the frequency observed in the larger arterioles: 0.014 ± 0.00073 Hz (SEM), $n=26$.

Conclusions: The vasomotion frequency observed in smaller retinal arterioles corresponds to the frequency found in other vascular beds, whereas the vasomotion frequency observed in large retinal arterioles was significantly lower. This may be due to differences of method. However, the findings support the notion that small and larger retinal vessels may have different roles for the regulation of retinal blood flow.

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Effects of aging on choroidal and optic nerve head circulatory responses caused by a postural change

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Purpose: The aim of the study was to delineate the effect of human aging on the sympathetically mediated circulatory response of the choroid and the optic nerve head (ONH) to the loading of cardiopulmonary baroreceptors from postural change.

Methods: Hemodynamic response to hypervolemic stress was measured in 10 healthy young males (mean age 22.8 yrs.) and 10 healthy old males (mean age 59.1 yrs.). The experiment consisted of a 10-min sitting period and a 10-min lying period. Blood flow in the choroids and ONH was measured with a laser speckle flowgraphy in the both periods. The speckle image obtained was analyzed to estimate a square blur rate (SBR) value, which was considered to represent the quantitative tissue blood velocity and the blood volume. Intraocular pressure (IOP), mean arterial pressure (MAP), stroke volume (SV), heart rate (HR), and plasma norepinephrine (NE) were also measured throughout the experiment.

Results: Change from sitting to lying posture, SBR in the choroid increased in both groups, however the magnitude of the increase was less in the old (16% increase in the young; $P<0.01$, 8% increase in the old; $P<0.05$). SBR in ONH and perfusion pressure (calculated as $2/3\text{MAP}-\text{IOP}$) were constant throughout the experiment in both subjects. Postural change decreased HR and NE, and increased IOP and SV significantly in both groups, and the changes were lower in the old than those in the young.

Conclusions: We conclude that the vasomotor tone of the capillary vessels is differently controlled between the choroid and ONH, and the choroidal vasodilatation is less in magnitude in the old.

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Does rinsing solutions osmolarities affect the corneal epithelium?

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Purpose: Different osmolarities of solutions applied to the eye cause burning to the eye. It is known in literature that solutions of 0 and above 600 mOsmol/kg will be burning to the eye. In eye burns the osmolarity of the cornea is much higher and osmolar trauma to the surviving cells will be as higher as the osmolarity of the rinsing fluid decreases. Therefore hyperosmolar fluids are on the market, but do the have harmful effects on healthy eyes?

Methods: 12 rabbits being in final anesthesia with healthy eyes were kept in anesthesia for 120 min. Each 4 eyes were applied regularly with balanced salt solution (Aqusia), isocorneal solution and Previn (R) every two seconds one drop for 120 minutes. 4 healthy corneas served as control. The eyes were then fixed in situ and excised. The corneas were prepared for SEM examination.

Results: Hypoosmolar rinsing of the cornea (Aqusia) showed corneal surface irregularity with pronounced desquamation of superficial cells with loss of microvilli. Isocorneal and hyperosmolar solutions did not provoke changes in the constitution of microvilli or pronounced desquamation. in previn with a hyperosmolarity of 850 mOsmol/Kg there was a slight dehydration visible on the superficial cells by means of pronounced nucleus visibility.

Conclusions: No harmful effect could be detected with hyperosmolar solutions. Longer lasting hypoosmolar rinsing causes epithelial damage. Isocorneal or hyperosmolar solutions are required to improve corneal epithelium stability during surgery and not harmful for the healthy eye. Water as first aid rinsing might add osmolar damage to the eye, nevertheless it is the best compromise if nothing else is available.

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Retinal venous diameter response changes with application time of chromatic flicker

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Purpose: Exposing the retina to flickering light causes retinal vessel dilation. The retinal venous response to green-blue and green-red flickering light with different exposure time was examined in our study.

Methods: In 10 young, healthy volunteers the Retinal Vessel Analyzer (RVA) was applied to assess retinal venous diameters. A baseline measurement was performed for 1 min. Then a flicker light switching between the colors red (600 - 720 nm) - green (530 - 570 nm) and blue (430 - 520 nm) - green (530 - 570 nm) with a frequency of 12 Hz was applied for 10, 20, 30 or 60 sec.

Results: With both tested stimuli a prompt vessel dilation in comparison to baseline could be determined in all volunteers. On average with red-green flicker this amounted to 2,5% \pm 1,1 (p<0,001) after 10 sec stimulation, to 2,9% \pm 1,1% (p<0,001) after 20 sec stimulation, to 4,5% \pm 1,8% (p<0,001) after 30 sec stimulation and 4,8% \pm 2,3% (p<0,001) after 60 sec stimulation. With blue-green flicker we found a dilation of 2,3% \pm 1,2% (p<0,001) after 10 sec stimulation, of 2,7% \pm 1,2% (p<0,001) after 20 sec stimulation, of 3,4% \pm 2,2% (p<0,001) after 30 sec stimulation and 3,8% \pm 2,1% (p<0,001) after 60 sec stimulation.

Conclusions: Flickering light of 12 Hz leads to a prompt and reproducible retinal venous dilation. A significant increase in magnitude of dilation could be noted with increased stimulus exposition from 10 sec to 30 sec (p<0,05). With 60 sec stimulus there is no statistically significant rise in dilation compared to 30 sec (p>0,1). We therefore think, that flicker application of 30 sec duration constitutes a feasible basis for examinations of retinal vein behavior, with good dilation magnitude and low patient inconvenience.

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Elevated levels of TGF- β 1 and TGF- β 2 in the tear fluid of patients with pseudoexfoliation and primary open angle glaucoma

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Purpose: Significantly elevated aqueous humor levels of transforming growth factor (TGF)- β 1 (0.15 ng/ml) in pseudoexfoliation (PEX)-syndrome/glaucoma and TGF- β 2 (1.9 ng/ml) in primary open angle glaucoma (POAG) and local production of both isoforms by resident cells at the filtering site are implicated in increased conjunctival scarring after glaucoma filtering surgery. In addition, TGF- β contained in the tear fluid may adversely affect the outcome of filtering surgery.

Methods: Quantitative determination of total human TGF- β 1 and - β 2 concentrations was performed with sandwich ELISA technique in 75 samples of unstimulated tears from 75 patients (22 POAG, 17 PEX glaucoma, 7 normal tension glaucoma (NTG), 7 ocular hypertension (OHT), 8 PEX-syndrome and 14 cataracts as controls).

Results: Levels of TGF- β 1 were significantly elevated in the basal tear fluid of PEX glaucoma (0.53 ng/ml) and OHT (0.46 ng/ml) patients as compared to controls (0.079 ng/ml). TGF- β 1 concentrations in patients with POAG, NTG and PEX syndrome were increased 2-4 fold as compared to controls. Concentrations of TGF- β 2 in basal tears were significantly elevated in patients with PEX syndrome (6.59 ng/ml) as compared to controls (4.33 ng/ml) and PEX glaucoma (3.73 ng/ml). In POAG, NTG and OHT patients, the concentration of TGF- β 2 was elevated only 1,3 fold as compared to cataract controls.

Conclusions: This is the first report about raised concentrations of TGF- β 1 and - β 2 in the basal tear fluid of glaucoma patients. This excess of both isoforms, continuously bathing the filtering bleb, may adversely influence conjunctival wound healing processes after glaucoma filtering surgery.

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Monocarboxylate transporters appear not to contribute to diabetic retinal neuropathy

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Purpose: Neurons rely partly on the transport of monocarboxylates like lactate and ketone bodies through defined transporters (MCT's) to meet their metabolic needs. Diabetes alters serum levels of monocarboxylates, and retinal neuropathy has recently been described early in the disease. The purpose of this study was to determine whether diabetes contributed to diabetic retinal neuropathy by altering specific MCT levels in the retina.

Methods: Diabetes was induced in Wistar rats by a single intraperitoneal injection of streptozotocin (62.5mg/kg). Rats were killed at 8 weeks, and retinal extracts analysed for changes in protein (western blot) and mRNA (RT-PCR) levels of PKCa, bFGF, MCT 1, 2 and 4. In other animals the retinae were processed histologically, and sections stained for breakdown of DNA ("TUNEL").

Results: Diabetic rats showed cataract formation, elevated blood glucose levels, and were ketotic as evidenced by abnormal weight gain. Retinal levels of MCT 1, 2 and 4 were similar in diabetic and control groups, however the retinopathy markers bFGF and PKCa were significantly elevated. Histological examination showed slightly elevated TUNEL staining in diabetic retinae, but retinal morphology appeared unaffected.

Conclusions: No evidence could be found to indicate that MCT 1, 2 or 4 are affected in the diabetic retina. Thus ketosis-induced downregulation of MCT's does not appear to be a likely mechanism of diabetic retinal neuropathy.

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Techniques and measurement errors in optic disc planimetry

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Purpose: To compare two different techniques of marking disc and cup borders and to study effects of computerised image enhancement in optic disc planimetry.

Methods: A single masked observer analysed digitised mono-scopical Zeiss optic disc photographs from 3 visits in each of 12 eyes from glaucoma patients. Using an image analysis program, disc and cup borders were marked: a. manually, tracing the borders using a digitizing tablet; b. with a semi-automatic subroutine where the operator only has to mark 12 plan points, one for each clock hour, and the time needed for marking, was noted. All photographs were also colour and contrast enhanced. Two different photographs and one duplicate from each visit were compared to determine inter-photographic and intra-photographic RMSE. To estimate any learning effects, RMSEs between duplicated photographs were regressed over the sequence in which they were measured.

Results: Median RMSE for intra and inter-photographic variability did not differ significantly between the manual or the semi-automatic marking methods, or between original and enhanced photographs. Mean duration for disc marking was smaller with the semi-automatic (6 minutes, SD: 0.25), than with the manual method (10.1 minutes, SD: 0.36), p < 0.0001. For each image, mean duration for enhancement was 1.1 minutes (SD: 0.1). RMSE decreased slightly but significantly over the time sequence in some manually measured series, but not with the semi-automatic method.

Conclusions: Semi-automatic planimetry was faster; had similar intra-observer variability as the manual method, and showed no learning effects. Image enhancement did not decrease measurement errors, but increased the analysis time.

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Does subfoveal choroidal neovascularisation influence scanning laser polarimetry with variable corneal compensator?

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Purpose: To investigate the influence of subfoveal choroidal neovascularisation (CNV) on macular imaging performed using scanning laser polarimetry (SLP) of the retinal nerve fibre layer.

Methods: SLP was performed on 22 consecutive patients with angiographically verified CNV, and on 23 healthy control subjects. One eye per subject was evaluated using the GDx Nerve Fibre Analyser. Regularity of the corneal retardation on the macular SLP images was assessed according to three criteria: (1) magnitude of the "macular ratio", defined as the ratio of mean retardation values along two axes (the axis with the maximum retardation and the perpendicular one, corresponding in healthy eyes to minimum retardation); (2) the values of GDx parameters which are independent of quadrant position (ellipse modulation and ellipse average); (3) the frequency of the regular "bow-tie" polarisation pattern.

Results: "Macular ratio" was significantly higher in the CNV group than in the control group ($p < 0.001$). Ellipse modulation did not differ between the groups, but ellipse average was higher in the CNV group ($p = 0.016$). The variance for each of these two parameters was significantly higher for the CNV group ($p < 0.001$ for both comparisons). A "bow-tie" pattern polarisation was seen in 23 of the 23 control eyes but only in 7 of the 22 CNV eyes ($p < 0.001$).

Conclusions: The results show that CNV influences the macular image obtained with SLP. This suggests that measurements with SLP may be disturbed for eyes with CNV when the customised corneal compensation method, which makes use of the macular retardation image, is employed.

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The high decrease of anti-oxidant enzymes in aged corneas

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Purpose: The purpose of this study was to investigate, whether the enzymatic pro-oxidant/anti-oxidant balance in the cornea was changed during aging.

Methods: The pro-oxidant enzymes (xanthine oxidoreductase/xanthine oxidase) and anti-oxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) were investigated in young, adult and aged corneas of normal eyes (rabbits and oxen) using histochemical, immunohistochemical and biochemical methods.

Results: Results show that as compared to young and adult corneas, the activities of anti-oxidant enzymes were highly decreased in aged corneas (all animals investigated). In contrast, the xanthine oxidase activity remained high or even increased.

Conclusions: It can be concluded that in aged corneas the imbalance between anti-oxidant and pro-oxidant enzymes appeared. It is suggested that the dramatic decrease in anti-oxidant enzymes might increase the danger from oxidative stress to the aged corneas.

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Glutamate concentrations in vitreous of patients with retinal detachment

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Purpose: Glutamate, an excitatory amino acid, is associated with ischemic damage to neurons. When glutamate is released in excess, or if re-uptake is ineffective, as may occur in ischemia, glutamate is neurotoxic. The aim of our study was to examine whether rhegmatogenous retinal detachment is associated with an increased level of intraocular glutamate.

Methods: Undiluted vitreous samples were obtained from 176 patients undergoing pars plana vitrectomy in our clinic between 1999 and 2003. Vitreous concentrations of glutamate were determined using high-pressure liquid chromatography (HPLC). The study group consisted of vitreous fluid samples from eyes with retinal detachment ($n = 114$). Controls consisted of vitreous fluid from eyes with an idiopathic macular hole ($n = 26$) or idiopathic epiretinal membrane ($n = 26$). A third group consisted of vitreous fluid samples obtained from patients with proliferative diabetic retinopathy (PDR) ($n = 10$).

Results: The level of glutamate in vitreous fluid from eyes with retinal detachment ($16.6 \pm 5.6 \mu\text{M}$) was significantly higher compared to the control group ($13.1 \pm 5.2 \mu\text{M}$) ($P < .0001$). The mean level of glutamate in vitreous fluid from patients with PDR ($19.5 \pm 6.5 \mu\text{M}$) was even higher, and also significant compared to the control group ($13.1 \pm 5.2 \mu\text{M}$) ($P < 0.0001$).

Conclusions: In the current study, rhegmatogenous retinal detachment was found to be associated with a significant increase in vitreous glutamate. Whether this results from a release by degenerative retinal neurons or from an impaired uptake of glutamate due the retinal ischemia associated with retinal detachment needs further study.

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Retinal oximetry: Reproducibility and sensitivity

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Purpose: Retinal ischemia plays a role in the pathogenesis of many eye diseases, e.g. diabetic retinopathy and glaucoma. We study the reproducibility and sensitivity of a spectrophotometric method for the measurement of optical density ratios (605/586 nm, 605/542 nm) in retinal blood vessels which correlate with the level of oxyhemoglobin in blood (Beach et al. J Appl. Physiol. 1999).

Methods: A fundus camera with an image splitter with 542, 558, 586 and 605 nm filters was used. A digital camera captured 4 images and a computer analysed the images. Optical density within and outside of vessels was measured and ODRs calculated. For reproducibility we analysed the same image 5 times and 5 consecutive images. For sensitivity we analysed arterioles and venules from one eye of 7 individuals (Mann-Whitney test).

Results: Reproducibility: When the same points on the same image are analysed five times the SD of the mean ranges from 5% to 11% for venules and 12% to 29% for arterioles. When 5 consecutive images from 7 individuals are analysed the average SD of the mean ODR's ranges from 15% to 57%. Sensitivity: The mean ODR605/586 for venules is 0.4553 ± 0.1256 (mean \pm SD) and 0.2344 ± 0.1571 for arterioles. The mean ODR605/542 for venules is 0.4925 ± 0.1413 (mean \pm SD) and 0.2614 ± 0.1381 for arterioles. The difference between arterioles and venules is statistically significant ($p < 0.0001$). The coefficient of variation ranges from 19% to 29% for venules and from 21% to 67% for arterioles.

Conclusions: The difference between ODR's for arterioles and venules demonstrates the technique's sensitivity. This technique detects a difference in the level of oxyhemoglobin in venules and arterioles of the retina. Its reproducibility is fair, but needs to be improved.

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Neuroprotective effects of topical brimonidine against retinal ischemia: electrophysiological studies

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Purpose: To study in adult rats functional alterations induced by retinal ischemia and neuroprotective effects of brimonidine.

Methods: The left ophthalmic vessels were ligated for 90 minutes (LOV). Rats received in left eyes 1 hour before ischemia, two 5 l drops of saline or 0.5% BMD. Flash electroretinograms (ERG) were recorded from both eyes 1, 8 or 12 w after LOV. Light flashes were presented for 5 ms from light emission diodes (LED) centered on the visual axis with intensities ranging 0.01 to 10 Cd/m². ERG a- and b-wave amplitudes were measured at different times after LOV.

Results: In unlesioned animals, ERG a- and b-wave amplitudes recorded in response to maximum light intensities were 119±/-19 V and 512±/-65 V, respectively (n=12). The vehicle-treated group of animals showed a-wave values that corresponded to 80%, 65% and 43% of control values at 1, 8 and 12 weeks after LOV, respectively (n=24). In the BMD treated-group, a-wave amplitudes corresponded to 93%, 78% and 80% of control values at 1, 8 and 12 weeks after the LOV, respectively. ERG b-wave amplitudes in vehicle- or BMD-treated animals at 1, 8 and 12 weeks represented 73 or 96%, 50 or 70% and 44 or 78%, respectively, of control values (n=24).

Conclusions: Brimonidine protects LOV-induced degeneration of outer retinal cells as assessed by functional techniques. The protection afforded by BMD lasts up to three months after ischemia. F.Seneca PI82-00540-FS-01, BF12002-03742, EU QLK6-CT-2000-00569, 2001-00385, FIS01-0050-02, SAF2001-0369 and Allergan Inc. unrestricted grant.

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In vitro effects of capsaicin on vanilloid receptors in bovine ciliary muscle

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Purpose: This study was designed to investigate the effect of capsaicin and to characterize the vanilloid receptors 1 (VR1) in bovine ciliary muscle.

Methods: Bovine eyes were obtained from a slaughterhouse. Ciliary muscle was quickly isolated as previously described by Lograno and Reibaldi, (1986). Ciliary muscle strips were set up under a tension of 200 mg in an organ bath and contractile responses were isometrically measured.

Results: Capsaicin (1 nM up to 0.1 nM) produced the concentration-dependent contractile responses of bovine ciliary muscle with EC₅₀ value of 14.8 microM. The capsaicin-induced contraction was antagonized by the VR1 receptor antagonist capsazepine (10 and 100 nM). In presence of calcium ionophore A 23187, the capsaicin-induced contraction increased significantly (P < 0.001). Moreover, the Ca⁺⁺ pump inhibitor thapsigargin (1 nM, 5 min) and the L-type calcium channel bloker nifedipine (1 microM, 10 min) inhibited the capsaicin-evoked contractile responses.

Conclusions: We conclude that the capsaicin induces bovine ciliary muscle contraction triggering Ca⁺⁺ mobilization from intracellular Ca⁺⁺ stores and utilizing also extracellular Ca⁺⁺.

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Macular Pigment and Veiling Glare

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Purpose: To see if veiling glare is reduced by macular pigment.

Methods: Macular pigment optical density (MPOD) was measured by heterochromatic flicker photometry with an instrument previously described (Mellerio et al, Current Eye Res. 25:37, 2002). Veiling glare was generated by a blue filtered tungsten light source with a spectral power distribution closely matched to MP spectral absorption. Subjects adjusted luminance of a yellow flashing LED target (0.2 degree diam), set at different horizontal eccentricities to the right of the glare source, until it was just visible. Only right eyes were used so the image of glare source was on the temporal retina. The luminance of the LED was taken to be that of the equivalent veiling glare luminance (EVGL).

Results: 19 male and 19 female undergraduates had significantly different MPOD of 0.55 ± 0.15 (mean ± sd) and 0.29 ± 0.08 respectively. EVGL decreased with eccentricity; there was no significant difference with gender. Scatter plots of EVGL at 1 deg eccentricity versus MPOD for males and for females showed no correlation (R² < 0.01 for both female and 0.01 male).

Conclusions: Macular pigment should absorb the blue scattered light but the lack of a correlation between EVGL and MPOD suggests that scattered light reaches the foveal photoreceptors without traversing the MP. Alternatively, other mechanisms in the eye might affect scatter, e.g. it has been shown that lens pigmentation is related to MPOD. Another possibility is that the method of determining EVGL is insufficiently sensitive to detect EVGL changes with MPOD variations. The importance to vision of MPOD and for understanding the origins of veiling glare are considered.

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Effects of brinzolamide on ocular hemodynamics in healthy volunteers

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Purpose: A prospective, randomized study to evaluate the effects of brinzolamide on ocular hemodynamics in healthy volunteers.

Methods: Thirty volunteers (m: 12, f: 18; mean age 28 +/- 8 years) were examined at baseline and after a 2 weeks treatment with brinzolamide or placebo. In video fluorescein angiograms (scanning laser ophthalmoscope, Rodenstock) arteriovenous passage time (AVP, dye dilution curves) was measured by means of digital image analysis. Retrobulbar blood flow velocities (peak systolic and end-diastolic velocity) and resistive indices (RI) of ophthalmic artery, central retinal artery, and temporal and nasal short posterior ciliary arteries were measured by color Doppler imaging (Sonoline Sienna Siemens). Automatic static perimetry was performed with a Humphrey Field Analyzer (Prg. 24-2) and contrast sensitivity was assessed by CSV 1000 (Vector Vision).

Results: Intraocular pressure was significantly decreased by brinzolamide (p<0.0001). Brinzolamide therapy resulted in a significant reduction of AVP (ANOVA, p<0.05), while placebo showed no change in AVP. Apart from an increase of RI in ophthalmic artery under placebo-treatment (p<0.05) there was no effect in retrobulbar hemodynamics in both groups. Neither brinzolamide nor placebo treatment resulted in changes of visual field indices.

Conclusions: Apart from the expected decrease of intraocular pressure brinzolamide resulted in a significant reduction of AVP and in no changes of retrobulbar hemodynamics. Since in glaucoma prolonged AVP is associated with disease progression the shortening of AVP might be beneficial in glaucoma therapy.

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Effect of UV-A and UV-B irradiation on metabolic profile of aqueous humour in rabbits analysed by 1H NMR spectroscopy

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Purpose: Ultraviolet radiation is known to increase the risk of cataract. Change of the metabolic composition of the aqueous humour might play an important role in this process. In this study, the effect of UV-A and UV-B radiation on the metabolic profile of aqueous humour from the rabbit eyes was investigated using high-resolution 1H Nuclear magnetic resonance (NMR) spectroscopy.

Methods: Both eyes of adult albino rabbits were irradiated with UV-A (366 nm; n=4) or UV-B (312 nm; n=4) rays for 8 min., once daily in 5 days. Three days thereafter the animals were sacrificed. Samples of aqueous humour from the irradiated and from control eyes (n=3) were then analysed by high-resolution 1H-NMR spectroscopy. After quantification, the metabolic profiles of the samples were tested statistically, using also multivariate methods (PCA and SIMCA).

Results: The concentration of several metabolites in the aqueous humour was found to be changed only after the exposure of the eyes to UV-B irradiation, not UV-A irradiation. After the UV-B irradiation, the concentration level of valine, isoleucine, formate, TMAO (trimethylamine-N-oxide), creatine and glucose was increased compared to UV-A irradiated eyes and control eyes. The concentration of ascorbate was decreased compared to UV-A irradiated eyes and control eyes.

Conclusions: Shorter wavelengths in the region of UV-B rays had a larger influence on the metabolic composition of aqueous humour than the longer wavelengths in the UV-A region.

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Diffuse luminance flicker increases blood flow in the human retina

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Purpose: It has been shown that diffuse luminance flicker increases optic nerve head blood flow, indicating a tight coupling between neural activity and blood flow. The current study aimed to quantify the changes in retinal blood flow during flicker stimulation.

Methods: In a group of 11 healthy volunteers, red blood cell velocity and retinal vessel diameters were assessed with bi-directional laser Doppler flowmetry and the Zeiss retinal vessel analyzer, respectively. Measurements were done in major retinal arteries and veins before and during flicker stimulation. Using retinal vessel diameters and red blood cell velocity, retinal blood flow was calculated for each condition. Flicker light consisted of square wave light flashes at a wavelength below 550nm and produced a retinal irradiance of 140µW/cm². Stimulation frequency was 8Hz.

Results: Retinal arterial diameters increased by +3.8±1.6% (p<0.001), diameter of retinal veins by +2.8±1.2% (p<0.001) during flicker stimulation. Red blood cell velocity increased by +48±22% (p<0.01) in retinal arteries and by +44±25% in retinal veins (p<0.01). Thus, diffuse luminance flicker increased calculated retinal blood flow by +59±20% (p<0.01) in arteries and by +53±25% (p<0.01) in retinal veins.

Conclusions: Our study demonstrates that diffuse luminance flicker increases both retinal vessel diameters and red blood cell velocity. These results indicate that flickerlight stimulation leads to an increase in blood flow in major arteries and veins.

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Effect of latanoprost and travoprost on blood-aqueous barrier integrity. A flare meter study

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Purpose: To study, with an objective method, the inflammation of the anterior segment of glaucomatous eye after treatment with latanoprost and travoprost.

Methods: Thirty patients with chronic open angle glaucoma ranged from 38 to 76 years (mean 64.0±12.2) were randomly assigned to travoprost 0.004% and latanoprost 0.005%. The study period was 16 weeks. IOP was measured every 2 weeks. Before and after 8 weeks and 16 weeks of therapy we studied the intraocular inflammation with a flare meter (Kowa FM-500) an instrument that is comprised of a He-Ne laser beam system, a photomultiplier mounted on a slit-lamp microscope and a computer. This flare-meter permits the objective determination of the flare and the number of cells in the aqueous of the anterior chamber.

Results: At the baseline IOP was 26.4±3.6 mm/Hg after two weeks the IOP for the travoprost group was 18.8±2.1 (p<0.001) and 19.4±2.8 (p<0.003) for the latanoprost group. The flare in the aqueous of the anterior chamber before treatment was 4.681±0.818 and after 16 weeks of treatment was for the latanoprost 11.838±3.218 (p<0.001) and 8.950±3.692 (p<0.001) for travoprost. The group of patients treated with travoprost had a statistical lower (p<0.035) flare when compared with the group treated with latanoprost.

Conclusions: The flare meter analysis shows that the eyes treated with travoprost have less significant broken of the blood-aqueous barrier and less inflammation of the anterior chamber. The use of travoprost might be safer when compared with latanoprost.

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Delayed-type hypersensitivity characterises ocular allergy to systemic 5-fluorouracil

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Purpose: To describe the ocular reaction to systemic administration of 5-fluorouracil (5-FU).

Methods: Administration of the second cycle of intravenous 5-FU chemotherapy for carcinoid tumour to a patient was associated with the development of a conjunctival reaction shortly after. Response to the first cycle of 5-FU chemotherapy had been unremarkable. Examination revealed a bilateral follicular conjunctivitis more prominent in one eye and concentrated in the deepest fornices. There were no corneal or intraocular features of infection. No evidence of systemic infection was found. Chlamydial scrape and viral serology was performed. A topical mast cell stabilising agent was prescribed as therapy.

Results: All investigations were negative. Delayed type hypersensitivity to 5-FU is felt to be the cause of the patient's conjunctivitis. This case shows a clear-cut relationship between exposure to systemic 5-FU and conjunctivitis, delay in onset of conjunctivitis till following second cycle of chemotherapy, a conjunctival reaction characterised by lymphoid follicles, and reversal of conjunctivitis with topical mast cell stabilising agents. Other possible causes of a follicular conjunctival reaction were excluded.

Conclusions: While systemic administration of 5-FU is known to rarely cause conjunctivitis, little data had existed on its specific ocular features nor on the mechanism involved to produce the reaction. This report shows that the conjunctivitis is follicular, is treatable with topical mast cell stabilising agents, and is likely to be a delayed-type hypersensitivity phenomenon. There are also wider implications for when 5-FU is administered topically.

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Can local low-dose chemotherapy reduce retinal neovascularization in mice?

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Purpose: Low-dose chemotherapy with doxorubicin has been shown to reduce cell number in cultures of human umbilical vein endothelial cells. It has also been shown to prevent tumor growth in mice with renal tumors when applied systemically for several times. In the low dose used, the chemotherapeutic has no direct effect on the tumor cells, but affects proliferating endothelial cells of the tumor vessels. We therefore investigated the effect of an intravitreal injection of low-dose doxorubicin on oxygen-induced retinal neovascularization in mice.

Methods: C57BL/6J mice were placed in 75% oxygen on postnatal day 7 (P7) and put back to room air on P12. On P14 they were treated with an intravitreal injection (2µl) of doxorubicin (1.8µM/5.35µM) in one eye and NaCl solution in the fellow eye (n=29/n=14). On P17 the animals were perfused intracardially with fluorescein-dextran. Retinal whole mounts were prepared and ischemic retinopathy was evaluated using a retinopathy score adapted from Higgins et al. (1999).

Results: There was no significant difference in retinopathy scores between treated and control eyes (p=0.25/p=0.21). This was the case for both doxorubicin concentrations used (1.8µM and 5.35µM).

Conclusions: One-time intravitreal application of a low-dose chemotherapeutic (doxorubicin) seems no option in the treatment of retinal neovascularization. Failure may be due to the missing continuance of treatment. Frequent application of a systemic low-dose chemotherapeutic might yield better results.

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Changes in choroidal blood flow during light/dark transitions are not altered by atropine or propranolol in healthy subjects

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Purpose: It has recently been reported that light/dark transitions lead to changes in choroidal blood flow. Several observations indicate that these changes in choroidal perfusion are triggered at least in part by neural mechanisms. In the present study we hypothesised that the choroidal blood flow response to changes in retinal illumination may be modified by either the muscarinic receptor antagonist atropine or by the beta receptor antagonist propranolol.

Methods: In 15 healthy subjects the response of choroidal perfusion was studied in a randomised placebo-controlled three way cross-over study using laser Doppler flowmetry and laser interferometric measurement of fundus pulsation amplitude.

Results: Before drug administration a transition from light to dark reduced both choroidal haemodynamic parameters by 8 - 12%. Neither propranolol nor atropine altered basal choroidal blood flow or choroidal blood flow responses to light/dark transitions.

Conclusions: Our data indicate that neither muscarinic nor beta receptors are involved in the choroidal blood flow response to changes in retinal illumination. Further studies are required to elucidate which mechanisms contribute to this blood flow behaviour of the choroid.

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Disturbances in flow regulation in diabetic retinopathy studied with the Retinal Vessel Analyzer (RVA)

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Purpose: To study changes in pressure autoregulation in diabetic retinopathy using the Retinal Vessel Analyzer (RVA).

Methods: Diameter changes in retinal vessels were recorded during rest and after the blood pressure was increased by stepwise increasing isometric exercise (lifting weights of 1, 1½, 2, and 2½ kg in straight arm). 51 normal persons with an age range between 26 and 67 years and 13 diabetic patients were examined.

Results: The maximal autoregulatory response occurred while lifting 2½ kg. This gave an age-independent increase in mean blood pressure of 21.7±1.3%. In normal persons this blood pressure rise gave a decrease in the autoregulatory response with age seen as a 4.6% (p=0.001) contraction in arteriolar diameter in younger individuals that diminished to a 0.4% (p=0.82, NS) contraction in diameter in the older age group. The results of the diabetic patients are under analysis. Results and traces from normal persons and diabetic patients will be shown.

Conclusions: The Retinal Vessel Analyzer is suitable for studying pressure autoregulation in diabetic retinopathy in vivo. Assessment of tone regulation in retinal vessels in diabetic retinopathy should be corrected for normal age-related changes in vascular tone dynamics.

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Computer-assisted analyses of fundus images in oxymetry

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Purpose: To develop a system that enables analysis of spectrophotometric measurements of oxygen saturation in the retinal vasculature.

Methods: The images are taken with a fundus camera through an optical device, which produces four images of the human retina, corresponding to four wavelengths: 542, 558, 586 and 605 nm. These images are automatically registered as pairs by evaluating the rotation and translation for each image. After the registration process, a user can choose a spot on the vasculature to examine the optical density ratios (605/586 nm, 605/542 nm) in retinal blood vessels. The system was used to examine the reproducibility and sensitivity of optical density ratios, which correlate with the ratio of oxy- and deoxy-hemoglobin in blood.

Results: To evaluate a registration of two images the system outputs a map containing vasculature edges from both images. Using this map, visual observation show that the registration process is accurate (mean square error is <1 pixel) for most of the images. Only in extreme cases does the registration process fail, i.e. where very few vessels appear on the images. The proposed algorithm has proven to be quite efficient in terms of execution time. The sensitivity measurements show that this technique is capable of detecting a difference in the level of oxyhemoglobin in venules and arterioles of the retina. The reproducibility is fair, although it needs to be improved.

Conclusions: The system is easy to use and provides reproducible results. This system may be useful in the study of ischemic diseases in the retina and optic nerve and the evaluation of drug and treatment effects.

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Detection of Plasminogen activator inhibitor-1 (PAI-1) mRNA in human ciliary processes and determination of PAI-1 in human aqueous humor

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Purpose: The present study was performed to detect the gene expression encoding for PAI-1 in human ciliary processes, to determine the quantity of PAI-1 and to measure PAI-1 activity in human aqueous humor and cell culture supernatants.

Methods: Total mRNA was extracted from human ciliary processes, which were cut off from freshly enucleated human eyes (n=8). Reverse transcribed PAI-1 mRNA was measured by real time PCR (TaqMan PCR). In addition, we determined the concentration of PAI-1 antigen (Ag) in supernatants of human ciliary processes in culture (n=8) and in aqueous humor of patients undergoing cataract surgery (n=18) by using an ELISA (Zymutest PAI-1 Ag). The activity of PAI-1 in human aqueous humor (n=30) was measured by using a specific chromogenic test (Coatest PAI-1).

Results: PAI-1 mRNA was localized in human ciliary processes. Depending on the time interval of cultivation the PAI-1 Ag levels ranged from 43.1 to 152.3 ng/ml. The quantity of PAI-1 Ag in human aqueous humor ranged from 0 to 4.56 ng/ml (mean 0.46 ng/ml). In contrast, PAI-1 activity was detected in all samples of human aqueous humor. The activity ranged from 6.5 to 19.0 AU/ml.

Conclusions: Our results indicate that PAI-1 is produced and secreted by human ciliary processes. PAI-1 is a main regulator of the fibrinolytic system. The presence of PAI-1 in human aqueous humor indicate that the fibrinolytic system is physiologically found in the anterior segment of the eye. Therefore, stimulation of PAI-1 production may be involved in intraocular inflammation and wound healing processes.

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Differences of intraocular temperature rising patterns and ocular injury by different wavelength of infrared exposure

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Purpose: To compare intraocular temperature (Temp.) and ocular injuries in rabbits exposed to near- and far- infrared rays.

Methods: The intra ocular temp. were measured during infrared ray (IR) exposure. The thermometer probes were inserted into the anterior chamber, lens, vitreous, and retrobulbar. The eyelid temp. (IRA group only), body temp., and room temp. were also measured. The eyes were exposed unilaterally to either IRA (35 mW/cm²), or an IRC (2.5 W/cm²). Using other rabbits, changes in the anterior segment were observed by a Scheimpflug image analysis system, and a laser flare cell meter.

Results: In the IRA, while the temp. of the eyelid rose from 30-37C to 50-65C immediately after exposure, the temp. rise of the anterior chamber showed an increase from 36-38C to 39-41C during exposure. The temp. of the vitreous, retrobulbar, and body were 38.6±0.1C, 38.2±0.1C, and 37.9±0.1C. In the IRA group, conjunctival edema was seen after the exposure, but no changes were observed in the lens. The ocular flare value increased from 11 to 350 photon count/m. sec. in the IRA group. In the IRC group, the temp. of the anterior chamber was 41-42C immediately after exposure. The temp. rising pattern of the vitreous was slower than that of the anterior chamber, and the highest temp. in the vitreous during exposure was below 40C. The retrobulbar temp. did not change during exposure. The cornea was severely damaged immediately after exposure, but no change was observed in the lens. The ocular flare value did not show big changes in the IRC group.

Conclusions: Although IRA and IRC exposure induced thermal effects to the eye ball, rising patterns of the intraocular temp. differed.

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Determination of aqueous concentration of ofloxacin following oral administration using capillary zone electrophoresis

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Purpose: Topical ofloxacin given into the conjunctival sac has been shown to penetrate the eye well with a high aqueous concentration. In this prospective study, using the method of capillary zone electrophoresis, we determined the aqueous level of ofloxacin following oral administration prior to cataract surgery.

Methods: One hundred and ten consecutive patients undergoing cataract surgery were enrolled into the study. One tablet of ofloxacin (400 mg) was given orally on the day of surgery. Cataract surgery proceeded with an instillation of povidone-iodine into the conjunctival sac and the patient was cleaned and draped. Aqueous humour samples were collected via a paracentesis and then transferred to Eppendorf tubes and kept in the fridge until collected for analysis using the assay method of capillary zone electrophoresis.

Results: The mean aqueous concentration of ofloxacin was 0.69 mg/l (range 0.18 - 1.81). This concentration is higher than the MIC₉₀ (the minimum inhibitory concentration required to inhibit growth of 90% of the bacterial isolates tested) for the majority of bacteria responsible for endophthalmitis. The MIC₉₀ for Staph epidermidis is 0.4mg/l and 87.3% of patients achieved an ofloxacin aqueous level of 0.4mg/l or more.

Conclusions: -Our results indicate that oral ofloxacin given preoperatively as a single dose (400 mg) achieved an aqueous level of 0.4 mg/l or more in 87.3% of 110 patients undergoing cataract surgery-Capillary zone electrophoresis is a useful method of assaying ofloxacin levels in aqueous humour requiring only a very small volume of sample

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Effect of a nifedipine-induced reduction of blood pressure on the association between ocular pulse amplitude and ocular fundus pulsation amplitude

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Purpose: According to the Friedenwald equation a change in ocular volume leads to a change in intraocular pressure. The relation between these two parameters is dependent on the rigidity of the eye. Animal studies indicate that the ocular rigidity may depend on arterial blood pressure. In the present study we hypothesized that this may also be the case in humans.

Methods: In 16 patients with systemic hypertension single dose nifedipine was administered to lower systemic blood pressure. Before and after this intervention blood pressure, intraocular pressure, pulse amplitude as assessed with pneumotometry and fundus pulsation amplitude as assessed with laser interferometry were recorded. The ratio of pulse amplitude and fundus pulsation amplitude was calculated as a measure of ocular rigidity.

Results: As expected nifedipine reduced mean arterial pressure by 17.3%. Administration of nifedipine reduced pulse amplitude, but did not change fundus pulsation amplitude. Accordingly, we observed a significant decrease in the ratio of pulse amplitude/fundus pulsation amplitude after administration of nifedipine (-15.1%; p < 0.001).

Conclusions: The reduced ratio of pulse amplitude/fundus pulsation amplitude is indicative of an altered ratio of pressure change/volume change during the heart cycle when blood pressure is lowered. This indicates that ocular rigidity is dependent on systemic blood pressure and that there is a contribution of choroidal vessel rigidity to the mechanical properties of the eye.

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Effect of C-peptide on ocular blood flow in patients with IDDM

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Purpose: It has generally been thought that C-peptide is biologically inactive. However, recent studies suggest that C-peptide might exert vascular effects and that the lack of endogenous C-peptide may be associated with the development of diabetic microvascular complications. The aim of the present study was to investigate the vascular response to systemic infusion of C-peptide in IDDM patients with no evidence of diabetic retinopathy, in particular the effect on systemic blood pressure and ocular blood flow.

Methods: The study was performed in a randomized, placebo-controlled, double masked, 2-way crossover design. 10 IDDM patients were included. C-peptide was administered intravenously at two different levels. Physiologic saline solution was given instead of C-peptide to control for placebo effects. On both study days euglycemic clamps were performed to maintain the blood glucose level at 100 mg/100ml. To assess retinal blood flow, laser Doppler velocimetry (blood flow velocities) and retinal vessel analyser (vessels diameters) measurements were performed. Laser interferometric measurements of fundus pulsation were used to assess pulsatile choroidal blood flow. Blood velocities in the ophthalmic artery were measured using color Doppler imaging.

Results: Eight patients, including 2 women and 6 men, completed the study according to the protocol and without adverse events. The systemic hemodynamic parameters remained stable during both study days. During the infusion of C-peptide no significant changes in any of the ocular hemodynamic parameters were observed.

Conclusions: Data of the present study indicates that exogenous C-peptide exerts no effect on systemic and ocular hemodynamic parameters in diabetics under euglycemic conditions.

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Free plasmin and streptokinase-plasmin complex differ in their ability to degrade fibronectin and laminin

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Purpose: Plasmin enzyme (PL) has been proposed as an aid to create posterior vitreous detachment (PVD), based on its ability to cleave laminin (LM) and fibronectin (FN), both known components of the vitreoretinal interface. PL is generated by plasminogen (PG) activation, either by direct proteolytic cleavage by urokinase (UK) or tissue plasminogen activator, or by rapid formation of a 1:1 complex with streptokinase (SK) and conversion to a streptokinase-plasmin complex (SK-PL), which can generate free plasmin catalytically by cleavage of PG. This study compares the efficacy of SK-PL, SK-PL activated free PL and UK activated plasmin (UK-PL) in cleaving LM and FN.

Methods: PG was isolated from human plasma by affinity chromatography. SK was added to PG in various molar ratios, generating SK-PL at ratios of 1:1 and above, and mixtures of SK-PL and free PL (SK-PL/PL) at lower ratios. Various doses of SK-PL, SK-PL/PL and UK-PL (Sigma) were added to purified LM and FN. PL activity was measured (S-2251). The mixtures were incubated at 37°C for 30min-22h, then processed for SDS-PAGE.

Results: SK activated PG rapidly and completely at all molar ratios. All enzyme preparations cleaved LM and FN, degradation of LM was generally slower than that of FN. SK-PL/PL was more effective in cleaving both LM and FN with increasing free PL content (decreasing SK-PL/PL ratio). UK-PL displayed similar cleavage as SK-PL/PL at the 1:10 ratio.

Conclusions: The activation mode of PG determines its efficacy in cleaving FN and LM. Lower SK to PG ratios generate increasing amounts of free plasmin, which is more effective in LM and FN degradation than SK-PL, and may therefore be more useful than SK-PL as a surgical tool to facilitate PVD.

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Electronic microscopy findings in rats inoculated with a silicone ferrofluid

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Purpose: To describe the ultra structural findings with transmission electronic microscopy in the area of inoculation of a silicone oil ferrofluid in the legs of a cohort of Wistar rats.

Methods: We inoculated subcutaneous 90 Wistar rats with a silicone ferrofluid. We coupled 9 control rats with no inoculation, 9 rats with subcutaneous inoculation of a medical use silicone; 9 rats inoculated with an aqueous ferrofluid; 9 with a subcutaneous magnet; 27 with a silicone ferrofluid in different solutions: 1/4; 1/8, 1/16; and 27 rats in which we placed a magnet besides the silicone ferrofluid. All the animals were distributed in groups of three and were sacrificed 7, 15 and 30 days after.

Results: We found a local chronic inflammatory reaction, with an increasing intensity as time goes by. We did not find statistically significant differences among groups with different solutions. We found hystiocytes containing ferrofluid either as iron pigments or as electrón dense clusters inside vacuolae with clearly discernable membranes.

Conclusions: Ultra structural results confirm our findings with light microscopy. Inflammatory reactions were limited to the inoculation site. Tisular necrosis was not observed. The study should continue in order to evaluate the silicone ferrofluid as vitreous substitutes in eyes of experimental animals.

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Glutathione and lipid peroxide changes in pseudoexfoliation syndrome

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Purpose: To determine the oxidative status in the aqueous humour of patients with pseudoexfoliation syndrome (PEX).

Methods: Aqueous humour samples, obtained during cataract surgery, of patients with PEX and normal age matched subjects were examined for changes in the levels of glutathione (GSH), glutathione disulphide (GSSG) and malondialdehyde (MDA), a product of lipid peroxidation. GSH, GSSG and MDA were determined by appropriate fluorescent assays.

Results: Preliminary results in PEX aqueous humour samples showed an approximately 40% increase of GSSG concentration, while GSH levels remained unchanged. The ratio of GSH/GSSG was 2 fold decreased in PEX samples approximately. In addition, MDA was increased by 55% as compared to the control.

Conclusions: High levels of GSSG, followed by decrease of the GSH/GSSG ratio, and high levels of MDA levels (lipid peroxides) are considered as typical indicators of high oxidative stress. The existence of these indicators in the PEX aqueous humour samples strongly suggests a role for oxidative stress in the pathogenesis and the progression of PEX.

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Heptanol disrupts communication between ciliary epithelial pigmented and non-pigmented cells

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Purpose: Electrical measurements indicate that heptanol interrupts gap junction communication between individual pigmented (PE) and non-pigmented (NPE) cells. To investigate this, we have studied the effects of heptanol with or without ouabain on the Na, K and Cl contents of paired PE and NPE cells.

Methods: Tissues from isolated rabbit iris-ciliary body were mounted in chambers. After equilibration for 40 min with or without heptanol 3 mM in both solutions, tissues were incubated for 30 min in Ringer's solution alone, with heptanol added, or additionally with ouabain 100 µM in one or other of the solutions. After incubation, tissues were rapidly frozen at -180°C. Sections (0.2-0.4 µm) were cut at -90°C, freeze-dried and transferred to an SEM with an energy-dispersive x-ray spectrometer. Individual pigmented (PE) and non-pigmented (NPE) cells were analysed in pairs for their Na, K, Cl and P.

Results: Heptanol alone had little effect on cell composition. In contrast to the effects of ouabain alone, where paired NPE and PE cells were affected to the same extents, with heptanol present ouabain in the aqueous solution or in both solutions produced much larger changes in anterior NPE than in PE cells. Posterior cells were much less affected.

Conclusions: The results indicate that heptanol indeed interrupts PE-NPE junctions, providing an opportunity for electron microprobe analysis of the sidedness of modification of ciliary epithelial secretion.

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Inhibitory effect of certain neuropeptides on the proliferation of human retinal pigment epithelial cells

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Purpose: To define the effect of the neuropeptides substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and secretoneurin (SN) on the proliferation of human retinal pigment epithelial cells

Methods: ARPE-19 cells were used. The cells were standardly cultured in DMEM medium and one thousand and two thousand cells were incubated with the peptides for three and five days. The proliferation was evaluated by an ATP lite assay. Furthermore, specific antagonists were incubated with an effective concentration of the peptides to find out whether the effect would be reversed.

Results: Each peptide exerted an inhibitory effect on the proliferation. The effect was weak averaging 10% to 15% but highly specific as the antagonists fully reversed the inhibition.

Conclusions: The results clearly indicate that retinal pigment epithelial cells are under neural control. These neuropeptides constitute the first endogenous inhibitors of retinal pigment epithelial cell proliferation and for SN this is the first biological effect in the eye. The results are relevant both physiologically and pathophysiologically. Physiologically the inhibitory effect may mean that the peptides cause the cells to maintain in a differentiated condition and prevent them proliferating in excess. Pathophysiologically the results are relevant in proliferative vitreoretinopathy. The authors hypothesize that the inhibitory effect diminishes when retinal pigment epithelial cells are swept out and actively migrate from their physiologic location between the neurosensory retina and the choroid. This hypothesis provides a better understanding about the initial steps in the pathogenesis of the disease.

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Identification of leptin in aqueous humor in diabetic and non-diabetic patients

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Purpose: To determine the presence of leptin in human aqueous humor and to analyze the influence in diabetes mellitus.

Methods: Leptin concentrations in aqueous humor and serum from cataract operated diabetic (n=11) and non-diabetic patients (n=8) were determined using a specific radioimmunoassay.

Results: In the control group, leptin concentration in aqueous humor was 22.6 ± 4.5 pg/ml and in serum 4.1 ± 1.7 ng/ml. In diabetic patients, leptin concentrations in aqueous humor were 32.6 ± 8.7 pg/ml (serum: 3.67 ± 2.0 ng/ml). There was a linear correlation between leptin serum and aqueous humor concentrations in normals ($r=0.75$, $p=0.003$). This relationship was not present in non-diabetic patients. Although higher leptin concentrations in aqueous were observed in patients with diabetes mellitus, the difference in comparison to controls was not statistically significant.

Conclusions: Leptin can be found in aqueous humor in all subjects investigated. Our results indicate a disordered balance of leptin concentrations in serum and aqueous humor in patients with diabetes mellitus. This may be related to a compromised leptin transport into aqueous humor in diabetic patients.

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Mathematical modelling of aqueous humor formation

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Purpose: Because the aqueous humor is of great importance to the normal functioning of the eye, the mathematical model is developed to analyse aqueous humor formation influence on the intraocular pressure. The model describes fluid flow through the number of barriers between the blood capillaries in the ciliary body and posterior chamber of the eye.

Methods: The mathematical model is based on analysis of eye physiology experimental data and contains the balance equations for fluid penetrating through the capillary wall, pigmented and nonpigmented epithelial layers due to hydrostatic, oncotic and osmotic pressure gradients. Also the passive and active transport of ions and solutes are considered.

Results: The presented model may be used as an instrument for numerical experiments simulating the changes of external conditions like protein and solute concentration and outflow parameters in the posterior chamber. Model allows to estimate the contribution of passive ultrafiltration and active ion transport to aqueous humor formation.

Conclusions: The mathematical description and computer modeling may be important in studying the eye fluid dynamics regulatory processes by testing different hypotheses of aqueous humor secretion (inflow) and fluid drainage from the eye (outflow) through the trabecular meshwork and the uveoscleral pathways.

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Methylglyoxal increase ubiquitin dependent proteolytic capabilities in lens epithelial cells

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Purpose: Methylglyoxal, a highly reactive dicarbonyl, reacts with proteins to form advanced glycation end products (AGEs) thus contributing to various AGE-associated complications particularly those related to diabetes, including cataract. The ubiquitin-proteasome pathway is a major mechanism for selective degradation of damaged proteins. The main objective of this study is to determine whether exposure of lens epithelial cells to methylglyoxal disrupts the function of ubiquitin-proteasome pathway by interfering with proteolytic capabilities or by increasing the amount of ubiquitinated substrates.

Methods: Human lens epithelial cells are treated with methylglyoxal at different times and concentrations. The levels of ubiquitin-conjugates are determined by western blot. The chymotrypsin-like activity is assayed in cytosolic extracts using a fluorogenic peptide (LLVY-AMC). Conjugation activity to exogenous substrates is evaluated by the ability of cell lysates to ubiquitinate [125]I-lactalbumin and the ability to degrade endogenous substrates is assayed by following degradation of p53.

Results: Treatment of lens epithelial cells with methylglyoxal increases proteasome activity and the endogenous ubiquitin conjugates. The levels of p53, a characteristic ubiquitin-proteasome substrate, decrease in cells treated with methylglyoxal.

Conclusions: Treatment of cells with methylglyoxal, increases proteolytic capability and ubiquitin conjugation activity. These suggest that methylglyoxal may up-regulate ubiquitin-proteasome pathway, presumably by increases the amount of substrates.

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Ollagenase used in the treatment of chronic dacryocystitis

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Purpose: To improve efficiency in the treatment and prevention of chronic dacryocystitis recurrences using collagenase.

Methods: The results of treating chronic dacryocystitis using collagenase produced by the Ufa "Immunopreparat" enterprise are presented. The preparation is made from the hepatopancreas of Kamchatka crab marketable species. It contains collagenolytic trypsin-like proteinases with high specific activity (no less than 20 u/ml). Collagenase also has high necrolytic and fibrinolytic activities. Our study comprised 48 patients (52 eyes) with chronic dacryocystitis. Collagenase was used to prevent the disease recurrences following transcanalicular laser endoscopic dacryocystorhinostomy. The lacrimal ducts were daily irrigated with 1% collagenase (10ml) for five postoperative days.

Results: It has been noted that postoperative inflammation rapidly subsided using enzymotherapy (at three days postoperatively). The patient was followed up for six months and no dacryocystitis recurrences were observed. There was no evidence of allergic reactions.

Conclusions: The analysis of the data obtained allows to conclude that collagenase appears to be an effective agent in the treatment of chronic dacryocystitis.

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New evidence for regional variation in ciliary epithelial transport

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Purpose: Ciliary epithelial transport may be region dependent. To investigate this, we studied the effects of ouabain on the epithelial cells in the posterior (towards retina) and the anterior region (towards iris) of the iris-ciliary body (ICB).

Methods: Tissues from isolated rabbit ICB were mounted in chambers. After equilibration, tissues were incubated for 30 min in Ringer's solution alone or with ouabain 100 μ M in one or other or both solutions. After incubation, tissues were rapidly frozen at -180°C. Sections (0.2-0.4 μ m) were cut at ~90°C, freeze-dried and transferred to an SEM with an energy-dispersive x-ray spectrometer. Individual pigmented (PE) and non-pigmented (NPE) cells were analysed in pairs for their Na, K, Cl and P.

Results: Ouabain caused much larger changes in anterior compared to posterior Na/P and K/P, without significant effects on the Cl/P. In the anterior epithelium, the Na/P and K/P following asymmetrical solutions showed considerable variation from cell to cell. In contrast, ouabain on both sides produced significantly greater gain of Na and loss of K, with reduction in data scatter. Paired NPE and PE cells showed the same changes in ions after ouabain.

Conclusions: The results provide new evidence that net transport across the ciliary epithelium is region specific. This specificity could reflect the isoform distributions of Na⁺, K⁺ ATPase. The increased turnover of Na⁺, K⁺ and Cl⁻ would permit a higher rate of aqueous humor secretion across the anterior region of the ciliary epithelium.

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Prolonging tear film break-up time using a new ocular lubricating drop that gels in the eye

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Purpose: Evaluate a new lubricating eye drop containing a novel gelling ingredient, HP-Guar, against ocular lubricants containing either carboxymethylcellulose or glycerin/polysorbate 80 on tear film break-up time (TFBUT).

Methods: Randomized, double-masked, three-way crossover, single-center trial. Patients were seen days 0, 7, and 14. At each visit, a different ocular lubricant was instilled and TFBUT was evaluated at baseline and 5, 10, 15, 20, 30, 45, and 60 minutes post instillation. A micropipette was used to instill a controlled amount (40 μ l) of test product in each eye. TFBUT evaluations were performed using 1 μ l of 2% preservative-free sodium fluorescein, instilled using a DET strip (Akorn, Inc.) and recorded with a digital imaging device.

Results: Fifty patients completed the study. The new lubricating eye drop containing Polyethylene Glycol 400, Propylene Glycol and HP-Guar (SYSTANE[®], Alcon) (a) extended mean TFBUT up to 30 minutes post instillation (an increase from baseline of 4.26 seconds at 5 minutes to 1.81 at 30 minutes); (b) extended mean TFBUT significantly more than the carboxymethylcellulose based lubricant up to 20 minutes post instillation (P<.001); (c) extended mean TFBUT significantly more than the glycerin/polysorbate 80 based lubricant up to 30 minutes post instillation (P=.033).

Conclusions: The new lubricating eye drop containing a novel gelling ingredient, HP-Guar (SYSTANE[®]), exhibited a lasting effect on TFBUT, extending TFBUT up to 30 minutes post instillation. Moreover, it was statistically superior to carboxymethylcellulose and glycerin/polysorbate 80 based ocular lubricants in extending TFBUT. This suggests SYSTANE's ability to stabilize TFBUT can provide long-lasting relief.

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Spatial variations in pre-corneal drug concentrations

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Purpose: The specific objective is to determine whether Ofloxacin concentrations are different in the upper and lower regions of the cornea when delivered by standard means to typical patients attending hospital. There may be a relative deficit in superior regions of the cornea when drugs are applied to the inferior fornix.

Methods: One drop of proxymetacaine anaesthetic followed by a drop of ofloxacin 0.3% was instilled into the eye of each upright patient. At 2.5, 10 and 20 minutes following Ofloxacin administration two strips of Schirmer's paper were touched simultaneously at the superior and inferior regions of the eye's cornea at the margins of the lid, thus contacting the superior and inferior marginal tear films. Analysis was by High performance liquid chromatography in accordance with accepted and published papers on ofloxacin concentrations in the eye.

Results: Results show very high concentrations found at two minutes after instillation for both upper and lower areas (mean 132 micrograms/ml). This very rapidly deteriorated and little remained at 20 minutes after instillation (mean 4.4 microg/ml). Comparing superior to inferior regions there was a substantially greater concentration at inferior regions 2 minutes after instillation (461 microg/ml v 86.7 microg/ml) but by 5 minutes after instillation this difference was insignificant (38 microg/ml v 53 microg/ml).

Conclusions: Although there may be initially greater concentration of applied drug in inferior regions compared to superior regions of the tear film, this difference very rapidly disintegrates.

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Optical coherence tomographic bio-morphometry of the optic nerve head: inter-individual reproducibility

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Purpose: The optical coherence tomograph III (OCT III) features a new evaluation technique which possibly allows the quantification of optic disc structure. Since for any new technique, its reproducibility has to be evaluated before it may be used for the detection of diseases, it was the purpose of the present study to examine the reproducibility of the OCT.

Methods: The study included 10 eyes of 10 persons who underwent 15 OCT examinations using the high resolution papilla scanning program which were performed by 3 independent examiners. A second set of 3 scans was taken by the papilla fast scan program. The variation coefficient was calculated as the ratio of the mean of the standard deviations divided by the mean of the means.

Results: The interindividual variation coefficient was for the repeated measurements of the optic disc area in high resolution mode 6.3±1.2%, for neuroretinal rim 8.3±0.5% and for optic cup area 18.6±1.9%. Using low resolution fast scan mode, values improved (Disc: 5.7±1.1%; Rim: 7.3±2.6%; Cup: 15.9±3.1%). Rim and cup measurements of optic nerve heads without cupping were evaluated incorrectly, probably due to software error.

Conclusions: Morphometric measurements of the optic disc by the OCT are prone to artifacts due to atypical disc configurations. Reproducibility improved by using fast scan mode. This data may be important for the interpretation of measured differences between normal subjects and glaucoma suspects or patients with manifest glaucoma.

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The effectiveness of brimonidin for the treatment of pediatric glaucoma

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Purpose: Brimonidine is a relatively selective agonist, which reduces intraocular pressure (IOP) by decreasing aqueous production and increasing uveoscleral outflow. Our study was designed to examine the hypotensive effects and safety of this medication in a heterogeneous group of children with glaucoma uncontrolled with otherwise maximal medical therapy.

Methods: We analyzed the IOP data and side effects of children with brimonidine therapy in our Department from March 1999 to March 2002. Brimonidine 0.2% was used for patients whose glaucoma was uncontrolled on maximal tolerated medical therapy. The study included 38 eyes of 27 patients with uncontrolled IOP and varied glaucoma diagnoses. The mean patient age was 9.3 years (range 5.5 to 16 years), with a mean follow-up on brimonidine of 21.6 months (range 12 to 36 months).

Results: The baseline IOP decreased from a mean of 23.1 ± 4.7 mm Hg to a mean of 20.5 ± 3.6 mm Hg (a mean decrease of 11.2% ± 9.5%, P = 0.037) on brimonidine after a mean follow-up of 21.6 ± 9.8 months. Most patients were on at least 2 other glaucoma medications. Eleven children experienced fatigue or somnolence after more than 12 months of brimonidine administration and symptoms completely resolved after brimonidine was discontinued. Most of these children were less than 6 years of age.

Conclusions: In selected children of our study brimonidine showed an impressive ocular hypotensive effect. However, brimonidine can cause somnolence and fatigue and should be used with caution in children younger than 6 years. A larger groups of children monitored for longer periods may further delineate the role for this drug in the treatment of pediatric glaucoma.

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A Swiss prospective study on the analysis of the intraocular pressure lowering efficacy of Travoprost monotherapy in newly diagnosed patients with an IOP of 21mmHg or more

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Purpose: The study evaluated the intraocular pressure (IOP) lowering effect of travoprost ophthalmic solution 0.004% in newly diagnosed ocular hypertensive and glaucoma patients with baseline IOP at 21 mmHg or more.

Methods: A 3-month open label, prospective, multi-center trial was conducted in Switzerland. This open-label study, with 1 and 3-month follow-up examinations, conducted at 219 sites, enrolled 1,590 patients, 440 of whom were newly diagnosed. Exclusion criteria were in accordance with product labeling. Demographics and baseline IOPs were collected and study medication dispensed at the initial visit. Patients were instructed to instill one drop of travoprost ophthalmic solution 0.004% q.d. in each affected eye at approximately 8 PM, and return for 1 and 3-month follow-up visits. Efficacy was assessed by mean IOP change from baseline at each follow-up.

Results: There were a total of 440 newly diagnosed patients, 351 of whom, completed all examinations with baseline IOPs of 21mmHg or more. The average baseline IOP of this patient population was 26.2mmHg (SD=4.3). The baseline IOPs decreased to 17.6mmHg (SD=3.2) at the 1-month follow-up and 17.7mmHg (SD=3.0) at the 3-month follow-up. The differences of 8.6mmHg at 1-month and of 8.5 at 3-months on TRAVATAN therapy were reductions of 33% and were statistically significant (p < .0001).

Conclusions: Results show that travoprost ophthalmic solution 0.004% significantly reduced IOP on average by at least 8.5mmHg, which is a reduction of 33%, in newly diagnosed patients whose baseline IOP was 21mmHg or more. Results show no significant loss of efficacy from the 1-month to the 3-month visits.

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Optic disc morphometry with optical coherence tomography. Comparison with scanning laser tomography

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Purpose: To compare morphometric optic disc measurements obtained by optical coherence tomography and by scanning laser tomography.

Methods: Thirty-three eyes of 22 Caucasian subjects (12 female and 8 male patients) were included. Mean age was 59.6 ± 16.8 years (mean \pm SD), mean refractive error was 1.3 ± 2.4 D. All eyes underwent optic disc assessment by optical coherence tomography (OCT3, Carl Zeiss Ophthalmic Systems-Humphrey Division, Dublin, CA, USA). Six cross-sectional scans of the disc were obtained in differing meridians. Of the six scans, morphometric data of the optic disc were computed. Of all eyes, 3 scanning laser tomographic images were obtained (HRT II, Heidelberg Engineering, Dossenheim, Germany) and morphometric data of the optic disc were computed.

Results: Correlation coefficient (Pearson) for the two methods was $R = 0.64$ ($P < 0.0001$) for the disc size, $R = 0.60$ ($P < 0.0001$) for the cup size and $R = 0.17$ ($P = 0.33$) for rim area. In 5 eyes displaying a small disc without cupping, the OCT3 measured a total cupping without rim. After correction for this artifact, correlation coefficient for cup area was $R = 0.91$ ($P < 0.0001$) and for rim area $R = 0.48$ ($P = 0.004$).

Conclusions: Morphometric optic disc measurements obtained by OCT3 correlate well with those of an established method, HRT II, if artifacts are considered.

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Correlations in optic disc morphometry with optical coherence tomography

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Purpose: Papillometry with OCT is a new method to quantify the morphology of the optic disc. Herein we evaluated, whether the measurements show associations with external factors.

Methods: 56 eyes of 44 Caucasian normal subjects (27 female) with a refractive error above -4 D and a minimal age of 25 years were included. Mean age was 59 ± 13.8 years (mean \pm SD), mean refractive error was $+0.75 \pm 1.5$ D. All eyes underwent optic disc assessment by optical coherence tomography, fast scan protocol (OCT3, Carl Zeiss Ophthalmic Systems-Humphrey Division, Dublin, CA, USA). Six cross-sectional scans of the disc were obtained in differing meridians. Of the six scans, morphometric data of the optic disc were computed.

Results: Mean size of the optic disc was 2.14 ± 0.39 mm₂, of the optic cup 0.68 ± 0.61 mm₂, and of the neuroretinal rim 1.42 ± 0.40 mm₂. There appeared no significant correlation of any of the morphologic parameters with age or refractive error (Pearson, $P > 0.1$). Similarly, there was no association with sex (Mann-Whitney, $P > 0.5$). Disc area was correlated with cup area ($P < 0.001$, $R = 0.79$) and a weak negative association existed with rim area ($P = 0.03$; $R = -0.3$).

Conclusions: Morphometric optic disc measurements obtained by OCT3 seem not to be associated with age or refractive error.

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Enlargement of parapapillary atrophy in stable and progressive open-angle glaucoma

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Purpose: Occurrence and size of parapapillary atrophy PPA (zone beta) has been associated with glaucomatous optic atrophy. Aim of the study was to assess the incidence of enlargement of PPA in stable and progressive open-angle glaucoma.

Methods: In a prospective glaucoma follow-up study 978 eyes with a refractive error above -8 D (141 normal eyes, 289 eyes with ocular hypertension (OHT), 354 eyes with open-angle glaucoma (OAG) and 194 eyes with normal-tension glaucoma (NTG)) were included. Optic disc photographs taken annually were evaluated. Follow-up was 1.5 - 10 years. Two examiners judged optic disc slides of follow-up examinations for enlargement of PPA and for morphologic signs of glaucoma progression in comparison with baseline examination.

Results: In 16 eyes an enlargement of PPA could be observed after 3.9 ± 2.6 (0.6 - 9.1) years of follow-up. This occurred in no normal eye, in 1 (.3%) OHT eye, in 12 (3.4%) OAG eyes, and in 3 (1.5%) NTG eyes. Enlargement of PPA developed in 5/102 (4.9%) progressive glaucoma eyes and in 10/446 (2.2%) stable glaucoma eyes ($P = 0.13$). If only eyes with a myopic refractive error exceeding -3 D were excluded, enlargement of PPA was observed in 5/81 (6.2%) progressive glaucoma eyes and in 3/354 (.8%) eyes with stable glaucoma ($P = 0.001$).

Conclusions: PPA (zone beta) may enlarge during the course of open-angle glaucoma and in moderate myopia. This happens significantly more frequent in progressive than in stable glaucoma in a range of refractive error above -3 D. Even in progressive glaucoma, however, this is a rare event. Therefore, enlargement of PPA is not a sensitive marker for glaucoma progression.

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Blue-yellow VEP under Cold-water Provocation in different Glaucomas

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Purpose: Vasospasm after a cold-water test is a risk factor in the pathogenesis of low-tension glaucoma. Investigations of the blue-sensitive pathway in glaucoma showed differences between normal subjects and patients in primary open-angle glaucoma. The aim of our study was to investigate changes of the blue-yellow VEP in normals and in different types of glaucoma due to cold-water provocation.

Methods: 24 patients with open-angle glaucoma (POAG), 25 with low-tension glaucoma (LTG) and 22 controls. VEP was measured before and after cold-water provocation. A two-channel Maxwellian view system was used to present in onset-offset mode blue stripe pattern superimposed on a yellow adaption field. VEP was measured before cold-water provocation as well as 2 minutes and 4 minutes after the beginning of cold-water provocation. The eye of the patient with the larger glaucomatous damage was included in the analysis. The amplitude and the peak time of the VEP onset were evaluated.

Results: The mean amplitudes of the patients with LTG decreased from $4.17 \mu V$ before cold-water test to $3.52 \mu V$ after 2 minutes and to $3.54 \mu V$ after 4 minutes cold-water test. This difference was statistically significant (t-test: $p = 0.01$). The other groups showed no decreases in amplitudes with cold-water provocation. Without cold-water provocation peak times in all glaucoma groups were significantly prolonged in comparison to controls. Cold-water provocation showed no influence on peak times in normals and patients.

Conclusions: Only the amplitude in patients with LTG showed a significant reduction due to the cold-water provocation in blue on yellow VEP. This reduction was present in 21 of 25 patients with LTG. The mechanism of this reaction is not clear.

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Changes in optic disc topography at different stages of preperimetric glaucoma disease.

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Purpose: To compare topographic parameters obtained with the Heidelberg Retina Tomograph (HRT II) depending on retinal nerve fiber layer (RNFL) exploration, with the Rodenstock Scanning Laser Ophthalmoscope (SLO).

Methods: 107 eyes from 107 subjects with normal conventional automated perimetry underwent full ophthalmologic examination, at least two 24-2 full threshold Humphrey perimetry, one optic disc topography with the HRT II and one evaluation of the RNFL with SLO. They were classified in three different groups: normal group (31 eyes with intraocular pressure, IOP, less than 21 mm Hg, normal RNFL evaluation and normal optic disc ophthalmoscopy) ocular hypertensive group (57 eyes with IOP higher than 22 mm Hg, normal RNFL evaluation and normal optic disc ophthalmoscopy) and preperimetric glaucoma group (19 eyes with IOP and localized RNFL defects or diffuse loss in SLO exploration). ANOVA test was used to compare global HRT II parameters among the groups of study ($p < 0.05$).

Results: Demographic characteristics showed no statistical differences between the groups. Several HRT parameters showed significant differences ($p < 0.05$) among groups: cup area, cup volume and mean RNFL thickness.

Conclusions: Optic nerve head parameters measured with HRT II can discriminate among different groups at early glaucoma stages. These results support the use of HRT II to detect the presence and quantification of glaucomatous damage.

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Comparative study between frequency-doubling technology and standard automated perimetry

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Purpose: To compare frequency doubling perimetry (FDP) results in glaucoma, ocular hypertensive eyes and control eyes classified upon conventional automated perimetry performance.

Methods: Four hundred and sixteen eyes of 416 subjects were included in the study. All subjects underwent full ophthalmic examination, 2 conventional automated perimetries (full threshold strategy, 24-2) and 1 FDP (C-20-5) with a Welch Allyn/humphrey Zeiss perimeter. The eyes were classified based on intraocular pressure, conventional automated perimetry and optic nerve head evaluation in: 92 ocular hypertensives without evidence of glaucomatous damage in the optic nerve head, 110 ocular hypertensive eyes with evidence of glaucomatous damage in the optic nerve head (preperimetric glaucoma), 122 glaucomatous eyes and 92 normal eyes. Statistical analysis was performed by means of ANOVA to compare performance on FDP in the groups of the study.

Results: Significant differences ($p < 0.001$) were found between the glaucoma group and others in MD, PSD, number of altered points in each quadrant and number of altered points at different probability levels in FDP.

Conclusions: Frequency-doubling perimetry effectively discriminates glaucomatous damage by means of several criteria.

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Clinical performance of NCT by Reichert AT550 in glaucomatous patients

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Purpose: The aim of this study was to assess the level of accuracy for measurements of intraocular pressure (IOP) obtained with a new non-contact tonometer (NCT) (AT550, Reichert), taking as reference Goldmann applanation tonometer (AT).

Methods: Forty-four eyes from 22 glaucoma patients undergoing medical treatment for different forms of glaucoma were recruited from an ophthalmological clinic in the north of Portugal. There were 11 male (64 ± 15.2 year) and 11 female (63 ± 16.2 year). Another 40 optometry students acting as control sample were evaluated. There were 13 male (24 ± 2.5 year) and 27 female (24 ± 2.5 year). NCT was performed first in order to avoid the effect of aqueous humor evacuation by anterior chamber compression with applanation tonometry. Two different examiners perform measurements with NCT and Goldmann AT, the last being masked for values obtained with NCT five minutes before.

Results: Agreement was assessed by plotting the differences against the means for NCT and Goldmann. IOP was significantly higher for glaucomatous patients when comparing with control sample using both instruments ($p < 0.05$). Within the glaucomatous population, mean values of IOP were 18.98 ± 2.77 and 19.08 ± 3.02 mm Hg using Goldmann and AT550, respectively. Plots of differences against means displayed good agreement (mean difference \pm limits of agreement, -0.09 ± 3.30); these value was not found to be statistically different from zero (t-test for dependent samples, $p = 0.709$).

Conclusions: Readings of IOP with the AT550 are clinically comparable to those obtained with Goldmann AT in glaucomatous patients. This feature validates this NCT not only for screening of IOP but to follow-up glaucomatous patients with a rapid, non-invasive method.

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Comparison of optic nerve head measurements made by HRT2 and OCT3 in ocular hypertensive patients

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Purpose: Evaluate the relationship between optic nerve head measurement made by the HRT2 and OCT3 in ocular hypertensive patients.

Methods: Prospective study in the glaucoma clinic. 30 eyes of 30 patients were imaged. A single operator performed all the imaging for the HRT2 first and then a different operator performed all the imaging for the OCT3. The Pearson correlation test was used for assessing the relation between measures obtained by the two modalities.

Results: 20 females and 10 males were imaged. Mean age 65 (range 45-77). Disk area had the poorest correlation between the 2 machines ($r = 0.46$). Significant correlation was seen between Cup Area ($p = 0.01$), Rim Area ($p = 0.05$) and Cup disc area ratio ($p = 0.01$) between the 2 machines, correlation coefficients being ($r = 0.792, r = 0.486, r = 0.652$) respectively.

Conclusions: Significant correlation is seen between the two machines with respect to their modality variable except for rim area. Both machines correlate well with respect to measuring Cup/Disc area ratio in ocular hypertensive patients.

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Discrimination of glaucomatous damage with scanning laser ophthalmoscope (SLO) and optic disc topography in ocular hypertensive subjects.

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Purpose: To determinate glaucomatous damage in ocular hypertensive subjects, depending on the presence of structural defects in the retinal nerve fiber layer (RNFL) and topographic parameters of the optic nerve head performed with Heidelberg Retina Tomograph (HRT II).

Methods: 70 eyes from 70 glaucoma suspects with intraocular pressure (IOP) higher than 21 mm Hg, normal conventional automated Humphrey perimetry and cup to disc ratio less than 0.6, without disc haemorrhage. All of them underwent RNFL exploration with blue argon laser (SLO) and optic disc topography with HRT II. Findings of localized RNFL defects or diffuse loss were considered abnormal, and each patient was classified as normal RNFL or pathological RNFL. Global topographic parameters and Moorfields regression classification were analyzed in both groups, results were compared with a Student t ($p < 0.05$).

Results: Fifty eyes presented normal RNFL and 20 had localized RNFL defects in SLO exploration. Demographic analysis did not show statistical differences between both groups. There were significant differences in some HRT II parameters: cup disc, cup/disc area ratio and cup volume.

Conclusions: SLO and HRT II can detect structural damage, even before than other functional examination techniques. RNFL should be evaluated in glaucoma early diagnosis.

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Electronic microscopy investigation on trabecular meshwork and Schlemm's canal of pseudoexfoliation syndrome and primary open angle glaucoma.

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Purpose: Electronic microscopy comparison between the structure of trabecular meshwork and Schlemm's canal and the size of the same canal in primary open angle glaucoma (POAG) eyes and pseudoexfoliation (PEX) syndrome.

Methods: The electronic microscopy investigations were provided on 4 trabecular meshworks and Schlemm's canals, taken from POAG eyes and 4 trabecular meshworks and Schlemm's canals from surgical pieces of trabeculectomy performed in patients diagnosed of (PEX) syndrome.

Results: We established that ultrastructural changes in (PEX) syndrome trabecular meshworks are more expressive than in POAG trabecular meshworks. We founded collagen degenerative processes loss of endothelial cells, accumulated material of pigment granules, tighter spaces of trabecular meshworks and morphologic injuries in the same places. We observed that the size of Schlemm's canals of (PEX) syndrome eyes is less than POAG eyes.

Conclusions: The electron microscopy changes observed in (PEX) syndrome trabecular meshworks and Schlemm's canals show morphological and structural alterations, which can help for clarification of (PEX) syndrome.

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Efficacy of brimonidine as an adjunctive therapy for patients with uncontrolled IOP in prostaglandin monotherapy

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Purpose: Introduction: Although a number of glaucoma medications are efficacious in the treatment of patients with ocular hypertension and glaucoma, a number of patients require intraocular pressure (IOP) lowering beyond that provided by monotherapy. Purpose: To determine the efficacy and safety of brimonidine 0.2% (ALPHAGAN) when used in combination with prostaglandins in patients with inadequately controlled IOP despite previous prostaglandin monotherapy.

Methods: Methods: This study is a retrospective analysis of one hundred patients with uncontrolled IOP and visual field instability after 6-12 months of prostaglandin monotherapy. Patients with IOP = 17 mm Hg and ≥ 24 mm Hg were included in the analysis. Patients received brimonidine 0.2% bid in addition to their existing regime of prostaglandin treatment for 4 months. Study visits were at 2 weeks, 2 and 4 months after initiation of adjunctive treatment.

Results: Results: Data from thirty-nine patients has been analysed to date. The mean baseline IOP following 6-12 months of treatment with prostaglandin was 20.6 mm Hg. Adjunctive brimonidine therapy with prostaglandin resulted in additional mean IOP reductions from baseline of 2.2 mm Hg (10.8%) after 2 weeks, 2.9 mm Hg (14.0%) after 2 months and 3.3 mm Hg (15.9%) after 4 months. Ocular allergy was reported in 4 patients (10.2%).

Conclusions: Conclusions: Brimonidine is an efficacious and safe adjunctive agent, providing superior IOP lowering in combination with prostaglandin compared with that provided by prostaglandin monotherapy. This additional reduction in IOP is likely to result from combining agents with different mechanisms of action.

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Frequency doubling technology versus conventional automated perimetry: a correlation study

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Purpose: To evaluate the correlations between visual field global indices of standard automated perimetry (SAP) and Frequency-Doubling Perimetry (FDP) in glaucomatous subjects.

Methods: A total of 101 control subjects and 102 glaucomatous subjects (IOP figures above 21 mmHg and glaucomatous appearance of the optic disc) were included in the study. SAP and FDP were performed in all the subjects by means of a Humphrey Visual Field Analyzer and an FDP perimeter (Zeiss-Humphrey Systems). The visual field indices of both perimetric tests were compared and correlations were elaborated.

Results: FDP mean deviation and corrected pattern standard deviation showed strong correlations to visual field indices of SAP: mean deviation ($r = -0.717$; $p < 0.001$) and corrected pattern standard deviation ($r = 0.665$; $p < 0.001$). Grading visual field defects in categories, both test showed a statistical significant parity to quantify visual field losses.

Conclusions: FDP visual field indices were significantly correlated to their respective visual field indices of SAP. This fact supports the usefulness of FDP to detect glaucomatous functional losses.

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Glaucoma damage criteria for frequency doubling technology

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Purpose: To determine the optimum criteria that best discriminate glaucomatous damage in frequency-doubling perimetry with the C-20-5 test.

Methods: The study included 211 eyes of 211 subjects classified by means of conventional automated perimetry, IOP and optic nerve head evaluation in 92 normal subjects and 119 glaucomas (68 early damage; 19 moderate damage; 32 advanced damage). Relative operating characteristic curves were performed for each probability level ($p < 5\%$, $p < 2\%$, $p < 1\%$ y $p < 0.5\%$) and points with best sensitivity-specificity balance were determined.

Results: Best criteria to define glaucoma by FDP C-20-5 are: 4 or more points with $p < 5\%$, 3 or more points with $p < 2\%$ and/or 1 or more points with $p < 1\%$, at any location.

Conclusions: The use of the proposed optimum criteria allows glaucoma diagnosis with an adequate balance of sensitivity and specificity.

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Intraocular pressure lowering effect of the combination of Latanoprost and Timolol as single therapy in open angle glaucoma

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Purpose: To evaluate the efficacy and safety of a fixed combination of 0.005% latanoprost and 0.5% timolol maleate administered once daily in open angle glaucoma patients.

Methods: 105 subjects with open-angle glaucoma were included in this study. The eligibility criteria to be included in the study were the following clinical status: 1) latanoprost once daily and an insufficient IOP level (previous-latanoprost group); 2) timolol twice daily and an insufficient IOP level (previous-timolol group), 3) unfixed combination of latanoprost and timolol and optimal IOP level (previous unfixed combination). After baseline, all the patients received the fixed combination of latanoprost and timolol once daily. Intraocular pressure and ocular side effects were recorded at baseline, and after 1 and 3 months of treatment.

Results: Compared to baseline measurements, the fixed combination latanoprost and timolol caused a significant ($p < 0.001$) IOP reduction in the previous-latanoprost group and also in the previous-timolol group. Mean diurnal IOP reduction was 4.71 mm Hg (19.5%) and 5.16 mm Hg (24.2%), respectively. The patients that changed from the non-fixed combination to the fixed combination of latanoprost plus timolol showed no significant differences in mean diurnal IOP after 3 months of follow-up. On an individual-based approach, 21% (6/28) of the patients showed a mild increase in mean diurnal IOP, but only 3 patients 10.7% raised their IOP figures above the target IOP.

Conclusions: The fixed combination of latanoprost and timolol once daily is an effective option to achieve an additional IOP lowering effect in latanoprost or timolol treatments in patients without adequate IOP control.

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Glaucoma's impact on quality of life

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Purpose: To evaluate the impact of the diagnosis of glaucoma itself on patients, to record their feelings and to verify their relationship with the medical equipe and the information they have on the disease and its therapy.

Methods: 130 glaucoma patients, at various stages of the disease, received a self-administered questionnaire of 25 questions, filled in the waiting room. Patients have been recruited in 3 different centers: one "Glaucoma Service" of a University Eye Clinic and two "General Ophthalmic Services" of a General Hospital.

Results: The analysis is still ongoing. The aim is to identify the impact of the glaucomatous disease on various subjects and to verify any difference between patients followed in a Glaucoma Service (a steady relationship with the same group of Glaucoma Specialists) versus patients from a General Ophthalmic Service (relationship with different doctors in different visits).

Conclusions: Quality of life is considered more and more important in the evaluation and treatment of glaucoma. Authors would like to present their data.

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Noncontact quantitative assessment of the anterior chamber angle (ACA) using the Orbscan system (OS)

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Purpose: To evaluate the validity of the OS for measuring the ACA width.

Methods: Eighty-four eyes with different ACA configurations underwent conventional gonioscopy (CG) -Spaeth system- and OS examination. The ability of the OS was tested with two extrapolation methods of the iris surface: the two-dimensional polynomial (TDP), and the plane.

Results: The TPD method provided a better correlate with CG than the plane method. However, the OS was unable to measure 2 of the 5 occludable angles by CG.

Conclusions: The polynomial approach of the Orbscan system may be used as a noncontact measurement tool of the anterior chamber angle, but has some limitations with occludable angles.

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One day glaucoma screening in a mid-sized German city

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Purpose: To investigate the efficacy of the "Glaukombus", a mobile glaucoma screening unit performing frequency doubling perimetry (FDP) and measure of the intraocular pressure (IOP).

Methods: The screening included a questionnaire to evaluate ocular and systemic diseases, a non-contact IOP measurement, and a single frequency doubling perimetry session (FDP; C-20-5 screening algorithm). The bus was situated in a pedestrian area of Aachen city on a working day for 8 hours.

Results:

Conclusions: A considerable amount of glaucoma suspects may be alerted by a screening program, as incorporated in the "Glaukombus". IOP measurements alone seem to be less effective.

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Optical outflow regulation - a new perspective of refraction surgery

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Purpose: Having conducted our research, we can now confirm the conditionless dominance of accommodation over outflow regulation, which is the key principle for understanding the mechanism of outflow. The second most important reason of ophthalmotonus disorders seems to be the shift in refraction due to the age related changes to cornea and lens.

Methods: And since MC is a servant of three masters, these are: accommodation, AH production and outflow regulation systems, it is tempting to try to use this natural physiological contradiction by changing a refractive power of lens or cornea by means of different types of refraction surgeries, causing the MC tonus to change just like it is required in order to normalize the IOP.

Results: All optical methods of outflow regulation will use the newly discovered mechanism of stationary retina positioning which is activated by an artificial myopisation or hypermetropisation of an eye. This mechanism changes the choroids blood saturation, which actually alters the tonus of MC and AH production rate. So the IOP can also be changed in the long term, which was detected by Russian ophthalmologists M.S. Remisov and L.V. Toropova during their experiment with glass atropine. It is also possible to improve the outflow facility by 10-15% while using green glasses, and decrease it by 30-40% while using red glasses.

Conclusions: For practical use it is necessary to develop as soon as possible the technique of appropriate refraction change or creation of an artificial astigmatism for different types of venous sinus position in the eye. For example, by changing the natural convergence and divergence of an eye, using light filters or different illumination, etc

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Optic disc topography in ocular hypertensives depending on glaucoma risk factors.

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Purpose: To compare global topographic parameters obtained with the Heidelberg Retina Tomograph (HRT II) in glaucoma suspects divided in three different risk levels.

Methods: Ninety eyes from 90 patients with intraocular pressure (IOP) higher than 21 mm Hg, normal conventional automated perimetry, normal retinal nerve fiber layer (RNFL) evaluation with Scanning Laser Ophthalmoscope (SLO) and no optic disc hemorrhage underwent full ophthalmologic examination: at least two 24-2 full threshold Humphrey perimetry, optic disc topography with HRT II, evaluation of the RNFL with a SLO and optic disc stereophotographs. Three risk groups were created: low risk (IOP between 22 and 24 mm Hg), moderated risk (IOP between 25 and 27 mm Hg, or cup to disc ratio between 0.6 and 0.8, or cup to disc asymmetry between both eyes between 0.3 and 0.4) and high risk (IOP >28 mm Hg, or cup to disc ratio >0.8, or IOP >24 mm Hg and cup to disc asymmetry >0.4). ANOVA test was used to compare the global HRT II parameters among the groups of study (p<0.05).

Results: Fifty three eyes presented low risk, 28 moderated risk and 9 high risk.

Demographic characteristics showed no statistical differences among groups. There were significant differences (p<0.05) in cup area, cup/disc area ratio, cup volume and discriminant function FSM.

Conclusions: Topographic optic nerve head analysis performed with HRT II can discriminate among different glaucoma suspects levels. There are structural differences between ocular hypertensives at early stages of the disease that should be evaluated.

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Plateau iris related to multiple ciliary cysts assessed by high frequency ultrasonography. Case report.

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Purpose: Clinical diagnosis of plateau iris is sometimes difficult. The high frequency ultrasonography of anterior segment is useful for the examination of angle and basal iris and allows sometimes to detect an unusual aetiology.

Methods: We report the case of a 43 years old male patient who presented two acute closed angle glaucoma in 1999, resolving with pilocarpine and having a clinical appearance of bilateral plateau iris. We examined him with I3 (Innovating Imaging Inc) ultrasonograph using a 20 Mhz probe for the anterior segment immersion examination and with the Paradigm UBM (50Mhz probe).

Results: Ultrasonography examination confirmed the anterior position of basal iris secondary to multiple anterior ciliary cysts, all around the pars plicata of each eye. The cysts had a thin regular and high reflective wall and no internal reflectivity. They were responsible for angle closure. Clinically a careful biomicroscopic examination revealed a mild iris protrusion only on horizontal meridians. This patient was treated by pilocarpine and timolol with a good result since four years. Discussion: By using ultrasound biomicroscopy, ciliary cysts are very frequent (15% of examinations) most frequently isolated, sometimes associated to a tumour. They rarely cause clinical complication. Some authors perform laser cystotomy or iridocystotomy and have good results.

Conclusions: High frequency ultrasonography for anterior segment allowed us a precise diagnosis and could guide the treatment for this patient. This examination seems to be useful for every young patients presenting acute or sub-acute angle closure glaucoma.

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Qualitative comparison of the glaucomatous optic nerve head atrophy using conventional digital 2D photography and stereophotography

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Purpose: Real colour documentation of the optic nerve head (ONH) is one of the most important methods to identify the chronic course of glaucomatous optic nerve damage. This study compared qualitatively the evaluation of ONH photographs using a time-multiplexing system in 3D to the conventional 2D photography, visualised on a computer monitor.

Methods: Twelve consecutive stereoscopic images from the Erlangen Glaucoma Register were scanned by a SprintScan35 Plus Film scanner (Polaroid, MA) in jpeg format (2D) and then converted by a special software (3D-PIX, Nuvision) in jps (3D). The same ONH images were shown in 2D and in 3D on the computer monitor to 22 subjects with different training grades. A standardised questionnaire evaluated semi-quantitative parameters like ONH stage, neuroretinal rim (NRR) configuration, visibility of retinal nerve fibres (RNF) and depth of cup.

Results: A significant better evaluation of the glaucomatous optic nerve head (ONH) atrophy using the stereoscopic method was achieved for the following parameters: NRR ($p=0.012$), depth of cup ($p=0.000$) and visibility of RNF ($p=0.001$). For the contrary 2D digitalized photography could identify significantly other glaucomatous features of the ONH: Parapapillary atrophy zone beta ($p=0.002$) and the stage of the optic atrophy ($p=0.010$).

Conclusions: The study shows that the glaucomatous ONH assessment of the 3D parameters depth of cup, visibility of RNF and NRR configuration is more precise than with 2D conventional photography. The evaluation of parapapillary atrophy zone beta and the stage of optic atrophy was better achieved, using the 2D method.

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Sensitivity and specificity of frequency doubling technology depending on optic nerve head topographic damage

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Purpose: To assess the diagnostic value of frequency-doubling perimetry (FDP) in a population of normal and glaucomatous subjects defined by structural parameters of Heidelberg Retina Tomograph (HRT II).

Methods: A total of 206 eyes of 206 patients were included in the study. All the subjects underwent a full ophthalmic examination, a topographic evaluation of the optic nerve head by means of HRT II and a FDP (C-20-5 test, Welch Allyn / Humphrey Zeiss perimeter). The criteria to define glaucoma were based on FSM and RB discriminant functions and Moorfields analysis. A statistical analysis was performed to evaluate the COR curves in the different subsets of patients defined by the HRT criteria. The areas under curve and best balance between sensitivity and specificity were elaborated.

Results: The best result of the analysis of the areas under curve was observed in the subset of patients based on RB. The worst results were observed in the subset defined by FSM criteria. Sensitivity and specificity values ranged from 70 to 80%.

Conclusions: Frequency-doubling perimetry showed a good diagnostic value to detect glaucomatous damage defined by structural parameters.

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Reproducibility of visual field parameters in terminal glaucoma (FDT versus 24-2 SITA)

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Purpose: To compare the degree of reproducibility of conventional automated perimetry (24-2 SITA) and Frequency Doubling Technology (FDT C-20 threshold) in patients with very advanced glaucoma.

Methods: 30 eyes with end-stage glaucoma (more than 75% and less than 100% of explored points had 0 dB of threshold sensitivity in 24-2 SITA) performed a FDT test (C-20). Both examinations were repeated one month later. We studied MD, DEP, foveal value, test duration, preserved points value sum, preserved points percentage in the best preserved quadrant and total percentage of preserved points. The Altman and Bland scatter was used to study the reproducibility.

Results: The Repeatability Coefficients for FDT and Humphrey respectively were as follow: MD: 1.98 and 1.48, PSD: 2.09 and 2.12, foveal value: 5.96 and 21.7, total percentage of preserved points: 19.44 and 10.33. Both MD and SMD parameters had a good reproducibility in Humphrey or FDT. Humphrey is less variable for the total percentage of preserved points.

Conclusions: With conventional perimetry, the evaluation of visual field progression advanced glaucoma is a complex task. We have previously reported that FDT showed greater performance than 24-2 SITA test for end-stage visual field defects evaluation, with the advantage of being less time-consuming. In end-stage glaucoma, both Humphrey and FDT perimeters are comparable in terms of reproducibility. Further studies are necessary to investigate the "ideal" test combining a low variability with a high reproducibility, to detect changes due just to disease progression, and not related to test variability.

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Perimetric learning with SWAP and WWP

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Purpose: To study perimetric learning with Short Wavelength Automated Perimetry (SWAP) and conventional White-White Perimetry (WWP) in perimetrically inexperienced normal subjects.

Methods: Ten eyes of 10 healthy subjects were tested 5 times each at 5 separate visits with Humphrey 30-2 Full Threshold SWAP and WWP. Mean Sensitivity was calculated for each field and also for each field divided into 3 eccentricity zones.

Results: The magnitude of training was larger for SWAP than for WWP ($p<0.0001$). For SWAP, the average Mean Sensitivity increased by 3.0 dB with from visit 1 to 5, and by 3.4 dB from visit 1 to 3. For WWP the corresponding figures were 1.0 dB and 1.2 dB respectively. The learning effect was slightly but non-significantly larger in the peripheral zones compared to the central ones. All subjects appeared fully trained after 3 test sessions with both SWAP and WWP.

Conclusions: Both SWAP and WWP showed significant learning effects. The magnitude of training was larger with SWAP than with WWP, but the same pattern was seen with both programs. The results indicate that SWAP and WWP required the same number of tests for subjects to be considered experienced.

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Short-wavelength automated perimetry and frequency-doubling technology in early glaucoma diagnosis

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Purpose: To assess the ability of frequency-doubling perimetry (FDP) and short-wavelength automated perimetry (SWAP) to detect early glaucomatous damage in ocular hypertensive subjects at high risk to develop glaucoma.

Methods: 223 eyes of 223 subjects suspected of having glaucoma with a normal conventional automated perimetry were included in the study. All subjects underwent a full ophthalmic examination, at least 2 conventional automated perimetries, 1 FDP, 1 SWAP and an optic disc topography with an HRT II (Heidelberg Retina Tomograph). The 223 eyes were divided into different risk levels depending on intraocular pressure, optic disc evaluation (stereophotographs) and/or HRT criteria. FDP and SWAP data were compared between these groups.

Results: Both perimetries (FDP and SWAP) showed focal visual field losses in 20-30% of the patients. In groups at higher risk, the percentage of abnormal results in these perimetries was higher.

Conclusions: FDP and SWAP can detect glaucomatous damage at early stages of the disease, before the onset of visual field losses in conventional automated perimetry.

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SLO C/D index compared with C/D photographic index

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Purpose: In our experience vertical and horizontal SLO C/D indexes are higher than biomicroscopically determined values. We tried to confirm and explain such differences.

Methods: We compared TopSS 2010 SLO disc morphological images, with the correspondent clinical photographic images, in order to determine the outer papillary limits. Using anatomical landmarks (vessels) we modify and remeasure C/D indexes with SLO.

Results: The SLO neuroretinal rim is much thinner than the clinical one if we use color coded and wire frame images. The C/D values are higher than those photographically determined. There is a good agreement between the changes in vertical and horizontal TopSS disc profiles and the location of photographic papillary outer limits.

Conclusions: Color coded and wire frame images in TopSS 2010 underestimated the actual papillary perimeter. An accurate determination of the papillary limits is obtained when using photographic images-related topographic profiles.

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The use of ginkgo-biloba and pufa in ocular hypertension. A frequency doubling perimetry study

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Purpose: To evaluate the efficacy of systemic treatment with ginkgo-biloba and omega-3 polyunsaturated fatty acid (PUFA) in improving the frequency doubling perimetry (FDP) indices in patients with ocular hypertension.

Methods: Forty patients with mean IOP 23.7 ± 1.8 mm/Hg who showed pathological values of mean defect (MD) and corrected pattern standard deviation (CPSD) with FDP were randomly divided in two groups. One group (group A) were treated with ginkgo-biloba (120 mg/day) and PUFA (240 mg DHA+340 mg EPA/day) the other group (group B) received a placebo. Before and after three months of treatment we made a frequency doubling perimetry.

Results: After three months of therapy in group A the MD decreased from -2.839 ± 1.470 dB to -1.715 ± 1.366 dB ($p < 0.005$) and the CPSD from 3.971 ± 0.441 dB to 3.218 ± 0.852 dB ($p < 0.005$). In group B there were no statistically significant variation in the MD end CPSD indexes.

Conclusions: Ginkgo-biloba and polyunsaturated fatty acids must be considered an efficacious supportive therapy in the prevention of glaucomatous optic neuropathy.

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Ultrasound biomicroscopy of a filtering bleb and intrascleral drainage pathway one and three months after trabeculectomy in primary open angle glaucoma

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Purpose: The aim of the present study is to investigate the extent, shape and ultrasound structure of a filtering bleb and intrascleral drainage pathway one and three months after trabeculectomy in primary open angle glaucoma (POAG) by using ultrasound biomicroscopy (UBM).

Methods: The filtering blebs and intrascleral drainage pathways of 23 eyes (17 patients) with POAG were investigated after trabeculectomy. One surgeon has done all surgical procedures - standard trabeculectomy with triangular scleral flap, without using Mitomycin C. One suture has been positioned on top of the scleral flap. The ultrabiomicroscopic investigations have been done by another ophthalmologist on the first and third month after trabeculectomy. The scanning of the blebs and aqueous drainage pathways have been produced over the radial meridians by UBM. IOP has been measured before every scan.

Results: By using the caliper system of the device we measured the variations in the size of the filtering blebs and the drainage routes under the scleral flap. We detected differences in the mean height of filtering blebs, thickness of drainage routes and IOP on first and third month after trabeculectomy.

Conclusions: UBM offers extra and more accurate data concerning internal structure of filtering blebs, which is impossible to gain by using slit lamp solely. The information about the internal structures and extent of the filtering blebs and drainage routes suggests how are functioning the blebs and determines our therapeutic decisions. The availability of well passable drainage route under the scleral flap is substantial for the good functioning of the filtering bleb and maintaining adequate IOP.

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Results of phacoemulsification and deep sclerectomy converted into phacotrabeculectomy

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Purpose: To study the success rate and complications of phacoemulsification and deep sclerectomy (P-DS) converted into phaco-trabeculectomy (P-T) after unintended perforation of the trabeculodescemet membrane (TDM).

Methods: Between January 2001 and January 2003, we performed 78 P-DS. Seven cases had to be transformed into P-T, because of a perforation of the TDM during the deep sclerocorneal dissection.

Results: The mean age was 79.3 years. Mean time of follow-up was 16.9 months (range: 7 to 28). The mean preoperative IOP was 21.7 mmHg (SD: 4.92) and it decreased to 3.6 (SD:3.5); 6.8 (SD:5.6); 13.4 (SD: 8.1); 12.8 (SD:3.5) and 12.5 (SD:3.6) mmHg at 24 hours, 1 week, 1, 6 and 12 months postoperatively. 100%, 85.7% and 57.4% of the eyes had a final IOP without treatment less or equal than 21, 18 and 15 mmHg respectively. The mean number of glaucoma medications preoperatively was 1.4 (range: 1 to 3). At the last visit no patient was under treatment. The mean pre and postoperative VA acuity were 0.28 (SD: 0.14) and 0.6 (SD: 0.25) respectively. VA was better than 0.5 in five eyes (71.42%). Hyphema occurred in 4 eyes (57.4%), choroidal detachment in 2 (28.57%) and fibrous uveitis in 1 eye.

Conclusions: The major intraoperative complication of DS is the perforation of the TDM during the deep sclerectomy dissection. It occurred mainly during the learning curve phase. This study shows that, when a P-DS is complicated with a perforation of the TDM, a good mid-term control of the IOP is obtained without antiglaucomatous treatment. However, immediate postoperative complications such as hypotony and hyphema are frequent. These results should encourage surgeons to learn this new filtration surgery.

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Scanning laser polarimetry in glaucoma: customised versus fixed-angle corneal polarisation compensation.

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Purpose: To investigate differences between scanning laser polarimetric (SLP) measurements of the retinal nerve fibre layer with customised (SLP-C), and fixed corneal polarisation compensation (SLP-F).

Methods: Both SLP-C and SLP-F were performed on 37 consecutive glaucoma patients and 14 healthy control participants with the GDx-Access device. Global perimetry indices were used for correlation with global SLP parameters. SLP parameters in the superior and inferior quadrants were correlated with mean sensitivities (MS) calculated for the opposite hemifield.

Results: Both SLP-C and SLP-F parameters were able to discriminate between the glaucoma and the control groups except for the ellipse modulation, which differed significantly between the two groups with SLP-C ($p=0.017$) but not with SLP-F ($p=0.056$). With SLP-C, inferior maximum thickness and ellipse standard deviation were lower in both groups ($p<0.05$ for each parameter) whereas superior maximum thickness was lower in the glaucoma ($p=0.006$) and tended to be lower in the control group ($p=0.053$). The Number was higher with SLP-C ($p<0.05$ in both groups). The other parameters did not differ significantly when comparing the methods, in either group. Only with SLP-C a significant correlation appeared between superior maximum thickness and inferior hemifield MS ($r=0.653$, $p<0.001$), and between inferior maximum thickness and superior hemifield MS ($r=0.420$, $p=0.023$). The other global and sectorial SLP parameters correlated significantly with corresponding visual field indices with both modes.

Conclusions: SLP parameters developed to enhance diagnostic sensitivity distinguished between healthy and glaucoma eyes with either mode. However, SLP-C provides more realistic results.

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Neuroprotective effects of topical brimonidine against chronic ocular hypertension induced degeneration of retinotectal afferents

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Purpose: To study chronic ocular hypertensive-induced alterations of retino-tectal innervation and effects of topically applied Alphagan-P*, Timoptic* or saline.

Methods: In adult rats, ocular hypertension (OHT) was induced in the right eye by laser photocoagulation following already described techniques (WoldeMussie et al., 2001; IOVS 42:2849). Right eyes were treated twice daily with 5 µl of Alphagan-P, Timoptic or 0.9% NaCl. Retino-tectal innervation was studied by comparing at 3 weeks retinal afferents to the left superior colliculus (SC) anterogradely labeled with Cholera Toxin B subunit (CTB) applied to the OHT eyes. For each animal, serial coronal sections through the entire right SC were analyzed for CTB-labelled retinal terminals using image analysis software to estimate the volume of the retino-tectal projection.

Results: The mean (\pm SD) volume of the retino-tectal projection was 4.10 ± 0.39 (n=8), 3.56 ± 0.23 (n=7), 3.56 ± 0.51 (n=8), or 3.87 ± 0.34 (n=7), for the unlesioned, vehicle-, Alphagan-P-, or Timolol-treated groups of rats, respectively.

Conclusions: OHT within three weeks results in loss of approximately 13% of the retino-tectal terminals, Timoptic treatment did not prevent retino-tectal degeneration, and Alphagan-P-treatment resulted in loss of 6%, providing a statistically significant protection against OHT-induced degeneration of the retinotectal projection. These results are consistent with previous studies (WoldeMussie et al., 2001; IOVS 42:2849) showing a similar degree of protection against OHT-induced retinal ganglion cell death. Research support: F.Séneca PI82/00540/FS/01, BFI2002-03742 and Allergan Inc.

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Is post-trabeculectomy hypotony a risk factor for subsequent failure?

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Purpose: Hypotony results in an increased break down of the blood-aqueous barrier causing an increase in inflammatory mediator release. We postulate that this release may lead to an increased risk of trabeculectomy failure through increased bleb scarring. This study was designed to try to address the question if hypotony within one month of trabeculectomy for POAG, is a risk factor for future failure of the filter.

Methods: We performed a retrospective, case notes review, of patients who underwent trabeculectomy for POAG between Jan 1995 and Jan 1996 at our hospital. Augmented surgery and combined phacotrabeculectomy procedures were excluded. We identified those with postoperative hypotony within 1 month of surgery. Hypotony was defined as an IOP < 8mmHg or an IOP of less than 10mmHg with choroidal detachment or a flat anterior chamber. We compared the survival times of the surgery in this group with a control group (did not suffer hypotony as described above), over a 5 year period.

Results: 97 cases matched our inclusion criteria, of these 38 (39.2%) experienced hypotony within 1 month of surgery. We compared the survival times in those patients who developed hypotony with those who did not using the log-rank test. This data provided evidence of a difference ($P=0.0492$) with patients in the hypotony group failing more rapidly than the control group.

Conclusions: Early post-trabeculectomy hypotony (within 1 month) is associated with reduced survival time of blebs.

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The prognosis of traumatic uveitis severity

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Purpose: To make a search for a prognosis of the severity of traumatic uveitis in the early periods after injury.

Methods: Thirty patients with penetrating eye wound within the first three post-traumatic days were entered in the study. The level of nitrogen oxide (NO₂/NO₃) end metabolites in the lacrimal fluid was determined using the method proposed by N.Yemchenko and co-workers (1994).

Results: With lacrimal NO₂/NO₃ amount of more than 1,0 mmol/l, the prognosis of the disease course is considered to be favourable, while with the amount less or equal to 1,0 mmol/l, the prognosis is poor. It is accompanied by chronic flabby uveitis resulting in subatrophy. Normal lacrimal values of the index studied were 2,39±0,2 mmol/l.

Conclusions: The use of the present prognostic method for traumatic uveitis allows to make a prognosis of the disease severity in the early periods after injury and possible complications of uveitis. It is also helpful in instituting proper pathogenetic therapy. The method suggested appears to be highly informative and reproducible. It is a noninvasive method with wide range of applicability in health institutions having a biochemical laboratory.

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Constant and variable findings in Fuchs Heterochromic uveitis: study of 100 cases.

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Purpose: To analyze the clinical features of Fuchs uveitis and the extend of heterogeneity of this disease.

Methods: Constant and variable findings were studied, based on patients diagnosed with presumed Fuchs' cyclitis between November 1994 and March 2003. Patients were analyzed for clinical presentations described as typical for Fuchs' cyclitis. Their frequency was assessed and classified as constant and variable findings.

Results: One hundred patients (54 women and 46 men) with 110 affected eyes, were included. The average age of the first presentation of ocular symptoms was 30.2±10.5 years, while the mean age of diagnosis of Fuchs' cyclitis was 37.3±10.3 years. Initial symptoms were: decrease of vision in 46, floaters in 23 and ocular pain in 9 eyes. The diagnosis of Fuchs' cyclitis was made on a routine ophthalmic examination in 22 patients. The frequency of variable findings was as followed: diffuse KP distribution in 101 eyes, vitritis in 99 eyes, unilateral manifestation in 90 cases, iris heterochromia in 83 eyes and lens opacities in 81 eyes. One patient with unilateral disease had an unaffected twin. Cataract surgery was performed in 21 patients and Amsler's sign was observed in 9 cases. The average number of variable findings was 4.22±0.83.

Conclusions: Our study's criteria such as constant and variable findings could be used for a diagnosis definition of Fuchs' cyclitis.

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Anti-tuberculosis treatment in corticoiddependent sarcoidosis uveitis

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Purpose: Sarcoidosis uveitis' treatment is still difficult because of a high level corticoiddependence. Immunosuppressive treatments is then necessary, but side effects are frequents. We report two cases of patients with granulomatosis uveitis with a high corticoiddependence, improved by an anti-tuberculosis treatment.

Methods: Two patients with clinical features of bilateral sarcoidosis uveitis lasting for several years. The diagnosis of sarcoidosis was confirmed by a skin biopsy from sarcoid lesions. In the two cases an immunosuppressive treatment was indicate because of a high level corticoiddependence, but the two patients had tuberculosis antecedents without recent evolution. A prophylactic anti-tuberculosis treatment was established.

Results: Ocular features improved with the anti-tuberculosis treatment and the corticoid treatment could be stopped.

Conclusions: These cases suggest that patients with sarcoidosis may have an allergic condition to an intracellular bacteria such as mycobacteria and anti-tuberculosis treatment must be instauraed before any immunosuppressive alternative.

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Human retinal S antigen cloned & expressed in eukaryotic and prokaryotic systems: comparison of immunoreactivities by ELISA

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Purpose: Retinal S antigen (RSAg) is probably the best studied candidate antigen for autoimmune uveitis. Anti-RSAg immunoreactivity has been shown in human uveitis patients and animal models of the disease. As most experiments have of necessity used non-human forms of RSAg, the findings can not necessarily be extrapolated to humans. Here we sought to circumvent the problem of limited supplies of human RSAg by cloning and expressing recombinant protein. We also tested the recombinant antigen's performance against bovine RSAg in ELISA studies using human uveitis and control sera.

Methods: Human RSAg inserts were produced from genomic DNA by PCR, and ligated into cut pcDNA4-TO-myc-HisA or pCR-T7-NT-Topo plasmids. The recombinant pcDNA4-TO-myc-HisA vector was used to transform a human kidney cell line (TREx 293) while the pCR-T7-NT-Topo vector was transfected into BL21 (DE3)pLysS bacterial hosts. RSAg containing a polyhistidine tag was expressed from both systems by induction with tetracycline (eukaryotic vector) or IPTG (prokaryotic). Protein purification was carried out and purified RSAg detected by a combination of PAGE and Western blotting. Indirect ELISA was carried out on panels of human RSAg (expressed in bacteria)/bovine RSAg/ His-tagged protein/negative control against sera from uveitis patients and healthy controls.

Results: RSAg was successfully expressed using both prokaryotic and eukaryotic expression systems. Higher yields were found with the former. ELISA testing failed to detect a difference in baseline divided readings between groups of 39 uveitis patients and 41 controls, using human (prokaryotic expression) or bovine RSAg. Bovine and human RSAg readings correlated ($r=0.37$, $p=0.0003$) but readings from human RSAg wells were found to be significantly higher ($p=0.048$).

Conclusions: This study shows that it is possible to produce high quality recombinant human RSAg, in either prokaryotic or eukaryotic systems. Higher levels of immunoreactivity were detected using human rather than bovine RSAg in ELISA. Although yields of eukaryotic human RSAg were low in this study, this could easily be overcome by optimising expression times/conditions.

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Peroxisome proliferator-activated receptor gamma agonist 15-deoxy-delta 12,14 prostaglandin J2 suppresses murine model of experimental autoimmune uveitis

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Purpose: Peroxisome proliferator-activated receptor (PPAR) gamma agonist 15-deoxy-delta 12,14 prostaglandin J2 (15PGJ2) suppresses immune responses in previous studies. Here, we investigate effects of 15PGJ2 in murine model of experimental autoimmune uveitis (EAU).

Methods: EAU was induced in B10RIII mice using IRBP 160-181 emulsified in Freund's complete adjuvant (CFA). Control mice received PBS and CFA. At day 15, draining lymph nodes and spleens were harvested for single-cell suspensions. Cells were cultured with 15PGJ2. Con-A and IRBP were used as stimulating agents. Proliferation assays were performed after 72 hours incubation ([³H] thymidine uptake assay). IL-10, IL-12 and IFN gamma were measured in supernatants by ELISA. Expression of CD69 and CD25 was assessed by flow cytometry.

Results: 15PGJ2: (a) inhibited proliferation of Con-A activated splenocytes and lymph node cells obtained from normal mice in a dose dependant manner. (b) suppressed proliferation of IRBP activated cells from immunized mice (c) reduced IFN gamma and IL-12 secretion and increased IL-10 secretion, and (d) 15PGJ2 down-regulated CD25 and CD69 expression.

Conclusions: Our results confirm anti-inflammatory properties of 15PGJ2. In addition, we show that 15PGJ2 down-regulates immune cell activation in EAU in vitro. Our data, together with other immunosuppressive properties of 15PGJ2 on RPE cells, suggest that 15PGJ2 may be a useful candidate for control of intraocular inflammation.

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TCRBV repertoire analysis in infectious and non infectious granulomatous uveitis.

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Purpose: To investigate the immune response implicated in infectious and non infectious granulomatous uveitis by analyzing phenotype and TCRBV repertoire diversity of the accumulating T cells in aqueous humor.

Methods: Eight patients presented with granulomatous uveitis were included. Three had an infectious origin (herpetic uveitis, ocular toxoplasmosis and borreliosis), whereas five were non infectious uveitis. Aqueous humor was obtained by paracentesis under topical anesthesia at the operating room. Intraocular T cells were studied by three-color flow cytometry using anti-CD4, anti-CD8 and anti-CD28 mouse monoclonal antibodies. Analysis of TCRBV repertoire was performed using specific semi-quantitative RT-PCR assays for each TCRBV family. In each case, ocular results were compared to those obtained in the peripheral blood collected on the same day.

Results: In all patients with non infectious uveitis, most cells recruited in the aqueous humor were lymphocytes expressing the CD4+ marker. Conversely, a higher rate of CD8+ was found in infectious uveitis. Most of the TCRBV families were expressed both in the peripheral blood and in the aqueous humor sample. Similar patterns, suggestive of a polyclonal repertoire, were also obtained when purified CD4+ or CD8+ T cells were analyzed.

Conclusions: Intraocular lymphocytes are predominantly CD4+ or CD8+ T cells in non infectious versus infectious granulomatous uveitis, respectively. Analysis of the TCRBV repertoire in patients presented with granulomatous uveitis showed a diversified response. So, the detected pattern do not suggest an intraocular antigen-specific recruitment, unless this clinical presentation is considered as an advanced stage of the immune process.

■ 382

Study of anti-smooth muscle and antimitochondrial antibodies in case of Uveitis

SEHAM S (1, 2)

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Purpose: To study antismooth muscle and antimitochondrial antibodies as tests of autoimmune diseases.

Methods: 34 cases of uveitis classified in three groups according to site of inflammation in the uveal tract and 10 normal control subjects were chosen from outpatients clinic of Research Institute of Ophthalmology. Clinical examination (slit lamp and biomicroscopy). Blood samples (full bloodcount, erythrocyte sedimentation rate, Rhomatoid factor, antinuclear antibodies, fasting blood sugar, antismooth muscles and antimitochondrial antibodies) were done.

Results: Antismooth muscle antibodies was positive in 40% of cases in panuveitis.

Conclusions: Autoimmune uveitis may occur as a part of systemic autoimmune diseases.

■ 384

Endothelin-1 and endothelin-converting enzyme-1 in chalazia

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Purpose: Foreign body giant cells, lymphocytes, plasma cells, and polymorphonuclear leukocytes are present within the lipogranuloma of chalazion. The aim of the present study was to investigate possible local endothelin-1 (ET-1) production by inflammatory cells and ET-converting enzyme-1 (ECE-1) expression in chalazion lesions.

Methods: Forty chalazia removed surgically from suffering patients were studied. Chalazia were fixed in Bouin's or formalin solution and embedded in paraffin. Chalazia sections were stained with hematoxylin and eosin for histological examination and incubated with specific antiserum for ET-1 and ECE-1 for the immunohistochemical technique. The EnVision peroxidase detection system was used and the immunoreactivity was visualized with 3-amino-9-ethylcarbazole (AEC) as a substrate. Negative controls were carried out omitting primary antibody.

Results: Histological analysis was carried out on chalazia at different stages of development, particularly chalazia with inflammatory cells or granulomatous structures. The epithelium of central duct and the alveoli did not immunostain for ET-1. Instead, ET-1 positive cells, specially neutrophils and macrophages were located in the stroma, around the alveoli, and within the lipogranuloma. The ECE-1 is present strongly in epithelial cells of ducts and scarcely in the secreting portion of the glands, suggesting a local ET-1 production by inflammatory cells.

Conclusions: Thus, the ET-1 and the putative presence of ET- receptors on neutrophils or macrophages may provide a local source of ET-1 which might act in an autocrine/paracrine role to support granulomatous structures.

■ 385

Demodex folliculorum and chronic blepharitis

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Purpose: Demodex folliculorum is not usually considered pathogenic given that it is not uncommonly found in eyelash hair follicles. Due to the fact that this mite has been reported with a high frequency in many chronic cases of blepharitis we aimed to report the incidence of infestation of this mite in healthy people and in patients with chronic blepharitis.

Methods: 105 subjects without clinical signs or symptoms of blepharitis were selected as control subjects. Patient groups included subjects diagnosed as having chronic blepharitis according to the clinical picture and biomicroscopy study of the ocular surface. Cilia epilated (mean of 9 eyelashes per subject) were examined for Demodex under a low-power microscope after storage in a moist chamber. The patients with overpopulation of Demodex were treated with ether lid scrubs and 2% mercury oxide ointment.

Results: The incidence of Demodex folliculorum of the control group was 0.15 mites/eyelash, while in the patients with chronic blepharitis we found a mean of 0.85 mites/eyelash. In both groups mites were found in all stages of growth. After four weeks of specific treatment the number of mites decreased drastically and the symptoms of itchiness and scaling of the lids were relieved.

Conclusions: Much controversy persists as to whether Demodex should be considered a direct pathogen in chronic blepharitis or whether it is a saprophytic inhabitant of eyelash hair follicles. In our study the incidence of Demodex folliculorum in patients with blepharitis was very high in comparison to normal subjects, showing a clear association between blepharitis and Demodex. The treatment with mercury oxide ointment has been satisfactory in controlling the infection in spite of the difficulty in its application and toxicity.

■ 387

Intravitreal triamcinolone acetonide for treatment of sympathetic ophthalmia

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Purpose: To evaluate the therapeutic effect of intravitreal triamcinolone acetonide for treatment of sympathetic ophthalmia.

Methods: A 47-years old patient suffered from sympathetic ophthalmia treated by systemic immunosuppressive therapy. He had developed Cushing's disease with steroid induced diabetes mellitus, arterial hypertension, obesity, and bone fragility. Despite th intensive systemic and local treatment, intraocular flare measurements were abnormally high, and intraocular pressure ranged between 5 and 8 mm Hg. Visual acuity measured 0.30. Under topical anesthesia, the patient received an intravitreal injection of 25 mg of triamcinolone acetonide, which was transconjunctivally applied through the pars plana.

Results: After the injection, systemic immunosuppressive treatment was reduced to 10 mg prednisolone per day and could not be stopped due to an insufficiency of the adrenal glands. Within the first 4 weeks after the injection, visual acuity improved from 0.30 to 0.50, and intraocular pressure increased to values ranging between 10 and 13 mm Hg. Flare measurements were reduced to a level of about 50% of the pre-injection values. Two months after injection, visual acuity remained at 0.50, intraocular pressure measured 12 mm Hg.

Conclusions: The results suggest that intravitreal injection of 25 mg of triamcinolone acetonide may be an additional tool in the treatment for sympathetic ophthalmia by decreasing intraocular inflammation and reducing systemic immunosuppressive therapy with its systemic complications. Care must be taken, not to miss the time when the intraocular concentration of triamcinolone acetonide decreases to a subtherapeutic level.

■ 386

PCR-based detection of bacteria and fungi implicated in severe ocular infections

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Purpose: To evaluate PCR-based assays to detect bacteria and fungi in ocular samples from patients with severe ocular infections.

Methods: Nested-PCR and real-time PCR assays were developed to amplify portions of the bacterial 16S or fungal 18S ribosome gene. Forty ocular samples from patients with presumed infectious endophthalmitis, keratitis or scleritis, including 27 vitreous humor, 5 aqueous humor, 6 corneal, 1 scleral, and 1 conjunctival samples, were tested by PCR assays, as well as by standard microbiological techniques. Results of the two diagnostic techniques were compared. Vitreous samples from 30 non-infected eyes served as negative-controls.

Results: PCR and culture results matched (either both positive or both negative) in 14 (35%) of the 40 samples from infected eyes. Twenty-six (65%) samples were PCR positive but culture negative. No culture-positive samples were PCR-negative. Among the 36 PCR-positive samples, 29 were positive for bacteria and 7 for fungi. Of the bacterial organisms, 18 were identified as Gram-positive bacteria, 11 as Staphylococci, 5 as Streptococci and 9 as Gram-negative bacteria. More specific identification to the species level was obtained in 15 samples. Of the 30 negative-control samples, none were positive by either culture or PCR. Positive detection of bacteria was obtained in 9 of 12 infected vitreous samples using real-time PCR, with the quantitative pathogenic load being approximately 1000-30000 CFU/ml of vitreous.

Conclusions: PCR-based assays offer overwhelming advantages over conventional culture techniques in the detection of bacteria and fungi implicated in severe ocular infections. Real-time PCR is a potentially valuable tool in view of its quantitative and rapid diagnostic capability.

The Ophthalmic Research Lecture

■ 1001

Age-related cataract in Asian and European populations: Racial differences and environmental influences

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For over ten years, my lens research has mainly focused on epidemiology. Since the late 1980s, increased solar UV irradiation to the earth caused by depletion of the ozone layer has given rise to UV induced human health problems. Since the early 1990s, I have carried out epidemiological surveys in Japan, Singapore and Iceland. The racial distribution of the subjects in these surveys was: 100% Japanese, 98% Chinese Singaporean and 100% Caucasian, respectively. The relative ambient UV irradiances (TOMS) in the survey places are 1 : 2.1 : and 0.4, and the average yearly temperatures are 15.1, 26.7, and 5.0 °C, respectively, in Japan, Singapore and Iceland. Our epidemiological studies began with the aim of proving the scenario of sunlight UV cataract, however, in addition to the initial aim, several subject specific age related lens changes have also been revealed. In the lecture, the following items noticed in the general population of the three subject groups will be discussed: decrease of lens transparency by ageing, prevalence of the three main types of cataract according to race, incidence and progression of lens opacification (presently Icelandic subject group alone,) race specific morphological changes of the lens and the anterior eye segment, localization of cortical opacification in different races and a search for risk factors. Regarding the scenario of UV as a risk for human cataract formation and / or progression, our data can still not substantiate absolute proof. In most cataract epidemiological studies, each investigator applies his or her own cataract classification system. This makes it difficult to compare data between groups, even though each study has obtained highly valuable information. Most of the data in the lecture have been converted to the recently proposed WHO unified cataract classification system. The significance of applying this system to future surveys will be stressed.

■ 1002

Genetics of ocular development: a clearing vision

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The completed sequencing of the human genome has allowed a rapid increase in the number of genes identified that, when mutated, underlie inherited monogenic human disorders. Much recent progress has been made in the identification of those underlying ocular developmental disorders such as congenital cataract, anterior segment dysgenesis and microphthalmia. This will be illustrated with reference to recent work on MAF, a basic region - leucine zipper (bZIP) transcription factor that is critical in mammalian lens development. There is heterogeneity amongst many ocular patterning defects with mutations in different genes causing identical phenotypes (genetic heterogeneity), an observation which complicates the processes of gene identification and mutation analysis. By contrast different mutations in the same gene may cause different disorders (allelic heterogeneity). This will be discussed with reference to the PITX2 gene and underlines the continued importance of accurate clinical characterisation of patients' exact ocular - and extraocular - phenotypes. Many of the proteins encoded by these genes are also expressed in the adult and are critical to tissue maintenance and repair. They are therefore attractive candidates for conditions of later onset (for example COL8A2 mutations in Fuchs endothelial corneal dystrophy). Hence the distinction between developmental or congenital conditions and those of progressive or late-onset may not be clear-cut. This therefore illustrates the importance of developmentally-expressed molecules in supposedly non-genetic, late-onset disease processes.

Herpes viruses: new patterns in eye disease

■ 1101

Genesis of ocular herpes disease: a love-hate relationship

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Purpose: The eye is a "privileged site" for herpes disease and to date it is not clarified why there is a tropism of herpes viruses for this anatomical area after the systemic primoinfection. Herpes viruses are old commensal viruses of the human species and are therefore reasonably well adapted to their human host. Depending on the herpes virus species, the geographical area and the socio-economic conditions the proportion of adult humans infected by these viruses ranges from 50% to nearly 100%. In a very large proportion of cases the relation is one of love, the virus reproducing itself silently and cosily, remaining mostly in a latent stage and not causing disease. This stage is maintained by a watchful immune system in as much as the viral strain does not have an excessively aggressive behaviour. However the equilibrium is put off balance in case of a failing immune system or by the occurrence of unusually aggressive strains giving way to a hate relationship at the origin tissue damage and disease. In the past a small proportion of ocular herpes cases, especially herpes zoster eye disease, had a deleterious evolution. In recent times however herpes disease has become increasingly destructive, not only causing corneal scarring but total retinal necrosis. The vast majority of these new herpes diseases including cytomegalovirus retinitis (CMV retinitis), acute retinal necrosis (ARN) and progressive retinal necrosis (PRN) are not the result of strain mutations but of a failing immune system especially at the cellular level.

Results: After reporting experimental work that has identified the main neuronal pathways herpes viruses use in the eye to spread centripetally as well as towards the periphery, this presentation will first focus on a situation, ocular sarcoidosis, where an overwhelmed cellular immune defense is successful compensated by the humoral system that increases its antibody production. It will further be shown that the necrotic herpetic retinopathies (NHR), whether ARN, PRN or intermediary forms are a spectrum of clinical entities resulting from the failure of the cellular immune system allowing the expression of the full hatred of herpes viruses, principally herpes simplex virus, varicella-zoster virus and cytomegalovirus. At the far end of this process we find CMV retinitis and PRN where there is unopposed killing of the retinal structures by the viruses. Fortunately, in parallel with the genesis of new destructive herpes diseases, highly effective antiviral agents became available. They were shown not only to reduce to a minimum the complications of classical herpes disease such as herpes zoster ophthalmicus but also to lead to the control of most of the new destructive entities restoring the balance in favor of the host.

Conclusions: Despite the fact that the evil forces have been pushed back, we nowadays increasingly depend on sophisticated weapons rather than natural defenses to fight disease in this troubled post- (herpes)-cold-war era.

■ 1102

Herpetic kerato-uveitis: clinical forms and management

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Purpose: To describe the different clinical forms of herpetic involvement of the anterior segment.

Methods: Review of the literature and personal experience.

Results: In the literature there is a confusing plethora of terms to describe the different clinical entities of herpetic involvement of the cornea and the anterior segment. Five different modalities of corneal involvement should be distinguished: (1) infectious epithelial disease (dendritic ulcer) (2) neurotrophic keratopathy (HSV induced surface disease) (3) necrotizing stromal keratitis (viral replication) (4) immune stromal keratitis (5) endotheliitis. Patients with HSV keratitis may develop a concomitant or subsequent iridocyclitis. In addition iridocyclitis may occur without prior history of keratitis. Segmental iris atrophy is characteristic of herpetic uveitis. Trabeculitis with acute severe pressure rise may develop. VZV causes a similar clinical picture. **Diagnosis:** Although the diagnosis of herpetic eye disease relies primarily on a thorough ophthalmic examination, laboratory techniques (PCR, Goldmann-Witmer) are helpful in selected cases. **Management:** Antivirals often in association with corticosteroids are the treatment of choice in herpetic kerato-uveitis. Adjunctive medical or surgical therapy is indicated for the treatment of complications (glaucoma, corneal scarring).

Conclusions: Although the clinical manifestations of herpetic eye disease of the anterior segment are well established, treatment is far from satisfying and HSV is still a significant cause of ocular morbidity.

■ 1103

Classical viral retinopathies : clinical presentation and molecular tools for the diagnosis

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Acute retinal necrosis (ARN) syndrome is a severe sight-threatening viral retinopathy that can involve both immunocompetent and immunosuppressed patients. ARN is a rapidly progressing viral retinitis characterized by a peripheral retinal whitening that progresses to necrosis over several days. Associated ocular findings include anterior granulomatous uveitis, arteritis and vascular occlusion, papilledema, severe vitritis and retinal detachment. Clinical diagnosis can be confirmed by detection of viral DNA in aqueous humor or vitreous body using polymerase chain reaction. Herpes simplex virus 1 and 2, varicella zoster virus and cytomegalovirus have been associated with ARN to varying degrees. Treatment with intravenous aciclovir or with intravenous foscarnet combined with intravitreal ganciclovir injections for more fulminant cases have been used successfully. A particularly virulent form of retinal necrosis due to VZV in AIDS patients has been described and named first progressive outer retinal necrosis (PORN) and more recently progressive retinal necrosis (PRN) or VZV retinitis. This affection is characterized by rapid bilateral constriction of the visual field and loss of vision within several days. Clinical findings include multifocal large deep retinal lesions, arteritis, lack of intraocular inflammation, very rapid progression, high rate of retinal detachment, and poor visual outcome. Prompt therapy with a combination of both i.v. foscarnet and ganciclovir and intravitreal injections of ganciclovir is indicated. Retinal detachment repair using vitrectomy and silicone oil tamponade results in anatomically reattachment, however the visual prognosis remains poor.

■ 1104

Emerging viral entities

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As with all viruses, survival of herpes viruses (HSV, VZV, CMV, EBV, HHV-8) requires survival of their host cells. Throughout the millions of years that herpesviruses have lived together with them, they have evolved means of combating both death of the host cell and rejection by the organism as a whole. Herpesviruses have performed this by incorporating series of genes that functionally are homologous to, or mimic, cellular genes. Viral mimicry can have an array of effects. Viral proteins interfere with normal cell metabolism and modulate the ocular immediate environment. Viral mimogenes may cross-react with corneal, uveal or retinal proteins causing autoimmune pathologies. Both, cytopathic and immunopathological mechanisms must be taken into consideration in ocular herpetic disorders. Clinical features of ocular viral diseases are quite well defined and will be reviewed. However, diagnosis is based on the analysis of ocular fluids by molecular techniques such as PCR or the evaluation of local antibody production. Emerging viral entities have been defined since the beginning of the new century. Ocular involvement may be bilateral, especially in immunocompromised patients. The use of highly active antiretroviral therapy in AIDS patients has dramatically decreased the incidence of CMV retinitis but cases of immune recovery uveitis have been reported in patients with a previous history of CMV retinitis. Furthermore, cases of CMV-associated anterior uveitis have been recently described in immunocompetent patients highlighting the major place of a specific antiviral therapy. Finally, the spectrum of viral retinopathies has been recently enlarged. The role of herpesviruses has been confirmed in atypical forms of posterior uveitis misdiagnosed as birdshot retinochoroidopathy, retinal vasculitis or behçet's disease resisting to high doses of corticosteroids. Non narcotising herpetic retinopathies are a new viral entity requiring both antiviral and anti-inflammatory drugs.

Herpes viruses: new patterns in eye disease

■ 1105

Update on Cytomegalovirus Retinitis

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CMV retinitis while less frequent than a decade ago still continues to manifest itself in immunocompromised patients with or without AIDS. It is often encountered as an AIDS defining infection and may be associated with significant visual acuity loss in at least one eye. Progression in AIDS patients is limited, provided that immune recovery occurs. The risk of progression is at its highest in the initial few months after introducing HAART therapy. CMV also manifests itself in immunocompromised patients following transplantation or even mild immunosuppression for autoimmune diseases. Depending on their overall medical status, one of several treatment modalities can be instituted from oral or intravenous therapy to ocular implants or intravitreal injections. Risk benefit of each approach will be discussed in the context of the patient's underlying condition and response to treatment.

■ 1111

From exfoliative syndrome to exfoliative glaucoma: an overview

IRKEC M

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Purpose: Significance of the PEX syndrome comes from its characteristic of being the most common identifiable cause of open-angle glaucoma. The purpose of this presentation is to evaluate the course of PEX syndrome to the development of capsular glaucoma and the features that might be helpful to predict patients with high risk for glaucomatous transition.

Methods: A literature survey has been done through major abstracting and indexing systems to find out epidemiological features of PEX syndrome and the incidence of patients developing PEX glaucoma. Additionally, a group of PEX syndrome cases with no significant findings of glaucomatous optic disc damage and visual field changes were evaluated for diurnal IOP variation and fluctuation and examined with scanning laser polarimetry for the changes in retinal nerve fibre layer.

Results: PEX syndrome is common all over the world, with significant differences in its prevalence. Almost 25% of the patients with PEX syndrome develop glaucoma, which has an insidious onset. In areas where PEX syndrome is common, it has been documented that from 12 to 35% of the patients developed capsular glaucoma. Our studies on the significance of diurnal IOP variation and fluctuation in patients with PEX syndrome pointed to early thinning of the retinal nerve fibre layer on scanning laser polarimetry.

Conclusions: PEX syndrome takes many years before the signs and findings of PEX glaucoma become manifest. PEX syndrome cases with high levels of diurnal IOP variation and fluctuation should better be evaluated with scanning laser polarimetry to detect early nerve fibre layer thinning, a finding that might be an early predictor of transition into PEX glaucoma.

■ 1112

Current concepts in the pathogenesis of pseudoexfoliative glaucoma

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Purpose: To give an overview on the pathogenetic mechanisms involved in glaucoma development in patients with pseudoexfoliation (PEX) syndrome.

Methods: Clinical-pathologic correlations.

Results: PEX syndrome represents a generalized fibrilopathy leading to progressive accumulations of an abnormal fibrillar material in many tissues. Active involvement of the trabecular meshwork in this matrix process may lead to the development of a rapidly progressing, hypertensive type of secondary open-angle glaucoma in about half of the patients. The primary cause of chronic pressure elevation in PEX eyes is considered the blockage of outflow channels by locally produced PEX material in the juxtacanalicular tissue of the meshwork with subsequent degenerative changes of Schlemm's canal wall. The amount of PEX material in the meshwork correlates with the severity of glaucoma and with the axon count in the optic nerve. Additional factors contributing to glaucoma development include pronounced melanin dispersion, increased aqueous humor protein concentrations, vascular factors, and connective tissue alterations in the lamina cribrosa. The adenosine receptor A3, which is involved in the regulation of aqueous humor production and intraocular pressure, is upregulated in the ciliary epithelium of PEX glaucoma eyes up to 30-fold. In addition, growth factors, particularly TGF- β 1, a proteolytic imbalance of matrix metalloproteinases and their inhibitors TIMPs, transglutaminase-mediated cross-linking processes, and increased cellular and oxidative stress may participate in the pathogenesis of this fibrotic matrix process.

Conclusions: Future therapeutic principles should be guided by the pathogenetic mechanisms underlying this frequent and specific type of glaucoma.

New developments in exfoliative glaucoma

■ 1113

Systemic associations in exfoliative syndrome

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Purpose: To summarise the systemic associations revealed in exfoliative syndrome and exfoliative glaucoma, and to evaluate their clinical significance.

Methods: Search of the peer-reviewed literature published in the last ten years, and review of the author's clinical research on exfoliative glaucoma patients.

Results: Exfoliative material has been shown not only in the intraocular tissues, but also in the orbit and in many extraocular tissues such as skin, vessel wall and visceral organs. Exfoliation syndrome was found associated with cardiovascular and cerebrovascular ischemia as well as neurosensory hearing loss, and an increased homocystein plasma level was detected in patients suffering from exfoliative syndrome. In our human studies the venous plasma endothelin-1 concentration did not differ between patients with exfoliative glaucoma and primary open-angle glaucoma or healthy control subjects, but the cutaneous capillary flow was found reduced, and the cold-induced and warm-induced changes of the cutaneous capillary flow were significantly slower in the exfoliative patients than either in primary open-angle glaucoma or in healthy subjects. In another study we found a mild parasympathetic cardiovascular neuropathy in exfoliative glaucoma.

Conclusions: These systemic alterations clearly show that the whole body is involved in exfoliative syndrome. However, it has not been clarified whether these alterations represent a systemic disease or only an altered condition without clinical significance.

■ 1114

Clinical features of pseudoexfoliative glaucoma

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Purpose: To evaluate differences in four separate European population groups (Hungary, Spain, Russia and Greece) in the clinical presentation and long-term follow-up of exfoliation glaucoma.

Methods: We consecutively reviewed charts of newly diagnosed exfoliation glaucoma patients that had at least five years of follow-up. Design: Retrospective, case series analysis.

Results: Two hundred patients were included in this study (50 from each site) with an average follow-up time of 6.0 ± 2.1 years. Patients in Hungary and Spain statistically presented at an older age (79 years) than Greek patients (67 years). However, Greek and Hungarian sites, at presentation, showed a greater number of patients with glaucomatous damage, more severe glaucoma and a higher untreated intraocular pressure (31.8-32.1 mm Hg). On long-term follow-up Greek, Russian and Hungarian patients also had the highest pressure (18.8-20.8 mm Hg) and the greatest incidence of progression (approximately 50%). In general, in the Greek and Hungarian patients glaucoma was more difficult to control showing a greater number of changes in medicines during the follow-up period, a greater number of medicines at the end of the follow-up period, and more progression. Hungarian patients also showed a trend for more surgeries per patient (1.5). In contrast, Spanish patients demonstrated the lowest intraocular pressure (17.6 ± 3.6 mm Hg) and the lowest rate of progression (28%) during the follow-up period and the fewest number of medications per patient (0.7) to control the pressure at the end of the follow-up period.

Conclusions: This study suggests that differences in exfoliation glaucoma prevalence, clinical presentation, and long-term follow-up, may exist between geographic populations.

■ 1115

New drugs in the treatment of exfoliation glaucoma

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Purpose: Exfoliation glaucoma (XFG) comprises over 50% of open-angle glaucomas in many European countries and its socioeconomic importance has increased considerably in recent years. The purpose of this lecture is to provide an update on the current knowledge with the newer drugs in XFG.

Methods: Surprisingly, to date there is very limited information with regard to the response and success rate of the newer medications in XFG (e.g. the timolol / dorzolamide fixed combination, latanoprost, bimatoprost etc.). Since glaucomatous damage occurs more rapidly in XFG and the rate of subsequent blindness is twice that seen with primary open-angle glaucoma, determining the most effective initial therapy and the best medical stepwise therapy in XFG is especially important to help control these subjects.

Results: This lecture will review recent studies investigating the efficacy of latanoprost and that of the timolol/dorzolamide fixed combination specifically in XFG and outline the rationale of an on-going study comparing the efficacy and tonographic outflow facility with bimatoprost compared with that of timolol in XFG.

Conclusions: It is important to determine the success of monotherapy with the newer medications in XFG to facilitate the future management of this important secondary glaucoma.

Visual field experiences in chiasmal lesions

■ 1141

Multifixation Campimetry

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Purpose: To describe multifixation campimetry and discuss its scope with special reference to neuro-ophthalmology.

Methods: Multifixation campimetry uses the patient's eye movements to facilitate visual field examination. Manual versions consist of a card with series of numbered fixation targets around a central stimulus. Computerised versions for use on a personal computer use an interactive, moving fixation target. Multifixation Campimetry On Line can be performed on the internet, with the facility to e-mail the results to an investigator for analysis. This test uses the previous stimulus as the fixation target and presents 42 stimuli in the central 24-degree field. The standard version of the test presents only black stimuli whereas an advanced version presents grey stimuli of increasing contrast, enhancing sensitivity. A basic version has also been developed for patients who cannot control their eye movements and this uses a variable fixation target, which the patient must identify.

Results: Multifixation campimetry produces similar results to conventional methods. Visual fields have been plotted in patients who could not be examined with conventional methods. The online version successfully enables patients to perform self-examination using a personal computer, at their own convenience.

Conclusions: Multifixation campimetry enables visual field examination to be performed when other methods are impractical and should facilitate the detection and measurement of chiasmal disease.

■ 1142

Humphrey versus frequency doubling perimetry in chiasmal disease

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Purpose: To evaluate the concordance between Humphrey perimeter (24-2 SITA threshold strategy) and frequency doubling technology (FDT) in chiasmal disease.

Methods: Ten patients with typical pattern of chiasmal visual field defect in 24-2 SITA were evaluated with FDT (C-20 screening test). Topographic correlation, percentage of affected points and duration of both tests were evaluated.

Results: The mean time inverted for visual field performing was 88.78 seconds (SD: 29.22) and 350.56 seconds (SD: 31.64) for C-20 and 24-2 SITA respectively (P= 0.000). The mean percentage of points with moderate or severe damage in the hemianopic side was 71.59 (SE: 33.58) and 76.02 (SD: 31.92) in C-20 and 24-2 respectively (P = 0.607). Pearson correlation coefficient for this variable was 0.644 (P = 0.033). Macular sparing was more common in FDT.

Conclusions: FDT could be useful for evaluating the visual field in chiasmal disease.

There are a good topographic correlation between both tests. In C-20 test, the presence of bitemporal hemianopsia only in the outer column, sparing the inner column of the hemianopic fields is highly suggestive of chiasmal disease. C-20 screening test is less time consuming (more than 4 minutes of time saving). This short time of evaluation for FDT is of great utility in some neurologic patients with difficulties for perimetry performance.

■ 1143

High-Pass Resolution Perimetry in patients with chiasmal and other lesions of the optic pathways

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Purpose: To present the HRP technique and to give a survey of the experience of HRP use in patients with chiasmal and other lesions of the optic pathways.

Methods: High-Pass Resolution Perimetry (HRP) uses high-pass filtered stimuli and the results are directly correlated with the number of functional retino-cortical neural channels. It has been extensively tested and there are some 200 published studies, most of them dealing with glaucoma and/or aspects of evaluation.

Results: The technique will be demonstrated and results from studies regarding chiasmal lesions, pseudotumor cerebri and other lesions in the visual pathways will be presented.

Conclusions: The HRP is reported to be a sensitive and specific perimetric technique in neuro-ophthalmic disorders, as good as or better than conventional perimetry. The method was strongly preferred by patients compared to conventional automated perimetry.

■ 1144

Rarebit microdot perimetry in neuro-ophthalmology

MARTIN L

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Purpose: To describe the rarebit microdot perimetry and report findings in chiasmal and other neuro-ophthalmic disorders.

Methods: The rarebit technique was recently developed and described by Frisén (2002). The rarebit principle relies on testing the retinal receptors with very small stimuli (microdots), less than 0.5 MAR, presented one or two at the time. The test is designed to evaluate the density of the matrix of retino-cortical channels. Since this matrix normally is complete, with no gaps between the receptive fields, a normal person will have a hit rate of nearly 100%. Loss of receptive fields gives a lower hit rate. The results are summarized in two measures, the mean hit rate and the number of locations with a hit rate below 90%. Fixation is encouraged by dynamically changing the location of the fixation mark.

Results: The technique will be demonstrated and results from studies regarding chiasmal lesions, periventricular white matter damage, and intrauterine growth restriction will be presented.

Conclusions: The rarebit perimetry technique seems to have high sensitivity for neural damage and to be well suited for neuro-ophthalmic diagnosis.

Corneal Innervation

■ 1151

Architecture and fine structure of corneal nerves

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Purpose: Purpose: Nowadays, corneal resculpturing techniques (PRK, LASIK, LASEK) are commonly used for correction of refractive errors. Unfortunately, these techniques damage the nerves that are considered to be essential in the process of wound healing. Therefore, description of the fine structure of control corneas is required.

Methods: Methods: Light (LM) and electron (EM) microscopy, whole mount immuno-cytochemistry (WMI) and in vivo confocal microscopy (IVCM) of human corneas. The data obtained with these techniques were combined with the current literature on corneal nerves.

Results: Results: Mammals have the highest density of nerve terminals in the apex. The human is an exception with similar densities in the mid-periphery. Following injury many epithelial cells and subbasal nerves are damaged. Despite regeneration control levels of sensitivity are not achieved. This is most probably due to a decrease in the density of nerve terminals as observed in EM. An advantage of WMI is the possibility to follow terminals as strings of beads and to study subbasal nerves in samples 10 times larger than IVCM and LM. The data obtained resulted in an adapted scheme of the subbasal plexus and in an estimate of 7000 nerve terminals per mm².

Conclusions: Conclusions: As corneal nerves are important in the process of corneal wound healing, it is a challenge to search for compounds that will stimulate their recovery after injury or refractive surgery.

■ 1152

Neuronal factors in the cornea

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Purpose: The cornea is considered to be the most densely innervated tissue outside the central nervous system. It contains both sensory as well as cholinergic and parasympathic fibers. These fibers are important for corneal epithelial function, consequently damage to corneal nerves due to a variety of diseases or therapeutic interventions can lead to neurotrophic keratitis.

Methods: Neurons and corneal epithelial cells support each other through the release of soluble factors such as growth factors. Several members of the neurotrophic growth factor family and other neurotrophins, opioid growth factors, neuropeptides and neurotransmitters are present in the cornea released by either neurons or epithelial/stromal cells.

Results: Neurotrophins play an important role in regulating wound healing and regeneration of corneal epithelium: Both nerve growth factor (NGF) and its high and low affinity receptors as well as glial cell derived neurotrophic factor (GDNF) and its receptors are present in the cornea where they enhance epithelial migration and MAP-kinase signaling. Other factors that enhance corneal wound healing are substance P (SP), calcitonin gene related peptide, acetylcholine or norepinephrine.

Conclusions: Therapeutically nerve growth factors or neurotransmitters could be used to enhance nerve recovery in various corneal diseases and to modulate wound healing. A first step towards clinical application was made when murine NFG or SP-analogues/IGF were used to treat patients with neurotrophic keratitis.

■ 1153

Stimulus detection by corneal sensory neurons: from nerve endings to sensations

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The existence of various functional classes of primary sensory neurons innervating the cornea and bulbar conjunctiva is now well documented. Differences are based on the form of energy to which they respond preferentially, in the membrane properties of their somas, axons and nerve endings and also in the conscious sensations evoked by the activation of each neuronal type. Neurons responding exclusively to mechanical forces represent about 20% of the total. A few of them with small receptive fields located in the limbal area have low mechanical thresholds and relatively fast conduction velocity (>20m/s, A-delta) while the majority have higher mechanical threshold, larger receptive fields and slower conduction velocities (<10m/s), but still in the A-delta range. The somas of mechanoreceptive neurons have fast action potentials, phasic discharges and marked inward rectification while their peripheral endings produce propagated impulses carried by TTX-resistant sodium channels. Polymodal neurons, the most abundant type (70%) are activated by mechanical stimuli but they also respond to temperature over 39°C and to exogenous chemicals (acids, capsaicin) and a variety of endogenous mediators (prostaglandins, BK). They conduct nerve impulses at velocities in the A-delta or the C range, produce wide action potentials with a hump and do not rectify; their nerve fibers also possess TTX-resistant sodium channels that sustain propagated action potentials. Polymodal nociceptor terminals branch extensively and action potentials initiated in one point invade antidromically other terminals. This releases neuropeptides contained in the endings. The third type of corneal neurons are cold-sensitive neurons. Their endings discharge spontaneously at corneal temperatures of 33°C, often in bursts and increase the firing frequency with moderate cooling. The somas of these neurons that belong to the A-delta and the C group, are highly excitable but do not fire spontaneously, produce fast action potentials and exhibit a prominent rectification. A fraction of these neurons also respond to capsaicin. The endings of cold-sensitive neurons do not conduct propagated action potentials. Selective stimulation of the different types of sensory neurons innervating the human cornea with mechanical, chemical (CO₂) and thermal (hot or very cold) stimuli using a gas esthesiometer, evoke distinct sensations of irritation while moderate cold stimuli elicit only cooling sensations. Qualitatively distinct sensations of irritation are evoked by stimulation of the cornea with combinations of stimuli of different modality, as a consequence of the variable activation of the different functional types of corneal sensory endings.

■ 1154

Corneal innervation in clinical ophthalmology

TERVO T

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Purpose: To discuss the significance of testing of corneal sensitivity of patients with corneal problems at the light of our observations made using in vivo confocal microscopy or esthesiometry.

Methods: Patients, who underwent PRK or LASIK or suffered from various corneal or ocular surface problems were examined by a Tandem Scanning in vivo confocal microscope (IVCM) or Belmonte non-contact aesthesiometer (BNCE).

Results: PRK or LASIK patients not only show dry eye symptoms but also relatively slow, probably incomplete regeneration of subbasal nerve fibre bundles (sbnf). These are readily visible by IVCM, and can be used as morphological hallmark for neural recovery. LASIK patients, who retain normal Bowman's layer rather than post-PRK patients show more complete morphological recovery but hardly any functional superiority over PRK patients. Despite two years of recovery LASIK patients may show subnormal sensitivity, and morphological features are often abnormal even 4 yrs after PRK: IVCM also revealed neural abnormalities in diabetes mellitus, postherpetic corneae, and in various hereditary dystrophies such as amyloidosis type II (Meretoja), Schnyder's dystrophy and recurrent erosion/basement membrane dystrophy, whereas posterior dystrophies such as pre-Descemet's or Fleck dystrophy patients tend to show normal nerves. IVCM also assists to understand complications due to refractive surgery. Sjögren's syndrome patients show normal subnfs but some activated keratocytes, which may correlate with pain and inflammation related to the disease.

Conclusions: Assessment of corneal nerves should always be done for patients with ocular surface symptoms or history of refractive surgery. Drugs supporting neural recovery may prove out to improve the control and/or velocity of corneal healing.

Corneal Innervation

■ 1155

Corneal innervation and morphology in primary Sjögren's syndrome

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Purpose: To analyze the in vivo morphology of different corneal sublayers and corneal nerves in primary Sjögren's syndrome.

Methods: Ten eyes of 10 patients with primary SS and 10 eyes of 10 sex- and age-matched control subjects were investigated. In vivo confocal microscopy equipped with a confocal microscopy through focusing system was used to investigate corneal morphology and to measure corneal sublayer thicknesses.

Results: Epithelial punctate staining with fluorescein was observed in 6/10 SS and 0/10 control cornea. Epithelial thickness did not differ between SS and controls. Confocal microscopy revealed patchy alterations or irregularities in surface epithelial cells of 6/10 SS corneas. Average corneal thickness was lower in SS (515.9 ± 22.0 μ m) than in controls (547.4 ± 42.0 μ m; $p = 0.050$, t-test). Accordingly, the mean intraocular pressure was lower in SS (13.9 ± 2.1 mmHg) than in controls (16.7 ± 2.9 mmHg; $p = 0.022$). The subbasal nerve plexus and stromal nerve fiber bundles were found in all corneas. No difference was present in nerve densities. However, in 4/10 SS eyes, the subbasal nerve plexus showed structures resembling nerve sprouting, suggesting ongoing active neural growth. None of the controls presented with such features. Signs of anterior keratocyte activation were observed in 5/10 SS patients.

Conclusions: In SS, the corneal surface epithelium was irregular and patchy. Anterior keratocytes frequently showed morphological features of activation. The subbasal nerve fiber bundles revealed abnormal morphology and the central corneal thickness was reduced due stromal thinning. The findings confirm epithelial, stromal, and neural abnormalities in the cornea of SS patients.

■ 1156

Trophic interactions between primary sensory neurons and corneal tissues

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Injury of trigeminal sensory nerves innervating the cornea causes disturbances in the corneal epithelium known as 'neuroparalytic keratitis'. On the other hand, damage of corneal nerves produce alterations in corneal sensitivity that possibly reflect changes in the functional properties of injured neurons. The nature of this mutual dependence between corneal sensory neurons and corneal tissues is still poorly understood. During development, survival of corneal neurons is critically dependent on the presence of neurotrophins. TrkA knockout mice showed a drastic reduction of the number of nerve trunks and branches in the corneal stroma and a reduced response to noxious stimuli. In normal adult mice, peripheral axotomy of corneal neurons induced changes in the expression of the proto-oncogene *c-jun* in the soma of corneal neurons but not in neuropeptide content. Immunocytochemical changes in the soma vary according to the type of corneal neuron involved (myelinated or unmyelinated) and the location of the lesion (nerve endings or parent axons). Co-culture of trigeminal ganglion neurons with corneal epithelium cells stimulate their mitotic rate. This effect was also obtained by addition of Substance P to corneal epithelium cell cultures while CGRP had an inhibitory action. Capsaicin, applied topically and by retrobulbar injection to the rabbit eye, but not the topical application of the NK1 antagonist, spantide, reduced the wound healing rate of corneal epithelium experimental wounds performed with n-heptanol. Likewise, corneal epithelial wound healing was delayed in mice lacking the expression of the Tac-1 gene (encoding for the whole preprotachykinin A family peptides), but only slightly retarded in animals lacking the expression of the NK1 receptor gene for the SP. This suggests that tachykinin peptides could act on the corneal epithelial wound healing mainly through a NK1-independent mechanism. Corneal epithelial wound healing was severely impaired in animals lacking the expression of the aCGRP gene, suggesting that this neuropeptide has a major role in wound healing "in vivo". This suggestion is also supported by the observation that a majority of corneal sensory nerves (around 60%) are CGRP-immunoreactive. Taken together, these results suggest that neuropeptides contained in the peripheral endings of corneal sensory neurons contribute to the maintenance of the integrity of corneal tissues and to their reaction to injury. Nevertheless, the cellular and molecular mechanisms that mediate the trophic actions of sensory neuropeptides on the cornea are still largely unknown.

■ 1161

Protective effect of pyruvate against oxidative stress and cataract formation in mouse lens

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Purpose: It has been shown that pyruvate protects rat lens against oxidative stress and diabetic cataract formation. Since the mechanism of cataractogenesis in humans is multifactorial, with only a minor role of aldose reductase (AR), unlike in rats, further studies were desired using AR deficient species. The present studies were hence done with mice lens which, akin to humans, is deficient in this enzyme.

Methods: Its effectiveness against oxidative stress was evaluated by organ culture. The ROS damage was assessed on the basis of the loss of cation transport activity and decreases in ATP and GSH. Its effectiveness against cataract was studied by maintaining diabetic mice on pyruvate-enriched diet.

Results: Addition of pyruvate to the medium protected the lens against oxidative stress as measured by its ability to maintain 86Rb^+ transport and levels of GSH and ATP, all adversely affected by ROS. In vivo, the incidence of cataracts at the end of 4 months in the diabetic group fed pyruvate was only 57%, against 82% in the control diabetics. There was also a significant drop in GSH with a concomitant increase in glycated proteins in the diabetics. In the pyruvate group, GSH and glycated proteins remained nearly normal.

Conclusions: The results demonstrate that pyruvate could be effective in preventing cataract formation under oxidative as well as glycemic stress. Since pyruvate is an endogenous compound, the findings are considered to be of therapeutic significance. The findings also indicate that the range of pyruvate effect could be much wider than ordinarily thought.

■ 1162

Protective effect of ascorbate against oxidative stress in mouse lens

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Purpose: High ascorbate of human aqueous has been hypothesized to be preventive against senile cataract formation. The concept is based on studies with rat lens, which however, unlike the human lens, is rich in aldose reductase (AR), acting by itself as an antioxidant. The applicability of these studies to human lens therefore needs further assessment with AR deficient species. These studies were hence done with mice lens, known to be AR deficient. They are also desirable due to the use of hyperbaric oxygen therapy in humans and consequent cataract development. Hence these investigations were done under high as well as normal oxygen tensions.

Methods: CD-1 mice lenses were incubated in 86RbCl and xanthine containing medium 199, at 37°C under air:CO₂ (95:5) or O₂:CO₂ (95:5). NaCl or sodium ascorbate (2mM) were added to controls and experimentals. ROS was generated by adding Xanthine oxidase and uricase. At 5 or 17 hrs, lenses were rinsed with saline and their radioactivity determined for calculating the distribution ratio of the ion between the lens water (CL) and the medium (CM).

Results: The CL/CM values in the lenses incubated without ascorbate were 50% of that in its presence, either in air-CO₂ or O₂-CO₂. ATP & GSH were also higher in the ascorbate group.

Conclusions: The results show that ascorbate prevented the ROS damage to the membrane transport. The attenuation was also apparent by higher GSH and ATP contents. Hence, ascorbate is effective in protecting the lens against oxidative insult even in a low lens AR situation, under normal as well as high oxygen tensions. The findings are consistent with the hypothesis that ascorbate at physiological levels is primarily anti-oxidative and not pro-oxidative.

■ 1163

Lens concentration of vitamin-E as a protective factor against ultraviolet radiation induced cataract.

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Purpose: to investigate if vitamin-E (a-tocopherol) levels in the lens correlates with levels of opacities in ultraviolet radiation (UVR) induced cataract.

Methods: 40 Sprague Dawley rats were divided into two groups with 20 animals in each. One group was fed with vitamin-E: 100 IU a-tocopherol/day. The solution was diluted in corn oil and 0,5 ml solution contained 100 IU a-tocopherol. The vitamin was intragastric administrated by intubation with a rubber catheter. The rats were fed once a day during 4 weeks and the other group was used as control. Both groups were unilaterally exposed to 8 kJ/m² UVR. One week after the exposure the rats were sacrificed, both lenses were removed, and forward light scattering was measured. Vitamin-E concentration in the lenses was measured by high performance liquid chromatography (HPLC). The animals were kept according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Results: Exposed lenses in the vitamin-E group showed superficial cataract. Meanwhile lenses in the control group (without vitamin E) showed superficial, central and equatorial cataracts as same as vacuoles. Forward light scattering measurement in the control group was higher than in the vitamin-E treated group. Difference between the groups was statistically significant. Vitamin E measurements in the lenses showed no difference between exposed and no exposed lenses. However vitamin E levels in the lens were significant higher in the treated group than in the control group.

Conclusions: in the rats an antioxidant like the vitamin-E seems to protect the lens against UVR damage. In the lens oxidative stress probably plays a very important roll in cataract development.

■ 1164

Antioxidant supplementation: a dominant strategy?

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Purpose: In Ontario, Canada, in a cohort of all people initially aged 50-54 years, can vitamin E(VE), REACT antioxidants, and/or AREDS antioxidant supplement for AMD reduce of risk of cataract, AMD and AMI?

Methods: Using reported risk reductions, incidence and prevalence data by age and sex from the Ontario Health Insurance Plan, Framingham, and Beaver Dam and yearly costs: VE: C\$27, REACT C\$ 150, AREDS C\$182.50, potential savings were calculated as the difference between the estimated medical costs for the untreated cohort and the same cohort if treated with antioxidants. Different scenarios were explored for direct and indirect cost savings.

Results: For the Ontario cohort, ca. 788,000, for VE, net cost was \$ 231million or \$2070 per cataract averted. For REACT, savings were \$37 million of \$364 per cataract averted. AREDS would reduce photodynamic therapy costs, saving\$431 million. Combining VE and AREDS supplementation potentially could save \$340 million. Adding prevention of AMI saves \$1.26 billion. Adding productivity saves \$9.6 billion.

Conclusions: VE and AREDS antioxidant supplementation appears to be a dominant strategy for cataract, macular degeneration and myocardial infarction. Applied to the whole Canadian population, the potential medical cost savings for AMD, AMI, and cataract are \$49.5 billion dollars direct costs, with potential indirect productivity savings \$377 billion. These values would be tenfold higher for the USA, because of the currency and population size differences. Averting the need for some cataract operations would reduce the need for scarce operating rooms: an estimate would be freeing approximately 20 operating rooms for Canada, the equivalent to the operating rooms of several large hospitals.

■ 1165

Age-related nuclear cataract: becoming clearer

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Purpose: Age-related nuclear cataract is associated with oxidation of protein sulphhydryl and methionine residues. This is most likely due to interaction of the crystallins with H₂O₂/superoxide. The older the person, the more yellow is their lens, and the amount of blue light transmitted to the retina is correspondingly diminished. In young lenses, UV absorption is accomplished by free UV filters. The concentration of UV filters being tryptophan metabolites decreases linearly with age.

Methods: We have demonstrated the existence of a barrier within the lens at middle age. Leading to a functional compartmentalisation of the metabolically active cortex and the nucleus. One consequence of the barrier, is an increased residence time of the UV filters within the lens interior. The kynurenine derivatives are intrinsically unstable at pH 7 and deaminate to produce reactive unsaturated ketones. These readily bind to proteins. Therefore UV filter breakdown results in the progressive accumulation of kynurenine and 3-hydroxykynurenine glucoside bound covalently to the structural proteins in the human lens. This post-translational modification, involves His, Lys and Cys residues, and occurs to the greatest extent in the nuclear proteins of older lenses. Glutathione intercept these reactive intermediates before they bind to the structural proteins. By contrast, ascorbate is completely ineffective.

Results: New data indicate that the properties of free and bound UV filters are different. While free UV filters absorb light and do not act as photosensitisers, irradiation of UV filters that are bound to proteins, results in the production of proteins peroxides.

Conclusions: In conclusion this finding suggests that the progressive accumulation of UV filters on the proteins in the centre of the lens that takes place, may facilitate the oxidation of these proteins following exposure of the eye to UV light.

■ 1166

Conformational change in bovine lens crystallins

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Purpose: To determine whether thermal and/or pH insult can induce conformational change in wild-type bovine lens crystallins.

Methods: Soluble bovine lens crystallins were dissolved in pH 2.0 or pH 7.4 buffer (10 mM glycine, 0.2% N3). Incubations were carried out at 37 °C or 65 °C for varying time periods. Change in protein conformation was confirmed by measuring the absorption maxima of the protein solution in the presence of ANS, and by the thioflavin-T amyloid assay.

Results: Stress promoted conformational change in wild-type bovine lens crystallins. Fibril formation was evident in the HMW, a-Low, b-High, b, gS and g fractions following 8 hours incubation in a pH 2.0 buffer at 65 °C. In gS and g crystallin fractions, fibril formation was more readily induced by thermal insult than pH. Fibril formation was consistently high in a-Low crystallin fractions under all conditions examined.

Conclusions: The pathogenic mechanism of fibril formation remains unclear. Thermal and pH insults can induce fibril formation in lysozyme and apomyoglobin. We have shown that combinations of heat and pH insult result in fibril formation in freshly isolated wild-type bovine lens crystallins. Conformational change in lens crystallins may result in a decreased protein solubility and hence contribute to lens opacification.

■ 1171

Getting your work published

DAMATO B

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It is a pity when good scientific work remains unpublished because the manuscript is not written properly. In this talk, tips for preparing and submitting your paper will be presented. Some useful sources of information will also be identified. A simple strategy for constructing the various sections of the manuscript will be outlined. This presentation is intended for novices and those who find it difficult to prepare their manuscript.

■ 1172

How to cope with statistics

KIVELÄ T

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A few pitfalls, seldom addressed during basic courses of statistics, await the unwary scientist who is starting clinical research in ophthalmology. Likewise, senior scientists who regularly receive manuscripts for review are aware of certain problems in presentation of research data that should be trivial to resolve, but which are surprisingly persistent. This presentation will clarify several of them in detail, based on examples from real life and from a clinical rather than mathematical point of view. Individual topics include proper ways of analysing data that is collected from both eyes of patients, proper ways of summarising and comparing visual acuities, recognising the often neglected yet common problem of making multiple comparisons, analysing data from follow-up and time-to-event studies, and knowing how to avoid false impression of precision, how to communicate effect size, how to differentiate statistical from clinical significance, and how to cope with statistically nonsignificant results when writing the manuscript. The need to find a statistician well versed in ophthalmic research already at the planning stage of a study is stressed.

■ 1201

What are high risk corneal grafts?

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Purpose: To clarify the concept "high risk corneal transplants".

Methods: A Medline search and a review of the subject in different textbooks on corneal disorders.

Results: Several risk factors are associated with a higher than normal incidence of graft failure after corneal transplantation. A distinction should be made between risk factors for non-immunologic graft failure (e.g. ocular surface disease) and risk factors for immunologic rejection of the donor cornea. Risk factors for immunologic rejection: previous graft failure- corneal vascularization- increased graft size or eccentric graft- chronic or recurrent inflammation- previous anterior segment surgery- anterior synechiae- young age.

Conclusions: There is no uniform and distinct definition of what a high risk corneal graft is. Most corneal surgeons use the term "high risk" when a regrant is carried out after previous graft failure or when the acceptor cornea has deep vessels in more than one quadrant.

■ 1202

HLA in high risk transplants

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Purpose: The glycoproteins encoded by the MHC (Major Histocompatibility Complex) are central players in the immune response. These proteins are optimized to bind peptides and complex with the T cell receptor. This complex initiates the specific T cell immune response. In addition, the MHC molecules can bind Killer Inhibitory Receptors (KIR) expressed on NK cells and by this mechanism modulate innate immunity. Of the many molecules encoded by the HLA complex that are polymorphic, matching and antibody detection has been shown to be clinically significant only for the class I molecules: HLA-A, B and C and for the class II molecules: DR, DP and DQ. Polymorphism of these genes is so vast that every month between 10 and 20 new alleles are reported.

Methods: There are two levels for HLA typing. One recognizes "antigen" typing or "two-digit" or "low resolution" typing and an other "allelic" or "four-digit" or "high resolution" typing. The former is performed using PCR and sequence specific primers or sequence specific probes, the latter by actually sequencing the polymorphic exons of the genes.

Results: Allelic matching is performed in bone marrow transplantation since there is a clearcut beneficial effect on survival. This practice however requires millions of potential donors. In organ transplantation such as kidney and heart grafting, low resolution antigen matching is common practice and a beneficial effect of matching is seen. For liver transplantation no effect of matching can be demonstrated.

Conclusions: For corneal transplantation in high risk patients it is recommended to perform low resolution HLA typing for better grafting.

High risk corneal transplantations

■ 1203

Beneficial effect of HLA-AB + DR matching on the survival of corneal allografts

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Purpose: Although HLA typing and matching have been used for 3 decades, that practice has been poorly implemented in corneal transplantation, mainly because of inconclusive or contradictory analytical results. Consequently, we studied the immune response of corneal transplant recipients to HLA histoincompatibilities in a large homogeneous population.

Methods: All corneal transplants performed by a single surgeon between 1976 and 1996 were studied. HLA-AB matching was used for recipient selection. All HLA typings were performed by a single experienced laboratory. Population genetic techniques were used to assess the validity of the HLA typings. Mono- and multivariate analyses were performed to identify the factors which significantly influence the survival of corneal allografts. Simulation studies were carried out to demonstrate the effect of mis-typed donor and recipient HLA-DR typings on analytical results.

Results: Retransplantation, degree of vascularization, HLA-AB and DR matching, endothelial cell count, graft size, recipient gender, and storage method were identified as significant factors by our monivariate analyses. A Cox proportional hazards survival analysis model identified degree of vascularization and HLA-AB and DR matching as significant prognostic factors when all immunological rejection episodes were used, $P=0.000001$. When only irreversible immunological episodes were used, panel reactive antibodies, retransplantation, and number of rejection events were also identified, $P=0.000001$. Simulation studies showed that the effects of HLA-DR matching are abrogated by poor HLA-DR typings.

Conclusions: Corneal allograft recipients have a normal alloimmune response to histoincompatibilities. Demonstration of that fact requires accurate HLA typings.

■ 1204

Clinical results of HLA matching in high risk transplants

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■ 1205

Immunosuppression in high risk transplants

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Purpose: To define a strategy for the management of High Risk Corneal Transplants.

Methods: Review of literature and personal experience of last 7 years.

Results: Corneal transplantation is the most common solid tissue transplant performed world-wide. Immunological rejection still accounts for over 33% of all graft failures. Previous grafts or intraocular surgery, stromal vascularisation, chemical burns, herpetic eye disease and active inflammation, render the transplant at high risk for rejection and the failure rate can be as high as 70%. Increasingly immunosuppression and other strategies are being employed to increase the rate of graft survival. These include aggressive control of ongoing inflammation, occlusion of corneal vessels, use of organ cultured and HLA/ABO matched donor tissue and systemic and/or topical immunosuppression. To standardise such regimes it is important to evaluate the degree of 'risk'. For this we have developed a scoring system for the risk factors which will be presented. Steroids, usually topically, remain the mainstay in the management of high risk corneal graft. Specific systemic agents include cyclosporin, tacrolimus, rapamycin and less commonly sirolimus and mycophenolate. All these drugs are potent immunosuppressants with significant side effects. They need close monitoring but when used expertly they do significantly improve graft survival with manageable side effects.

Conclusions: Our experience, which is mainly with the use of tacrolimus, shows that significant improvement in survival of high risk corneal and limbal allografts can be achieved. The duration for which immunosuppression should be continued still remains unanswered.

■ 1241

Is recognition visual acuity a safe criteria to stop patching?

OTOS

Stabilization of recognition visual acuity is mostly the criteria for the termination of the occlusion therapy; however opinions vary on the exact time. The question is whether only the level of recognition visual acuity a sufficient criteria to quit the therapy. We did a prospective study to determine the change in recognition visual acuity and the nerve conduction delay with neutral density filters during the occlusion treatment, with a follow up of 6-40 months and seen that conduction delay still persists after the recovery of the visual acuity to 20/20. In decision of stopping the patching treatment predicting reading ability would also be helpful. We studied two groups of children with amblyopia, first group consisted of children with at least 0.2 logMAR difference between the two eyes and second group consisted of children whose vision was equalized with patching and were under maintenance therapy. Eye movements were recorded monocular for each eye and binocularly during reading a standardized text with best correction. We compared the peak saccadic eye movement velocity and time difference between completion of eye movement. We believed the symmetry of the reading capacity displayed the stability of the visual acuity.

■ 1242

Amblyopia therapy: Quantifying the dose-response effect

FIELDER A

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Purpose: Three aspects of amblyopia therapy will be discussed against the background of the recent research. First, how monitoring of occlusion therapy by objective dose monitoring has provided insight into the occlusion dose-response relationship. Over 75% of the response to occlusion occurs within the first 4 weeks of treatment and the response is greatest in children under 4 compared to those over 6 years of age.

Methods: Second, I will consider the definition and measurement of outcome in the treatment of unilateral amblyopia. This includes defining the optimum outcome acuity and also that improvement should be quantified, not in terms of lines of improvement, but by the proportion of change induced by the treatment - ie the proportion of the deficit that is corrected by treatment. Thus, the contributions of individual interventions to outcome, such as refractive adaptation and occlusion, can be precisely determined.

Results: Third, I will discuss the latest age at which treatment for amblyopia might be considered.

■ 1243

Electronic recording of patching for amblyopia study (ERPAS): preliminary results

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Purpose: One third of the treatments for amblyopia fails, mainly because of non-compliance. Therefore, in this prospective randomised study, predictors for non-compliance are being identified and the effect of a programme to enhance compliance will be assessed.

Methods: Since July 2001 all newly diagnosed amblyopic children in The Hague have been recruited by 8 orthoptists. Since January 2003 additional children have been recruited in Frankfurt, Leicester, Liverpool and Bristol. Compliance is measured electronically during 1 week every 3 months with the Occlusion Dose Monitor, distributed by house-visits. Both the socio-economic and ethnic background and the decrease in the quality of life during patching is assessed. After 6 months reasons of failure or success of the occlusion therapy are identified with the Patching Success Questionnaire. In addition to standard orthoptic care half of the included children receive a compliance-enhancing programme developed for all children irrespective of their ethnic or socio-economic background, consisting of a self-explanatory cartoon. It is supported by a direct reward system administered by the parents. The control group receives a colouring plate.

Results: So far 285 children have been recruited in The Hague of whom 236 have been included, mean age was 4.4 years. Overall compliance averaged 72%. Of the included children 48% were of foreign origin. Only 24% of the parents find amblyopia a serious condition. The exact aim of treatment was unclear for 23% of the parents and fitting the therapy into their daily routine was difficult for 77%. 12% of the children were teased when wearing the patch and 25% of the parents did not like the look of their child whilst wearing a patch.

Conclusions: CR: none, the Occlusion Dose Monitor was developed in the public domain in 1996-1997 Support: Health Research and Development Council of the Netherlands.

■ 1244

New aspects of amblyopia treatment

GOTTLÖB I

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Purpose: Amblyopia treatment is not standardised. In this study, we compare the different patterns of amblyopia treatment in several European countries. We also investigate the effect and compliance of patching in amblyopia by objectively monitoring occlusion and to establish whether there is a correlation between effective treatment and improvement of visual function.

Methods: Questionnaires were sent to orthoptists in the UK, Germany, Switzerland and Austria asking for their preferred choices of treatment of amblyopia. Newly detected strabismic amblyopic children in our department aged between 3-8 years (mean age 4.05 years) were randomly divided into three groups, with a prescription of 6 hours, 3 hours and 0 hours occlusion per day. Patching was monitored with a thermo-sensitive occlusion dose occlusion monitor (ODM) with follow-up for 3 months. Patients were assessed every 3 weeks with logMAR visual acuity.

Results: The number of hours of occlusion prescribed per week was significantly higher in German speaking countries than in the UK and orthoptists in the German speaking countries treat amblyopia up to an older age. In the ODM study, in the 6-hour group the average real ODM time was 4.1 hours. In the 3-hour group the average real ODM time was 2.1 hours. Compliance with the originally prescribed patching time was more variable in the 6-hour group. The mean increase in visual acuity was 3.8 logMAR lines for 6 hours patching, 1.7 for 3 hours and 1.7 for the 0 hour group. The mean increase in visual acuity was significantly better for 6 hour patching compared to 3 hour patching ($P < 0.05$). There was a linear relation between effective hours patching per week and the increase in visual acuity.

Conclusions: Orthoptists in the German speaking countries treat patients more intensively. We have shown with the ODM study that there is a problem with compliance with patching therapy. Six hours occlusion was more effective than 3 hours occlusion. However, there was little difference between the 3 hours patching and no patching.

■ 1251

Prevalence, annual blindness rate and causes of blindness in diabetic patients in Århus County, Denmark

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Purpose: To describe the prevalence, annual blindness rate, and causes of blindness in diabetic patients in the Danish county of Århus (population of approximately 645,000 persons), during the first ten years after introducing screening for diabetic retinopathy.

Methods: Data were obtained from a database of 7527 diabetic patients (1949 ~ 90% of the type 1 and 5459 ~ 40% of the type 2 diabetic population) but containing all patients from the county who had been treated or who had experienced visual loss due to diabetic retinopathy. Patients who had developed visual acuity less or equal to 0.1 in one or both eyes each year between 1993 and 2002 were selected, and the causes of legal blindness were noted.

Results: The prevalence of blindness was 0.06% for type 1 and 0.11% for type 2 diabetic patients registered in the database. Between 1993 and 2002 the annual blindness rate decreased significantly for type 1 diabetic patients ($P=0.004$) and increased significantly for type 2 diabetic patients ($P=0.013$). In type 1 diabetic patients with visual loss 66.2% of the cases were due to PDR and 4.0% was due to diabetic maculopathy, whereas in type 2 diabetic patients with visual loss 18.0% of the cases was due to PDR and 18.5% was due to diabetic maculopathy. Another significant cause of legal blindness among type 2 diabetic patients was AMD (21.9%).

Conclusions: During the past ten years screening, diagnosis and treatment of diabetic retinopathy in Århus county has received increased attention. In this period there has been a decreasing incidence of blindness due to diabetic retinopathy secondary to type 1 diabetes, but not to type 2 diabetes. AMD is a significant cause of legal blindness among patients with type 2 diabetes.

■ 1252

The prevalence and causes of visual impairment and blindness among elderly Danes

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Purpose: Purpose: An epidemiological study of prevalence and causes of bilateral visual impairment and blindness in a cohort of aged, urban individuals in Denmark.

Methods: Methods: Data originated from the cross-sectional eye survey conducted during the years 1986-1988. The study population consisted of 1000 randomly selected residents aged 60 to 80 years in Copenhagen, Denmark. The participants underwent an extensive ophthalmologic examination. A participation rate of 96.9% was achieved. Data were reanalyzed for causes of low vision using the following parameters: Visual impairment, defined as a best-corrected visual acuity in the better eye (VA) between 20/40 and 20/200; blindness was defined as VA of 20/200 or worse; low vision was correlated to possible causes.

Results: Results: The age-standardized prevalences of visual impairment and blindness were 2.90% and 1.06%, respectively, and the prevalence rates rose significantly with increasing age. Cataract was the most frequent cause (50%) and age-related macular degeneration (AMD) was the second most frequent cause (34.40%) of visual impairment. Among persons aged 65 to 69 years diabetic retinopathy, AMD and cataract were equally important causes of visual impairment. However, AMD was the leading cause of blindness, accounting for 60% of all blind persons. Glaucoma, myopic macular degeneration, cataract and retinitis pigmentosa were the second most common causes, each accounting for 10% of all bilaterally blind persons.

Conclusions: Conclusion: Visual impairment and blindness were associated with increasing age. Cataract and AMD were the leading causes.

■ 1253

CHARGE association - A condition with severe visual impairment, various etiologic factors and birth defects arising early in pregnancy

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Purpose: CHARGE association (CA) consists of a non-random association of malformations: coloboma (C), heart (H), atresia of choanae (A), retarded growth and/or development (R), genital hypoplasia (G), ear anomalies and/or deafness (E). The causes remain unknown in most cases. We wanted to describe the malformations and functional deficits, find etiologic factors and identify critical time periods for the maldevelopment in patients with CA.

Methods: A multidisciplinary study of 31 Swedes with CA was undertaken. The clinical files were analysed and a clinical evaluation of ophthalmic findings, hearing, balance, speech, oral and swallowing and neuro-psychiatric function was performed.

Results: 28 patients had isolated or combined coloboma of the iris, uvea, retina and optic disc, and 9 patients had additionally microphthalmus. Most anomalies were bilateral. 20 patients had severe visual impairment. Additional abnormalities included ears, cranial nerves, lip-jaw-palate, choanae, teeth, vertebrae, esophagus, trachea, lung, heart, kidney, genitals and intestines, hearing impairment and autism. Assisted fertilization, diabetes, infections, bleedings, drugs, hypertension, prenatal diagnosis, alcohol and smoking during pregnancy were reported among the mothers. Analysis of possible critical time periods suggested that most malformations were produced mainly during week 5 and 6 post fertilization.

Conclusions: Severe eye malformations and visual impairment and a wide variety of birth defects and functional deficits were found in CA. No clear etiologic factors were identified. The birth defects seem to arise early in pregnancy.

■ 1254

Recurrent corneal erosions - a large family with autosomal dominant inheritance

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Purpose: To describe the phenotype and the preliminary heredity pattern of a large family with recurrent corneal erosions as a main symptom and development of corneal keloids later in life.

Methods: A six generation pedigree could be constructed including 171 family members following interviews. A total of 44 were affected of whom 37 are alive. 28 family members were clinically examined, 27 of those were affected. Preliminary molecular genetic studies were made from blood-samples from three patients. Polymorphic markers of telomere and centromere of the TGFBI, GSN, KRT3 and KRT12 were employed.

Results: The disease starts as early as 7 months of age and the majority before 5 years of age. Symptoms of recurrent erosions is dominating with frequent attacks. The intensity of the symptoms subsides somewhat in middle age. About 2/3 of the patients develop corneal keloids at the age of 40 to 50. Six of the affected patients had been grafted. Recurrence in the graft happens within a year but is peripheral. Molecular genetics analysis and phenotype analysis allows the conclusion that the disease has not been described before.

Conclusions: A to our knowledge not previously described cornea dystrophy is presented. The dominating sign and symptoms are recurrent erosions having an early onset. Later, keloids of the cornea may develop sometimes leading to corneal grafting.

■ 1255

Expression of the Olfactomedin Protein Noelin in Ocular Tissues and Cultured Trabecular Meshwork Cells

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Purpose: Noelin is a member of the olfactomedin family, which is mainly expressed in the central nervous system and is involved in neurogenesis. The olfactomedin domain of noelin has a high homology to that of myocilin. Since mutations in the olfactomedin domain of myocilin are causative for some forms of primary open angle glaucoma, we investigated the expression of noelin in human and rat eyes.

Methods: mRNA expression of Noelin in rat ocular tissues at various ages as well as in human ocular tissues was investigated by RT-PCR and northern blot analyses. PCR products were cloned, sequenced and used as probe for northern blot analyses. In addition cultured trabecular meshwork (TM) cells were incubated with TGF- β 1 [1nM/ml] and dexamethasone [10⁻⁷M] for three days.

Results: By northern blot analyses of human retina, a specific band for noelin was observed. In addition, noelin was also detected by RT-PCR in the sclera, cornea and TM. In rat tissues, a strong hybridization signal was seen in the retina. A significant lower expression was observed in the sclera and optic nerve. In the RNA of eyes from 1-day-old rats, a marked signal for noelin was detected with almost similar intensity as in adult animals. Incubation of mouse TM cells with TGF β had no effect on noelin expression. In contrast, dexamethasone decreased the expression of noelin significantly.

Conclusions: In ocular tissues, noelin is expressed in substantial amounts. The tissue specific expression and the onset during development differs from that of myocilin. Nevertheless, noelin might share some functions with myocilin and might be involved in similar pathogenetic processes.

■ 1256

Components of signal transduction chains are present in choroidal neovascular membranes

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Purpose: Age-related macular degeneration (AMD) as the leading cause of blindness in elderly persons has been well documented at the clinical and histological level. But the underlying molecular mechanisms are almost unknown. Therefore, our experiments aim to get insight into the regulatory processes taking place in the retinal pigment epithelium (RPE) and resulting in the formation of choroidal neovascular membranes (CNV membranes).

Methods: Cell culture of normal (ARPE-19) and CNV-associated RPE for finding genes differentially expressed in both cell types by quantitative RT-PCR; immunohistochemistry for verification of the differences and describing the expression pattern.

Results: The ephrin receptor EphA7 is expressed in CNV membranes but not in normal RPE. Its ligand ephrin A1 as well as the interacting protein AF-6 are found both in CNV membranes and in normal RPE. Similarly, expression of the VEGF receptor R1 is enhanced in CNV membranes compared to normal RPE, whereas its ligand VEGF-A is expressed in both cell types.

Conclusions: It seems possible that the enhanced expression of the receptors EphA7 and VEGF-R1 in CNV membranes is completing the respective signal transduction pathway. In functional experiments this may turn out as a switching mechanism towards CNV membrane formation. Moreover, determining a (transcription) factor which activates the EphA7 gene in CNV membranes will offer the possibility to find an upper level regulator for CNV formation. In this way, investigating the regulation of differentially expressed genes from normal and CNV-RPE provides access to the regulatory network of AMD.

■ 1257

Screening mice for retinal degeneration

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Purpose: Retinal degeneration is a major cause for human blindness. Novel mouse model systems reflecting the human disorders are necessary to characterise the functional aspects of retinal degeneration for therapeutic approaches.

Methods: To identify mice with gene mutations causing retinal degeneration we established a high throughput electroretinography (ERG) method which allows a clear identification of affected individuals. The eyes of some mice were examined by histology.

Results: We have established the wild type baseline for several mouse strains; as a positive control for hereditary retinal degeneration we used C3HeB/FeJ mice. During these investigations, we identified a group of mice from strain 129/SvJ (129/SvJblind) showing no ERG response and several animals with lowered b-wave amplitudes in ERG among the CD-1 outbred stock. In the group of 129/SvJblind mice we detected the same retroviral insertion associated with the Pde6brd1 allele that is known to be causative for retinal degeneration in C3H mice. For the hereditary abnormal ERG response in CD-1 mice (Aey 20) this retroviral insertion was excluded. In contrast to the total loss of photoreceptor cells resulting in no ERG response in C3H mice, Aey 20 mice show variability in the ERG response which correlates with their retinal morphology. Aey 20 was established as a mutant line with a suggestive recessive mode of inheritance; mapping of the Aey 20 mutation is in progress. Additionally, we screened 700 offspring of ENU-treated C57BL/6J males. Among these animals we detected 5 animals with putative hereditary retinal degenerations. Genetic confirmation of these variants is in progress.

Conclusions: ERG is an appropriate method to detect novel murine mutations leading to retinal dysfunction. Supported by the German National Genome Research Network (01GR0103)

■ 1261

The Oqual Mark III, a device for the objective, quantified assessment of the optical performance of the human lens in vivo

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Purpose: The assessment of the quality of an eye-lens is frequently made on a qualitative basis, yet a quantitative assessment (Q.A.) may be desirable for example if a decision has to be made as to whether to operate for cataract in the presence of a potential retinal defect. It may also be of value in medico-legal issues. Thus if the Q.A. is consistent with the patient's visual acuity (V.A.), the departure of the latter from normal is most likely to be due to the lens, promising a useful outcome of an operation.

Methods: A device for an objective assessment of the image-forming quality of the in vivo lens was described 10 years ago (Weale RA: The Oqual: A new device for measuring the optical quality of the anterior segment of the human eye. *Exp Eye Res* 1992;55:507-510.), and has been further developed through two stages. The current form (patent pending) involves the projection into the eye of a periodic target with multiple periodicities. This is achieved by means of a simple attachment to existing slit-lamps. The resulting fourth Purkyne image is captured opto-electronically, and suitable software enables one to determine the modulation transfer function of the image. A simple transformation enables one to compare the resultant Q.A. with the patient's V.A.

Results: The method has made it possible to obtain quantitative indices for lens quality, and to enable one to make meaningful comparisons with a patient's V.A.

Conclusions: The new device provides a useful diagnostic facility supplementing and improving existing diagnostic procedures, and may provide an effective adjunct to methods of classifying cataracts.

■ 1262

The colour content of retinal fundus images may reflect the cumulated exposition to hyperglycemia in diabetic patients

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Purpose: The morphological appearance of the ocular fundus is one of the key parameters used for the diagnosis and management of retinal disease. However, optical imperfections in these refractive media result in blurring, low luminance and contrast, and changes in the colour composition of the image which can be seen as an increasingly yellowish appearance with age. The introduction of a method for quantifying this age-related change in colour content may help diagnosing and grading pathological changes in the eye lens secondary to ocular and systemic disease such as diabetes mellitus.

Methods: 102 digitized fundus images from 102 normal persons (mean age = 50.4, range 7.0 - 94.3 years) was used to build a model for estimating the age from the colour content of the images. The method was applied to fundus photographs from five diabetic patients.

Results: Estimation of age from the fundus images could be done with a standard deviation of 8.2 years. This variation could be reduced considerably by analysis of repeated photographs from the same examination. In the diabetic patients the age estimated with method was significantly higher than the true age.

Conclusions: It is concluded, that the colour content of fundus images can be used to predict the age of photographed individuals, and that deviations in the colour content from what would be expected from age might be an indication of external causes of increased light absorption in the lens than age, for example the cumulated exposition to hyperglycemia in diabetic patients.

■ 1263

The distribution of cataract types in diabetics and nondiabetics, a densitometric study

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Purpose: Diabetes mellitus type 2 represents a higher risk in cataract formation. We wanted to know which types of cataract appear in diabetics.

Methods: 46 diabetic (group I) and 83 nondiabetic patients (group II) who had to undergo a cataract operation were screened of exclusion and inclusion criterions. After that the photographic documentation of the opaque lens followed using the Topcon SL-45 (Scheimpflug). Densitometric analyses of each image were also performed.

Results: We could not find out significant differences relating to the metabolic disorder. Nevertheless it is remarkable that there is a higher appearance of percentage in the opacity of the deeper anterior cortex (group I = 8,7%; group II = 3,6%) as well as in the anterior and posterior cortex (group I = 13,0%; group II = 8,4%) comparing diabetics with nondiabetics.

Conclusions: Diabetics exposed a higher appearance of percentage in the opacity in the area of cortex than nondiabetics without detecting a significant. Therewith we could show positive associations between the existence of diabetes mellitus type II and a higher risk of opacities of the cortex.

■ 1264

Changes of lens epithelial cell parameters in different transparency of lens layers in diabetics and nondiabetics

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Purpose: We wanted to know if morphological changes in the epithelium of cataract lenses are different in dependence of the density in variant lens layers comparing diabetics with nondiabetics.

Methods: We examined 97 patients with and without diabetes mellitus type 2 (70 women, 27 men) who had to undergo a lens extraction because of cataract or in the case of vitrectomy. In accordance with Scheimpflug photographs (Topcon SL-45) of the anterior eye segment and linear microdensitometric analysis, the 10 different lens layers were identified. The central fragments of the lens capsules were analysed by light microscopy for different cell parameters.

Results: Concerning the total population the epithelium cell density decreases while the density of the anterior capsule rises ($n = 97, p = 0,042$) without any additional differences in sex or metabolic status. With loss of transparency in the anterior zone of disjunction we found an increase of the mean cell area within the cataract population of women ($n = 40, p = 0,019$) while within the group of noncataracts the mean nuclear area ($n = 7, p = 0,017$) and the mean nuclear volume ($n = 7, p = 0,013$) rises. Looking at the morphological alterations when the opacity increases in the anterior superficial cortex there was a lower nucleus-plasma-ratio for female cataract patients ($n = 51, p = 0,027$).

Conclusions: The changes of the lens epithelium by loss of transparency of the outlying layers are remarkable. In general we can predicate that a reduction in cell density by an increase in opacity walk along with a greater cell area that is represented in a lower nucleus-plasma-ratio. A comparison between diabetics and nondiabetics did not result in significant differences.

■ 1265

Optical formula for prediction of postoperative outcome in accommodative IOL implantation

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Purpose: Accommodative IOLs are used with increasing frequency in cataract surgery. However, postoperative accommodation amplitudes are sometimes not satisfying. Accommodation in accommodative IOLs is based on an anterior shift of the IOL due to contraction in the corpus ciliare. In addition to postoperative fibrosis, operation related problems and lack of anterior shift of the IOL, optical variables are important and predictable factors in the selection of candidates for accommodative IOL implantation.

Methods: Thin lens approximation optics were used to obtain a formula containing several variables: axial length (AXL), mean keratometry (Km), anterior chamber depth (ACD) and presumed anterior shift of the IOL (d(acd)). The influence of these different variables was separately analysed.

Results: As expected, accommodative amplitude (AA) was negatively influenced by longer AXL, higher Km and shorter ACD. AXL seems to be the most important factor, with an AA varying from 2.5 D to less than 0.5 D for a Km of 42 and AXL of 20 mm to 28 mm respectively. d(acd) is also a major factor influencing AA. Km and ACD influenced the AA less. These findings confirm results of other authors.

Conclusions: The obtained theoretical values and formula could guide selection of possible candidates for accommodative lens implantation. The results corresponded to the results of a preliminary clinical trial of accommodative lens implantation.

■ 1271

So-called "classic" and "predominantly classic" choroidal neovascularization

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Fluorescein angiography is still the major tool to identify subretinal new vessels in age related macular degeneration and most of the clinical studies on treatment are based on this technique. Well defined or so-called "classic" CNV is characterised by a net of vessels that are clearly to be seen, during the initial sequences of the fluorescein angiography. These vessels leak profusely in the late phases. Before the advent of ICG angiography the cases without identifiable CNV but with irregular late fluorescence and where the presence of a serous detachment or of haemorrhages strongly suggested the presence of subretinal new vessels were considered as eyes with "occult" CNV. ICG angiography thus may transform "occult" into "identifiable" CNV. The question is if this differentiation is still needed. The difference in behaviour corresponds to different histological characteristics as shown by Lafaut et al. (2000). CNV in ARMD probably starts as sub-RPE fibrovascular tissue and corresponds at that moment to "occult" membranes. It becomes "classic" or "mixed" when it grows through the RPE and proliferates into the subretinal space. In young individuals choroidal new vessels, mainly the consequence of inflammatory processes, grow in the subretinal space (type 2 of Gass). They are thus always of the classic type. The prognosis of surgical removal is far better as the RPE is still healthy and engulfs partially the fibrovascular tissue. It is thus possible to remove the membrane without tearing the RPE. In ARMD subretinal new vessels are usually of type 1 of Gass (beneath the RPE). When they transform into type 2 of Gass, they remain adherent to the RPE and their surgical removal will cause an extensive RPE defect. As well longterm follow-up as clinical trials suggest that it still makes sense to differentiate 'classical' from "occult" CNV.

■ 1272

So called "occult" and predominantly occult CNV

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Occult choroidal new vessels (OCNV) have been identified on the basis of FA, a few years after the so called "classic" CNV. Many years later, two different types of Occult CNV were recognized with or without association to Vascularized Pigment Epithelium Detachment (PED). Most of the patients with OCNV could not benefit from thermal laser treatment except for extrafoveal "occult in the notch". Photodynamic therapy results have led to recognize new stratification of patients: (1) Occult only; (2) Predominantly Occult, and (3) Minimally Occult (or classic predominant). The different results of PDT management are still incompletely explained but are probably related to the nature of vascular proliferation (density of the network, blood flow, RPE cells and/or fibrous tissue proliferation ...). PDT with verteporfin is indicated for Occult only and for Minimally Occult. Results are statistically significant (after 2 years) if patients present with recent symptoms or with documented extension and/or hemorrhages. Results are (even more) significant in Minimally Occult (or Classic Predominant). Results are still in evaluation for Occult predominant (or minimally classic - VIM Study). Controlled clinical studies are ongoing about management with anti-angiogenic drugs isolated or associated with PDT.

From diagnosis to treatment of ARMD

■ 1273

Vascularized pigment epithelium detachment revisited

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Purpose: Vascularized pigment detachment is a specific feature of the European classification of AMD. Its occurrence has been observed in privileged cases as overlying occult choroidal new vessels. Since the dissemination of ICG angiography vascularized PED has been subdivided into at least three major clinical pictures: polypoidal vasculopathy, chorioretinal anastomosis and choroidal new vessels in a PED. Chorioretinal anastomosis are still in the center of passionate controversies: is the origin in the retinal or in the choroidal circulation? What is the direction of the blood flow in these anastomotic vessel? CNV hidden on FA, associated to a PED reveal on ICG either as a network on the border or within the PED, or as a hot spot within a plaque. The signification and the prognosis of these different features are not elucidated today.

Methods: A limited prospective study was performed in the department in order to obtain an identification on the less damaging and/or most successful treatment approach, as none is available at present. Specifically, chorioretinal anastomosis in eyes with AMD features were randomly observed, treated with photodynamic therapy or with laser photocoagulation based on ICG-SLO findings or with successively the two treatments.

Results: Ten eyes were included in each group with a recent onset of visual symptoms, minimal follow-up had to be of 6 months to be herein analyzed. Early trend favored laser photocoagulation without photodynamic therapy despite the possible occurrence of RPE tears in both cases. Best results seem to be achieved when the PED is less than 1/2 disc diameter.

Conclusions: The conjunction of precise classification of vascularized PED and the increasing number of treatment modalities might result in major improvements.

■ 1274

Retinal angiomatous proliferation (RAP) and retinal-choroidal anastomosis (RCA)

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Purpose: To assess the connection between intraretinal angiomatous proliferation (RAP) and choroidal circulation in RCA.

Methods: 124 eyes of 100 patients (mean age: 79 +/-5; 24% M; 76% F) affected by RCA underwent fluorescein and indocyanine green angiography (FA, ICGA); 97 eyes (78.2%) have been studied by dynamic indocyanine green angiography (Scanning Laser Ophthalmoscope - SLO). Furthermore, 64 eyes (51.6%) have been examined by Optical Coherence Tomography (OCT).

Results: In 24% of the cases studied, RAP was bilateral. Drusen were present in 78% of the cases. Serous Neuroepithelial Detachment (SND) were observed in 91% of the cases, while Pigment Epithelium Detachment (PED) represented 47% of the cases. Only in few cases FAG and ICGA revealed angiographic retinal images that might be referred to the presence of an anastomosis between RAP and choroidal circulation.

Conclusions: Results suggest that intraretinal angiomatous proliferation is not usually connected with choroidal circulation, also at the advanced stage. This confirms the existence of two clinical types of exudative age-related macular degeneration (ARMD). In the first one the vascular complex has an intraretinal origin, while in the second one it has a choroidal origin.

■ 1275

Latest update on therapeutic advances in AMD

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Several treatment modalities have been explored in managing AMD from laser/PDT, to inhibiting vascular growth factors, choriocapillary inflammation, or use of dietary supplements. Clinical trial results are available for a number of these approaches and will be discussed. While each has shown promise, it is likely that adequate treatment response will depend on a combination of approaches. A forerunner of this approach is the combination of PDT with intravitreal triamcinolone. While current work has mainly concentrated on the treatment of predominantly classic or occult membranes, future work will have to define the exact time point at which each modality is most suited. Present and future prospects will be discussed.

■ 1301

Anatomy of extraocular muscle pulleys

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Purpose: Constraint of rectus muscles by pulleys but through which muscles may slide when contracting is suggested to explain the absence of sideslip during ocular rotations. The anatomy of pulleys is described in this report and their proposed function critically reviewed.

Methods: Orbital segments containing single rectus muscles with attachments including periosteum intact were prepared from human dissection room cadavers and similar preparations of medial and lateral recti from perfuse-fixed cynomolgus monkeys. Tissues were embedded in resin, examined histologically in longitudinal section and montages were produced from one micron thick sections.

Results: Equatorial thickening of muscle sheaths is unequal on the global and orbital surfaces (mean ratio 1:6 for medial recti and 1:4 for other recti in both species). Whereas global aspect thickening is often slight, it is regularly substantial and extends further forward beyond the muscle insertions on the orbital aspect, forming a dense plate within the intermuscular membrane. The plate varies between recti, the medial rectus being much the thickest containing substantial numbers of smooth muscle fibres. In monkeys it also contains a cartilage disc and a nictitans gland. Smooth muscle is sparse or absent from the plates of other recti. The inferior rectus plate is continuous with the suspensory ligament of Lockwood and the embedded inferior tarsal smooth muscle. Numerous fine fibrous strands attach the plates of the horizontal but not the vertical recti to the orbital wall (periosteum).

Conclusions: Marked asymmetry, variable content and orbital attachments of the thickened muscle sheaths opposite the equator are inconsistent with the notion that they constitute rings through which the muscles slide.

■ 1302

New Aspects on Choroidal Non-Vascular Smooth Muscle Cells in the Human Eye

MAY CA

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Purpose: To study the development and innervation of non-vascular smooth muscle cells (NVSMC) in the human eye.

Methods: Choroidal whole mounts and serial sections of human donor eyes, ranging between the 13th week of gestation and 95 years, were stained with antibodies against smooth muscle alpha-actin, myosin, neuronal nitric oxide synthase, vasoactive intestinal peptide, tyrosin hydroxylase, vesicular monoaminergic transporter 2, vesicular acetylcholine transporter (VAcHT), neuropeptide Y (NPY), substance P, calcitonin gene related peptide, calretinin, galanin, and synaptophysin.

Results: 1. Smooth muscle alpha actin positive cells not related to vessels (NVSMC) were first observed in the 20th week of gestation as single cells, forming a substantial network only after birth. In the first months the cells were more oriented parallel to the vessels and gradually changed to the three-dimensional network in adult eyes. 2. About 20% of the NVSMC showed neuronal contact only to NPY and VAcHT positive nerve fibers.

Conclusions: The development and formation of NVSMC in human eyes occurs mainly after birth. The type of innervation in our study is different from what claimed in the literature.

■ 1303

Bves, a novel adhesion molecule, is expressed in the developing and adult eye

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Purpose: Bves (blood vessel epicardial substance), is a novel adhesion molecule cloned from a subtracted library enriched for heart-specific gene products. However, our immunofluorescence analysis during embryonic development has revealed Bves to be expressed in a variety of epithelial derived tissues including the eye. This study is initiated to determine the expression pattern of Bves in the embryonic and adult eye in order to gain insight into the role of Bves in the eye.

Methods: Immunohistologic studies are carried out on chicken embryos at various developmental stages, staged Black Swiss mice, and adult human corneas using polyclonal antibodies for Bves. Immunohistologic studies for Bves expression are also performed using an in-vitro model of corneal healing.

Results: In the chick embryos, Bves is first expressed in the ependyma of the diencephalon, which evaginates laterally. Bves is also expressed on the surface ectoderm cells of the lens and cornea placodes. Interestingly, the expression of Bves is retained in the adult retina, lens, and cornea. In the cell culture model of corneal wound healing, confluent corneal epithelial cells demonstrate Bves at the cell surface. When the confluent epithelial sheet is wounded, Bves is removed from the surface of cells at the wound edge where cell-cell contact is lost. However, as cells migrate and proliferate to fill in the wound, Bves is again expressed at the cell surface.

Conclusions: This study demonstrates the expression of Bves during the early phases of eye development and in the adult eye. It appears that Bves plays a role in the movement of epithelia. Taken together, these results suggests roles for Bves in the development, maintenance, and/or regeneration of epithelial structures of the eye.

■ 1304

Human corneal cellular response to the synthetic stromal matrix replacements

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Purpose: Purpose: Polyurethane-based biomaterials have been used as heart valves. Our aim is to determine the feasibility of using such materials as potential corneal stromal replacements.

Methods: Material and Methods: Composite collagen-bound polyurethane corneal stromas of 3 different formulations were moulded into corneal stromas with different degrees of rigidity vs flexibility. These were classified as 1) very flexible (but also soft) 2) very rigid; 3) a combination of flexible with some rigidity. These polymers were sutured onto human donor rims and then organ cultured. Cell migration from the human corneal rims into the matrix was examined.

Results: Results: Within 3-5 days, half the polymer surface was covered by epithelial cells that have migrated from the human corneal rims. After air-lifting the cultures at day 5, the epithelial cells stratified but did not cover entire surface. Morphology of these epithelial cells was comparable to those of control intact human donor corneas. Stromal cells migrated into corneas that were soft but not the two more rigid ones. The migrated endothelial cells retained a monolayer configuration but underwent morphological changes. Irregular cell sizes and blebs were observed.

Conclusions: Conclusion: Polyurethane-based materials showed some biocompatibility. However, surface modification is most likely required to optimize cell migration and survival on and within these matrices. The in-growth of stromal cells occurred only in the softer matrices, correlating with previous reports from keratoprosthesis studies that the packing and pore sizes of the materials are important for anchorage into host tissues.

■ 1305

Extraglandular lacrimal gland tissue in the eye region. Comparison of clinical and histopathological diagnoses.

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Purpose: To establish the distribution of histologically verified lacrimal gland tissue (LGT) in lesions excised from extraglandular sites and to compare the histological and clinical diagnoses.

Methods: Retrieval of cases from the files of the Eye Pathology Institute, Copenhagen, Denmark in the years 1953-2003. Sections containing lacrimal gland tissue from extraglandular sites were examined and data on clinical diagnosis and location were compared.

Results: 150 lesions were collected. Fifty-seven (38%) lesions had ectopic LGT. Of these 31 (21%) were in complex choristoma and 26 (17%) were solitary ectopic LGT. Accessory LGT accounted for 33 (22%) specimens. Prolapsed lacrimal gland was found in 60 (40%) cases. The majority of the lesions (50%) were located in the temporal bulbar conjunctiva. The referral clinical diagnosis covered a wide spectrum of pathological lesions. The most common clinical diagnoses were non-specific tumour (32%), non-specific cyst (16%) and dermoid lesions (12%). Only two cases were pre-operatively diagnosed as ectopic LGT. Out of the 60 cases with histologically demonstrated prolapsed lacrimal gland, only three cases had this diagnosis clinically.

Conclusions: Lacrimal gland prolaps was the most common finding. The prime location of LGT was in the temporal bulbar conjunctiva. The referral clinical diagnosis was often wrong. Surgeons should keep in mind the possibility of prolapsed lacrimal gland or ectopic LGT.

■ 1306

Local Production of Secretory IgA in All Zones of the Normal Human Conjunctiva

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Purpose: Production of secretory IgA is a major mucosal immune defence mechanism. At the ocular surface its source is usually located exclusively in the lacrimal gland (LG). We found a large body of evidence based on histological, immunohistological and ultrastructural data that shows proteins of IgA and its transporter secretory component (SC) also in the conjunctiva and lacrimal drainage system and now aimed to verify the local production at the mRNA level.

Methods: Specimens were obtained from different regions of the conjunctiva and from the LG of 10 eyes from five macroscopically normal body donors. Primers for IgA and SC mRNA were constructed according to published sequence data and reverse transcriptase (RT-) PCR was performed with standard reagents.

Results: All normal tissues of the peritarsal orbital conjunctiva with a high density of plasma cells showed a single broad band of RT-PCR products of the expected size for the chosen sequences for IgA and SC. To exclude the possible involvement of accessory LG tissue we also analysed upper and lower bulbar conjunctiva with the same results. This was validated by using LG tissue as a positive control and muscle tissue as a negative control. The latter showed the absence of RT-PCR products for IgA and SC but presence of GAPDH (a house-keeping enzyme).

Conclusions: Previous findings of IgA and SC proteins are verified here by the respective mRNA. Since this occurred in all regions, it excludes a possible contamination with accessory LG tissue but verifies a local production of IgA and SC in the normal human conjunctiva. It further underlines that the LG, conjunctiva and lacrimal drainage system form an "Eye-Associated Lymphoid Tissue" (EALT) as a functional unit for ocular surface immune defence.

■ 1311

Endocannabinoids and neuroprotection

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Purpose: The endocannabinoid system is a valuable target for drug discovery, because it is involved in the regulation of many cellular and physiological functions. The endocannabinoid system consists of the endogenous lipids anandamide and 2-arachidonoylglycerol and the cannabinoid CB1 and CB2 receptors as well as the proteins for their inactivation. The endocannabinoid system has also been found in the retina. It is thought that (endo)cannabinoid-based drugs may potentially be useful to reduce the effects of neurodegeneration. In this presentation we review recent developments in the neuroprotection induced by endocannabinoid system.

Results: Exogenous and endogenous cannabinoids have been shown to exert neuroprotection in a variety of in vitro and in vivo models of neuronal injury via different mechanisms, such as prevention of excitotoxicity by cannabinoid CB1-mediated inhibition of glutamatergic transmission, reduction of calcium influx and subsequent inhibition of deleterious cascades, anti-oxidant activity, and inhibition of TNF- α formation. We will show that the release of endogenous endocannabinoids during neuronal injury constitute a protective response.

Conclusions: If this endogenous neuroprotective function of the endocannabinoid system can be extended to the eye, it might form an interesting drug target to reduce neuronal damage of the optic nerve.

■ 1312

Neuroprotection against retinal ischemia, axonal transport studies

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Purpose: To study neuroprotective effects of brimonidine (BMD) on axonal transport alterations induced by ischemia in adult rat retinal ganglion cells (RGC).

Methods: Left ophthalmic vessels (SLOV) were ligated for 90 min. Rats received in left eyes 1 hour before ischemia, two 5 μ l drops of saline or 0.5% BMD. Retrograde transport was investigated by comparing 1 or 2 weeks after ischemia (a.i.) RGCs labeled with fluorogold applied to both superior colliculi either 1 week before, or 1 h or 1 week a.i. Orthograde transport was investigated by comparing 2 months a.i. retinal afferents to the superior colliculus anterogradely labeled with Cholera Toxin B subunit (CTB) applied to the ischemic eyes.

Results: In vehicle animals, 15% of the RGCs that survived 7 or 14 days a. i. had their retrograde transport impaired. In the BMD-treated groups of animals, RGC densities amounted to 90% of the RGC population 7 or 14 days a. i. and were comparable to those obtained in contralateral non-ischemic retinas. The density of CTB-labeled profiles in the SC of the vehicle rats represented less than 50% the area occupied by CTB-labeled profiles in unlesioned rats, in BMD-treated rats CTB-immunoreactivity occupied an area of 86% of that observed in unlesioned rats.

Conclusions: Retinal ischemia causes RGC loss, alters retrograde axonal transport in a proportion of surviving RGCs and results in massive loss of retino-tectal afferents. BMD rescues RGCs from ischemia-induced cell death, preserves retrograde axonal transport in surviving RGCs and protects against ischemia-induced degeneration of the retinotectal projection. Research Support: F.Séneca PI82/00540/FS/01 and Allergan Inc. unrestricted grant.

New developments in Neuroprotection

■ 1313

Apoptosis and neuroprotection

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Purpose: Glaucomatous retinal changes are increased five fold in Alzheimer's (AD) and Parkinson's disease (PD). Loss of noradrenergic locus coeruleus (LC) neurons occurs early AD and PD and results in decreased forebrain noradrenaline (NA) with retention of alpha-2 adrenergic receptors. We sought the mechanisms that coordinate neuronal loss in AD, PD and glaucoma.

Methods: Laser scanning microscopy was used with immunocytochemistry and mitochondrial membrane potential measurements together with protein chemistry in cultured neurons, rat retinal organotypic cultures and rat retinas after optic nerve (ON) crush.

Results: We have found evidence that NA axons terminate on RGC alpha-2 receptors via the ON. NA depletion increases AD-like cortical neuronal loss and RGC layer cell loss after optic nerve (ON) crush. We found that brimonidine, an alpha-2 receptor agonist, reduces apoptosis in cultured neuronal cells after a variety of insults. Brimonidine anti-apoptosis was also found for rat RGC layer cells after ON crush and in retinal organotypic cultures after BDNF withdrawal or selective mitochondrial complex I inhibition mimicking Leber's Hereditary Optic Neuropathy. Alpha-2 receptor agonists maintain mitochondrial membrane impermeability and thereby prevent the release of apoptosis signaling factors. Alpha-2 receptor activation opposes decreases in BCL-2/BCL-XL and increases in mitochondrial BAX by inducing anti-apoptosis signaling provided by phosphorylated protein kinase B (Akt).

Conclusions: We propose that alpha-2 receptor agonists compensate for lost NA, which normally provides neuronal anti-apoptosis. Accordingly, in PD and AD, and possibly glaucoma, in which NA is depleted, selective alpha-2 receptor agonists facilitate neuronal survival and should slow the progression of the diseases.

■ 1314

Alpha2agonists in neuroprotection

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Purpose: Laboratory studies show that brimonidine, a selective alpha-2 adrenergic agonist, can enhance the survival of retinal ganglion cells (RGCs) and other neurons in the retina and optic nerve after stress and injury. These studies were done to determine how alpha-2 agonists are neuroprotective.

Methods: Neuronally differentiated PC-12 cells in culture were used to identify pathways that enhance neuronal survival. PC-12 cells were initiated into apoptosis by NGF and serum withdrawal. The ocular hypertensive rat was used to test whether the alpha-2 receptor pathway activated by brimonidine could protect RGCs stressed with elevated IOP.

Results: Addition of brimonidine (10⁻⁵ to 10⁻¹¹M) to NGF-deprived PC-12 cells enhanced survival 55% to 83%; an alpha-2 receptor antagonist blocked this effect. In the ocular hypertensive rat (OHTR), IOP was elevated =2-fold. After 3 weeks, OHTRs treated with vehicle (control) lost 35% of their RGCs. OHTRs receiving constant brimonidine treatment (1mg/kg/day by osmotic pump) lost only 15% of their RGCs.

Conclusions: Activation of alpha-2 receptors by brimonidine raises the resistance of neurons to stress and injury by enhancing intrinsic survival pathway(s), demonstrated by maintenance of mitochondrial pore closure and Bcl-2 protein levels. These data suggest possible mechanism(s) by which brimonidine maintains survival of RGCs in eyes with chronically elevated IOP.

■ 1315

Clinical implication of neuroprotection

GANDOLFIS

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■ 1341

Ophthalmodynamometric assessment of the central retinal vein pressure in patients with increased cerebrospinal fluid pressure or increased orbital pressure

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Purpose: Using a new Goldmann lens associated ophthalmodynamometric device, it was the purpose of the present study was to determine the central retinal vein collapse pressure in patients with increased cerebrospinal fluid pressure or increased orbital pressure.

Methods: The clinical interventional study included two female patients. A 13-years old patient complained of head-ache and vomiting and presented with the diagnosis of increased intracranial pressure. A 45-years old patient presented with increasing exophthalmos with the diagnosis of endocrine orbitopathy. For both patients, a Goldmann contact lens fitted with a pressure sensor was put onto the cornea. Pressure was asserted onto the globe by pressing the contact lens, and the pressure value was noted at the time when the central retinal vein started pulsating. The measurements of both patients were compared with the measurements obtained in ten normal persons.

Results: For the patient with the diagnosis of cranial hypertension, the central retinal vein collapse pressure was higher than 90 mm Hg. A direct measurement of the cerebrospinal fluid pressure showed a value of higher than 100 relative units. For the patient presenting with the diagnosis of endocrine orbitopathy, central retinal vein collapse pressure was 40 mm Hg, what was markedly higher than the values obtained in the 10 normal subjects and which ranged between 4 mm Hg and 20 mm Hg.

Conclusions: As measured by a new ophthalmodynamometer with direct visualization of the central retinal vessels during the examination, the central retinal vein collapse pressure measurements were markedly higher in patients with increased cerebrospinal fluid pressure and increased orbital pressure than in normal subjects. The results suggest that this new type of ophthalmodynamometry may possibly be helpful for the examination of patients with increased brain pressure and orbital pressure.

■ 1342

Cavernous sinus thrombosis secondary to MRSA septicaemia

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Purpose: To report the first case of cavernous sinus thrombosis due to Methicillin Resistant Staphylococcus Aureus (MRSA) infection. Also, to emphasise that MRSA infection may be acquired in the community.

Methods: Single observational case report.

Results: We present the case of a 35-year-old female IV drug user presenting to our hospital with heroin overdose and septicaemia. Following initial resuscitation she regained consciousness but remained confused with GCS of 8/15. She was febrile with temperature of 39.2°C. There was marked bilateral proptosis, severe eyelid swelling with erythema and conjunctival chemosis. Pupils were pinpoint and did not react to light. Ocular movements could not be assessed due to the patient's agitated state but appeared limited. Cavernous sinus thrombosis was suspected and the patient was intubated for an urgent CT scan of the brain and orbits, which confirmed the diagnosis of CST. High dose intravenous ceftazadime, metronidazole and flucloxacillin were commenced. Blood cultures were positive for Methicillin Resistant Staphylococcus Aureus (MRSA) and an antibiotic regimen of high dose vancomycin and rifampicin was commenced on the Intensive Therapy Unit. The patient was also anticoagulated. She had not had any prior hospital contact. The source of infection was not identified but the subsequent CT scan indicated multiple intracerebral abscesses and multiple cavitating abscesses of the lungs with a large pleural effusion. The patient died 9 days after admission to hospital.

Conclusions: MRSA infection can be acquired in the community and should be born in mind by ophthalmologists when dealing with infections. We describe the first case, to our knowledge, of cavernous sinus thrombosis secondary to MRSA sepsis.

■ 1343

Rehabilitation of patients with homonymous hemianopia

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Purpose: Patients with homonymous hemianopia may detect flicker in the hemianopic visual field. During flicker rehabilitation, the sensitivities in the trained areas of the hemianopic visual field reached those in the normal half field (Hyvärinen, Raninen & Näsänen, 2003). After flicker sensitivity became symmetric, magnetic evoked fields (MEF) showed activation in the temporal and occipital brain regions contralateral to affected hemifield (Vanni, Raninen, Näsänen, Tanskanen & Hyvärinen, 2001). Our aim was to study psychophysical and MEF responses during rehabilitation of two men with homonymous hemianopia.

Methods: Two men with homonymous hemianopia participated in experiments. They had a session twice a week. Flicker was used as stimulus at 10 and 30 degrees eccentricity along the horizontal axis. Seven different frequencies between 1-35 Hz were used. MEFs for checkerboard stimuli were recorded before training and every two months during it.

Results: Before training there was a 1.5 log unit difference between the sensitivities of the two hemifields. After 5.5 months of training, flicker sensitivity of the trained areas of both subjects became equal to that in the corresponding points of the normal visual field. In addition, after rehabilitation MEFs emerged in the ipsilateral lateral occipital (Subject 1) and contralateral temporal (Subject 2) areas for pattern reversal stimulation of the affected hemifield.

Conclusions: Visual function in homonymous hemianopic visual field may improve as a result of regularly repeated training using luminance flicker detection task. Improved function in psychophysical tasks was reflected in MEFs where new activity was recorded during stimulation of the trained areas of the visual field.

■ 1344

Stimulus influence on the development of smooth pursuit eye movements in infants

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Purpose: Visual pursuit is an indicator of maturation of the visual system. The aim of our study was to investigate visual pursuit in infants using stimuli of different sizes, structures and velocities.

Methods: Eye movements of 34 healthy, full-term infants between 3 and 18 months of age were recorded by infrared photo-oculography. For stimulation of visual pursuit, two targets were used: a black "dot" of 1.2 deg of visual angle and a square of 4.7 deg with a black and white checkerboard pattern. We also compared a cognitive (face) and a non-cognitive stimulus. The stimuli moved horizontally at velocities of 7.5, 15 and 30 deg/sec. We evaluated the longest smooth pursuit, saccadic frequency, velocity gain, the average velocity of smooth pursuit, and the percentage of attention time during each sequence.

Results: The longest duration of smooth pursuit increased with age. It was significantly lower at the highest stimulus velocity. Saccadic frequency decreased with age. It was significantly higher with the larger stimulus and increased with stimulus velocity. Gain decreased with stimulus velocity. The average velocity was neither influenced by the child's age nor the stimulus size. The attention time was significantly higher with the larger stimulus and at a medium stimulus velocity of 15 deg/sec. There was no significant difference between recordings with the cognitive and the non-cognitive stimulus.

Conclusions: These results show that the quality of smooth pursuit improves significantly within the first 18 months of age and is highly dependent on stimulus size and velocity, whereas, the recognition of the target does not seem to have a major influence on visual pursuit quality.

■ 1345

Pupil blown by a puffer...

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Purpose: To illustrate a novel case of ipratropium bromide spacer inhaled pupil dilation and present a review of published reports, pharmacokinetic and pharmacodynamic mechanisms involved.

Methods: A photographic presentation of two CT and MRI investigated episodes of spontaneously resolving pupil dilation after spacer inhaled ipratropium bromide. These episodes were reviewed in a basic meta-analysis of all published cases of ipratropium related pupil dilation. Identified mechanisms are discussed.

Results: All published cases of ipratropium bromide pupil dilation occurred with the combined simultaneous use of salbutamol. Fisher exact test (two tailed) $p < 0.005$. Previous reported administration have been nebulised and not spacer inhaled routes.

Conclusions: Ipratropium bromide used in combination with salbutamol can cause pupil dilation by either nebuliser or spacer inhaled routes.

■ 1346

Disturbed eye and eyelid movements in hemifacial paralysis patients

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Purpose: Eye and eyelid movements were recorded during different types of blinking in hemi-facial paralysis patients (Bell's palsy). Bell's palsy is a predominant unilateral facial paralysis, the aetiology is largely unknown although there are indications for a viral infection. Patients with a complete knock-out of the facial nerve, not inducible in the first two weeks of the affection, were selected for this study and followed during their blink recovery.

Methods: Patients were recruited to participate in this study. From the onset of the affection, patients were clinically observed and the direct magnetic search coil technique simultaneously with OO-EMG (lower eyelids) were used in order to register eye position, eyelid movements and orbicularis oculi (OO) muscle activity. Kinematics and neurophysiological aspects of lid saccade, spontaneous, voluntary blinking and different types of reflex blinking were registered in a weak electromagnetic field (Rommel set up). Registrations of eyelid movements and OO-EMG were made over a period of one year with 6 weeks intervals. Eye movements were recorded every 3 months.

Results: The first OO-EMG activity was observed in these patients after 4 months, caused by re-innervation of OO muscle by fibres of the facial nerve. After 9 months, OO-EMG recorded on the affected side was larger in all types of blinking than on the healthy side, resulting in a longer blinking duration. Although OO muscle activity increased the downward eyelid movement remained reduced to 30%. Velocity profiles of down and upward lid movements remain reduced at the affected side, even after one year. During different types of blinking the eyes make a characteristic nasal-downward and lateral upward movement. From the onset of the affection eye movements are aberrant during blinking, on the healthy side the eye showed normal kinematic characteristics, whilst on the affected side the eye moves abnormal. In almost all cases the horizontal component of the "abnormal" eye movement is larger than normal and strongly dominant. Eye movements elicited after an acoustic click of 100dB/5ms showed on the affected side less horizontal dominance, whilst the largest horizontal aberration was measured during forceful voluntary blinking. The recovery pattern is the fastest of eye movements elicited during reflex blinking, they showed the less deviation in their kinematics. Still after one year the Bell's Palsy patients showed blink recovery at the affected side by improvement of eyelid movements and a more effective OO muscle fibre activity.

■ 1351

Animal models of retinal vasculitis

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The posterior segment of the eye is endowed with a rich vasculature, which either primarily or secondarily in association with the wider spectrum and phenotype of posterior segment inflammatory conditions, is involved in the pathogenesis of uveitis and retinal vasculitis. Classically, experimental models of posterior segment inflammation, such as experimental autoimmune uveoretinitis (EAU), mimic posterior uveitis of which one component is retinal vasculitis. Our increased understanding of the underlying immunobiology of uveitis and our present ability to image in real-time the retinal vasculature during EAU has enlightened us in the pathogenesis of retinal vasculitis and uveitis. This includes the intimate role of retinal vascular endothelium and perivascular space and resident macrophage and microglial cells during inflammatory responses, where strong comparisons can be made to CNS and inflammatory responses therein.

■ 1352

Classification of retinal vasculitis and its differential diagnosis

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Retinal vasculitis is a sight-threatening inflammatory eye disease that involves the retinal vessels. Inflammation of the retinal vasculature may occur as a manifestation of an infectious disease, as part of a systemic inflammatory disease, as a complication of neoplastic disorders, or as a part of an isolated retinal disease, and can result in severe and permanent visual loss. The search for a cause in patients with retinal vasculitis requires appropriate systemic evaluation and testing. Discrimination between the infectious or noninfectious etiology of retinal vasculitis is important because the treatment is different.

■ 1353

Idiopathic retinal vasculitis: appraisal and new management principles

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Purpose: This study was undertaken to determine whether visual prognosis of retinal vasculitis associated with tuberculoprotein hypersensitivity (Eales disease) could be improved by appropriate medical and surgical treatment.

Methods: The authors retrospectively studied 30 patients (46 eyes) who were treated from 1992 to 2001. Recorded data included patient age, sex, race, medical history, medications, results of the ophthalmologic examination, results of diagnostic laboratory evaluation, and details of systemic and surgical treatments. The mean follow-up was 10.6 months.

Results: Nineteen patients (23 eyes) who presented with active periphlebitis received systemic steroids and antituberculous therapy. Extensive full panretinal photocoagulation was performed in 21 eyes that presented with new vessel formation and peripheral capillary closure with or without vitreous hemorrhage. Vitrectomy and endolaser panretinal photocoagulation was necessary in 15 eyes, for severe nonclearing vitreous hemorrhage in 11 eyes, and vitreous hemorrhage with traction retinal detachment in 4 eyes. Complete regression of the disease was achieved in all eyes. Vitrectomy resulted in a significant visual improvement with 14 of the 15 eyes (93.3%) achieving = 20/200 visual acuity. Overall, the distribution of visual acuities among eyes improved from presentation to final follow-up, with 36.4% of eyes having 20/40 or better acuity at presentation as compared with 63.6% of eyes by final follow-up.

Conclusions: These results suggest that aggressive treatment of Eales' disease with systemic steroids and antituberculous therapy, full panretinal photocoagulation and early vitrectomy, when necessary, may result in improving the anatomic and visual outcome.

■ 1354

Infectious vasculitis: update on new clinical entities

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The etiological work-up of retinal vasculitis remains a major step before the initiation of a therapeutic strategy. Infectious agents play an important role in the pathogenesis of retinal vasculitis. It is important to define if uveitis is due to direct infection of the eye or a secondary immune reaction. Direct cytopathic effects have been reported during viral infections whereas immune-related mechanisms are involved in different forms of bacterial uveitis. The use of molecular techniques such as PCR, applied to ocular fluids is particularly interesting, allowing a direct etiological confirmation and an well-adapted therapeutic management. Characterisation of the infecting organism at the species level on the one hand and linkage of clinical data with pathogenically relevant immune parameters on the other, shall lead to a more precise understanding of the pathogenesis and the individual clinical course of intraocular inflammatory disorders. Early diagnosis and therapy of an acute bacterial, viral or parasitic infection restrict the extent of the lesions and prevent ocular and general late manifestations. New data have been presented in the field of non-necrotizing viral retinopathies but also different bacterial conditions such as Lyme disease and tuberculosis. Diagnosis of previously well defined infectious agents but also emerging diseases is a real challenge before the use of conventional immunosuppressive regimens but also new therapeutic regimens such as anti-TNF molecules.

■ 1355

Retinal vasculitis : Diagnosis and management

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Retinal vasculitis is frequently associated with intermediate and posterior uveitis involving more often the veinules but also arterioles and capillaries. Retinal vasculitis is diagnosed on biomicroscopic examination completed by fluorescein angiography that demonstrates vascular leakage and staining of vessels walls. When severe, retinal vasculitis can lead to occlusion and retinal ischemia. Retinal vasculitis can be associated with various conditions such as systemic diseases (Behçet disease, sarcoidosis...), infectious disorders (syphilis, Lyme borreliosis, viral retinitis, tuberculosis...), and primary ocular diseases (birdshot, Eales, idiopathic retinal vasculitis...). Not all the patients require treatment. In cases of non infectious uveitis when ocular inflammation is mild, ocular function is good, patients may simply be observed. It is important to remember that once started, it may be required for several years so drugs toxicity should be taken into account. Treatment is started for patients with visual loss related to inflammation or macular edema or when vasculitis becomes occlusive with retinal ischemia. Systemic corticosteroids are effective but relapse can occur when the doses are tapered. Immunosuppressive drugs can be helpful in corticosteroid dependent patients as corticosteroid-sparing agents. The efficacy of new drugs like interferon alpha or anti-TNF alfa are evaluated in several trials but the results are already promising.

■ 1356

Choroidal vasculitis: appraisal and classification

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Purpose: Determination of choroidal vasculitis was not possible so far. When talking of intraocular vasculitis it always meant retinal vasculitis very well demonstrated by fluorescein angiography. By allowing to detect fluorescence beyond the retinal pigment epithelium, indocyanine green angiography (ICGA) has made it possible to analyse the choroidal vessels. Our aim was to characterize choroidal vasculitis in posterior uveitis using ICGA.

Methods: Charts of active posterior uveitis patients with a specific diagnosis that had undergone dual fluorescein and ICG angiography were reviewed. The type of inflammatory involvement of the choroidal circulation at entry and the treatment response on follow-up angiograms were analysed.

Results: A total of 129 patients were analysed. Choroidal vasculitis could be subdivided into two main patterns: (1) primary inflammatory choriocapillaropathy and (2) stromal inflammatory vasculopathy. The first pattern consisted of hypofluorescent areas up to the late phase of angiography characteristic for choriocapillaris non-perfusion and included entities such as multiple evanescent white dot syndrome (MEWDS), acute posterior multifocal placoid pigment epitheliopathy (APMPPE), multifocal choroiditis (MC), amipiginous choroidopathy and serpiginous choroidopathy. The second pattern consisted of fuzzy indistinct appearance of vessels in the intermediate angiographic phase and diffuse choroidal hyperfluorescence in the late phase indicating inflammatory vasculopathy of larger choroidal vessels. This pattern was found in all cases of active Vogt-Koyanagi-Harada disease, ocular sarcoidosis & tuberculosis and birdshot chorioretinopathy. In Behçet's uveitis of recent onset, choriocapillaris perfusion delay and fuzzy choroidal vessels without diffuse late choroidal hyperfluorescence was found. In posterior scleritis, enlargement of vorticos veins was an additional ICGA sign. Stromal inflammatory vasculopathy always responded to anti-inflammatory therapy. A third group of patients with severe retinal or choroidal inflammation presented associated secondary inflammatory choriocapillaropathy angiographically identical to primary involvement.

Conclusions: Characterisation of inflammatory involvement of the choroidal vessels is now possible using ICGA, showing either predominant inflammation of the choriocapillaris or predominant inflammation of the stromal choroidal vessels with or without secondary choriocapillaritis. ICGA will be unavoidable for the correct evaluation and follow-up of posterior inflammation with suspected choroidal involvement.

Chaperone function in the lens

■ 1361

X-ray structures of small heat shock proteins

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Purpose: To understand how large assemblies of small heat shock proteins can bind non-native substrate proteins in the light of their known temperature-dependent rapid equilibrium between sub-assembly species and the complete oligomer.

Methods: Monodisperse populations of small heat shock protein oligomers were crystallised in order to solve their tertiary and quaternary structures by X-ray crystallography.

Results: Analysis of the molecular coordinates shows that the interface regions that hold the eukaryotic small heat shock protein oligomer together involve the N-terminal domain, the conserved alpha-crystallin domain and the ordered C-terminal extension.

Conclusions: During rapid disassembly of the oligomer, considerable unfolding of the hydrophobic N-terminal region must occur concomitant with exposure of hydrophobic regions at the edges of the beta-sandwich structure of the alpha-crystallin domain. These regions would therefore be candidates for binding hydrophobic regions of non-native substrate proteins.

■ 1362

Alpha-crystallin and sHSP function

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Purpose: The alpha crystallin core domain is a structural hallmark of small heat shock proteins, sHSP, that act as molecular chaperones (Arrigo & Muller, 2002 Small Stress Proteins) and can be expected to be important for normal functions of sHSP. Subunits of sHSP assemble to form large functional complexes and the assembly sites on lens alpha crystallin are important in the establishment of the transparent structure of normal lens cells.

Methods: Protein peptide arrays have been used successfully to define the primary sequences of epitopes for antibody specificity and application of the peptide array technology identified four peptide sequences as interactive domains for dimerization assembly of active complexes of human alphaB crystallin. The interactive domains were mapped to a homology model of human alpha B crystallin.

Results: Two of the domains were identified in the alpha crystallin core domain. A third is in the C-terminus and a fourth is in the N-terminus of the alpha crystallin subunit. The secondary structure of the interactive domains involved beta strands 3 and 8 of the core domain that contributed to an immunoglobulin fold described on the basis of X-ray diffraction studies of wheat HSP 16.9 (van Monfort et al 2001, Nat Struct. Biol 8:1025). The C- and N-terminal domains appeared to contribute to the assembly of the multimeric complex. Considering the small size of the sHSP subunit, it is reasonable to suggest that the structural domains used for complex assembly are involved in functional interactions with cytoskeletal elements and chaperone target proteins.

Conclusions: Cellular transparency may be the result of normal interactions between alpha crystallins, a sHSP, and the major structural components of lens cytoplasm. Supported by EY04542 from the NEI.

■ 1363

Nuclear role for alpha B-crystallin?

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Purpose: To determine the nuclear location of sHSPs alphaB-crystallin and HSP27 and thus anticipate their nuclear function.

Methods: Confocal immunofluorescence microscopy coupled with the transfection and biochemical analysis of the nuclear compartment.

Results: In this study, the small heat shock protein (sHSP) chaperones, alphaB-crystallin and HSP27 are identified as nuclear speckle components in a variety of unstressed cells. We selected the astrocytoma cell line, U373MG to demonstrate the immunolocalisation of alphaB-crystallin and HSP27 in nuclear speckles (inter-chromatin granules). Whilst alphaB-crystallin was preferentially localised to speckles, HSP27 was also seen in spots within the nucleolar compartment indicating a subtle difference between these closely related sHSPs. We then examined a variety of transformed, immortalised and primary cells expressing endogenous alphaB-crystallin as well as some cells with ectopic alphaB-crystallin expression and all consistently showed alphaB-crystallin in nuclear speckles. The nuclear localisation of the sHSPs was confirmed biochemically and a series of transfections undertaken to determine the mechanism of nuclear transport and possible function.

Conclusions: Finding sHSPs in the nucleus suggests a constitutive function for these sHSP chaperones in this compartment not previously appreciated. These data extend the chaperone function of these sHSPs to include transcriptional processes.

■ 1364

Role of alpha-crystallins in cell growth and cell death

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Purpose: Alpha-Crystallins are chaperones containing the C-terminal conserved domain of small heat shock protein family, and can bind and sequester denaturing proteins thereby preventing their non-specific aggregation. We have analyzed the functions of the alphaA and alpha B-crystallin in the lens epithelium using knockout mice.

Methods: Wild type, alpha A-crystallin^{-/-}, alpha B-crystallin^{-/-} mice were used in this study (generously provided by Dr. Eric Wawrousek). Seven day old mice were injected with bromodeoxyuridine to label DNA in proliferating cells. Immunofluorescence with specific antibodies to BrdU was used to detect proliferating cells. Tubulin was detected by immunolabeling and confocal microscopy was used to visualize the microtubules. Cultured lens epithelial cells were also utilized.

Results: We have found a decreased growth phenotype in cultured alphaA knockout lens epithelial cells and in vivo lens epithelium. Lack of alpha A-crystallin results in increased cell death. Cells appear to die during mitosis in the alpha A knockout lens epithelium. In contrast to phenotype of alphaA-crystallin^{-/-} cells, we found that lack of alpha B results in genomic instability of lens epithelial cells, stabilization of p53 protein, and hyperproliferation.

Conclusions: Lack of alphaA or alpha B crystallins leads to cell destabilization of the lens epithelium. The effects of the two crystallins are distinctive. We propose that these proteins are associated with the tubulin cytoskeleton and assist the proper completion of mitosis.

■ 1401

OCT findings after photodynamic therapy

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Purpose: To understand the mechanisms of action of photodynamic therapy (PDT) in patient with subfoveal choroidal neovascularization (CNV) due to age-related macular degeneration, the authors evaluated the retinal response after treatment with serial optical coherence tomography (OCT).

Methods: PDT was performed on fifteen eyes of fifteen consecutive patients who presented with subfoveal CNV due to AMD, and serial evaluation with OCT was performed 1,7 and 15 days after therapy.

Results: In the first day, OCT showed an increase in the subretinal fluid in all eyes, increase in the intraretinal fluid in 2 eyes and increase in both subretinal and intraretinal fluid in 4 eyes. At 1 week, reduction of subretinal fluid was observed in all eyes. Four out of 6 eyes showed reduction of intraretinal fluid. In the fifteen day, OCT showed persistent intraretinal fluid in one eye.

Conclusions: Serial OCT evaluation of patients with subfoveal CNV due to AMD suggests that the initial response after PDT was the marked increase in the subretinal fluid. At 1 week, reduction of subretinal fluid was observed.

■ 1402

Predictive factors of successful single PDT session in subfoveal choroidal neovascularization

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Purpose: The purpose of this study was to evaluate the criteria of successful treatment by photodynamic therapy (PDT) in subfoveal choroidal neovascularization (CNV).

Methods: Retrospective study of 548 patients who underwent PDT for subfoveal CNV in a tertiary reference retinal center between September 2001 and March 2003. Criteria of successful treatment were patients who received only one single PDT session and had an improvement of visual acuity of 2 lines or more on ETDRS charts. Age, sex, follow-up and CNV etiologies were recorded.

Results: There were 548 patients who underwent PDT for subfoveal CNV. Twenty four patients received one single PDT session resulting in an improvement of 2 lines or more on ETDRS charts. Mean age was 67 years (range 54 to 88 years). There were 15 women and 9 men. Mean follow-up was 8.5 months. Twenty patients had subfoveal CNV secondary to myopia and the other four presented occult subfoveal choroidal neovascularization. None of the patients corresponding to successful criteria presented classic subfoveal CNV.

Conclusions: Myopia and to a lesser extent occult choroidal neovascularization appear to be positive predictive factors of good response to photodynamic therapy

■ 1403

Preliminary results of occult choroidal neovascular membrane treatment with argon laser using a digital membrane localization system

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Purpose: To analyse the results of two years of follow-up after laser treatment of occult choroidal neovascular membranes (OCNV) with a system that provides more precise location on the retinal plane of the area to be treated.

Methods: Fourteen eyes of patients with age-related macular degeneration (AMD) were treated with laser argon using a system comprised by a server, a communication channel and one or more user computers. The server contains a program that uses mathematical techniques and digital image processing to transfer the points marked by the ophthalmologist to the high resolution indocyanine green (ICG) image (1024x1024) to the fundus colour photograph (640x480) for photocoagulation.

Results: All the patients had OCNV. Twenty per cent of the membranes treated were extrafoveal and 78% juxtafoveal. Baseline best corrected visual acuity (VA) ranged from hand motion to six to ten. In 30% of cases, initial VA measurement was precluded by the presence of a central scotoma. In 78% of cases, the membrane regressed or stabilized after treatment. In 21% of cases the membrane continued to grow and required retreatment. After retreatment, 71% of the membranes regressed, 21% stabilized and 7% progressed. VA after 2 years of follow-up was the same as or better than before treatment in 75% of patients.

Conclusions: Our method makes it possible to determine the exact location of an OCNV on the retinal plane by providing the ophthalmologist with a situation plan of the membrane on the fundus photograph at the time of treatment. This makes for greater precision and certainty in laser treatment and favours photocoagulation of membranes close to the foveola.

■ 1404

Submacular surgery after photodynamic therapy

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Purpose: To evaluate the results of surgical excision of subfoveal choroidal neovascular membranes after photodynamic therapy (PDT).

Methods: Five eyes of 5 patients with subfoveal choroidal neovascularization secondary to idiopathic choroidal neovascularization (3 eyes), pathologic myopia (1 eye) and ocular histoplasmosis syndrome (1 eye) were reviewed. Standardized protocol refraction, visual acuity testing, color photographs, fluorescein and indocyanine angiograms were used to evaluate the results of both PDT and membrane excision. Follow-up ranged from 12 to 24 months with a mean (\pm SD) of 18.8(\pm 5.2) months.

Results: PDT was performed in all cases as a primary therapy (2 PDT in 3 cases, and 1 PDT in 2 cases). With PDT, best-corrected visual acuity (BCVA) worsened in all patients and progression of lesion was noted. All patients underwent subsequently pars plana vitrectomy with subfoveal membrane excision. All patients regained good visual acuity after surgery. No recurrence of choroidal neovascularization has been detected during follow-up.

Conclusions: These results show that subfoveal surgery should be considered for subfoveal choroidal neovascularization secondary to other causes than age-related macular degeneration which do not respond to PDT. The improvement in visual acuity also provides indirect evidence that PDT did not disrupt the underlying retinal pigment epithelium.

■ 1405

RETAANE™ (anecortave acetate) trans-scleral delivery: posterior juxtasceral administration and safety

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Purpose: To evaluate the clinical safety and feasibility of administering the angiostatic cortisene, anecortave acetate, onto the outer scleral surface as a posterior juxtasceral depot using a specially designed cannula.

Methods: Anecortave acetate has been evaluated for treatment of subfoveal choroidal neovascularization through a unique trans-scleral route of delivery. The Independent Safety Committee overseeing these activities has periodically reviewed clinical safety data from these studies. In the recently completed 24-month dose-response study, patients were randomized to anecortave acetate or placebo treatment, with optional re-treatment at 6-month intervals. In a completed 6-month study, the safety of a single administration of either anecortave acetate or placebo was assessed in patients following initial Visudyne PDT™. Systemic safety was assessed by periodic general physical examinations. Ophthalmic safety was assessed by periodic detailed ophthalmic examinations including angiography. Three multi-national studies are now comparing anecortave acetate to Visudyne™ PDT or placebo treatment.

Results: More than 900 posterior juxtasceral administrations of anecortave acetate or placebo have been given to over 600 patients. Adverse events reported to date have been typically mild, transient, reported in all treatment groups, and generally assessed as unrelated to study medication. Adverse events possibly related to depot administration include ptosis, ocular pain and subconjunctival hemorrhage.

Conclusions: Anecortave acetate can be safely administered and re-administered at 6-month intervals by a trans-scleral delivery system using the unique technique of posterior juxtasceral application. No clinically relevant adverse events related to either anecortave acetate or the administration procedure have been identified to date.

■ 1406

Intravitreal Triamcinolone Acetonide as Treatment for Exudative Age-Related Macular Degeneration

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Purpose: Prospective comparative non-randomized clinical interventional study to analyze visual acuity after intravitreal triamcinolone acetonide (TA) for progressive exudative age-related macular degeneration (AMD).

Methods: Study included 187 consecutive patients with progressive exudative AMD, divided into study group (n=115) receiving intravitreal TA (25 mg) and control group (n=72) without treatment. At baseline both groups did not vary significantly ($p>0.10$) in visual acuity, age, refractive error, gender. 25mg of intravitreal TA was injected in the operating room with the patient draped for intraocular surgery. Mean follow-up was 6.0 ± 4.2 months.

Results: Visual acuity increased significantly ($p=0.03$) in study group and decreased significantly ($p=0.01$) in control group 1 month after study begin. Change of visual acuity was significant ($p=0.001$) between study and control group. In study group, patients with increase in visual acuity of > 1 Snellen line was significantly ($p=0.001$) greater than in control group, and number of patients with decrease of > 1 Snellen line significantly ($p=0.007$) less; similar visual results after 3 months. Increase of best visual acuity during follow-up in 72 (62.6%) patients in study group, indicating significant ($p<0.001$) increase of 1.14 ± 2.89 Snellen lines. Forty-three (37.4%) patients had increase in visual acuity of > 1 Snellen line; in control group, best visual acuity changed by 0.32 ± 0.78 Snellen line. Difference between study and control group concerning increase of best visual acuity was significant ($p<0.001$).

Conclusions: After 25 mg of intravitreal TA in patients with exudative AMD, visual acuity increased at 1 month and 3 months after injection.

■ 1407

Anecortave acetate (RETAANE™) trans-scleral delivery: clinical trials status*SOLIBRANE G (1), ROBERTSON S (2)**(1) CHI Creteil, Creteil, (2) Alcon Research Ltd, Fort Worth TX*

Purpose: To evaluate the angiostatic cortisene, anecortave acetate, for treatment of subfoveal choroidal neovascularization (CNV) following posterior juxtасcleral administration with a specially designed cannula.

Methods: In four ongoing randomized clinical trials, anecortave acetate or placebo is administered as a posterior juxtасcleral depot. Safety data from periodic physical examinations and detailed ophthalmic evaluations are evaluated by an Independent Safety Committee. Clinical efficacy is being assessed by logMAR visual acuity evaluations and evaluations of fluorescein angiographic lesion changes over time.

Results: Month 12 data analyses demonstrate that RETAANE™ 15mg is statistically superior to placebo ($p=0.0323$) for stabilization of vision (less than 3 logMAR line change from baseline) in both the analysis of all patients (79% of the RETAANE™ 15mg group versus 53% of the placebo group) and in the subgroup analysis of patients with predominantly classic lesions (84% of RETAANE™ 15mg treated patients versus 50% of placebo treated patients, $p=0.01$). There is also a trend favoring RETAANE™ 15mg over placebo for inhibition of classic CNV when percent changes in area from baseline are compared. Longer-term data from an analysis of the 24-month study are being analyzed.

Conclusions: RETAANE™ 15mg is clinically efficacious for treatment of subfoveal CNV lesions secondary to AMD. Additional phase III clinical studies have been initiated to compare RETAANE™ 15mg to Visudyne® PDT and to obtain additional placebo-controlled clinical efficacy data, in the USA, Canada, the EU, New Zealand, Australia and South America.

The ins and outs of the aqueous humor

■ 1411

Electron probe x-ray microanalysis of ciliary epithelium

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Purpose: An understanding of the roles of ion pathways in ciliary transport requires knowledge of cellular composition under different conditions. We have used X-ray microprobe analysis to determine cell Na, K, Cl and P contents in individual rabbit pigmented (PE) and non-pigmented (NPE) cells.

Methods: Segments of isolated rabbit iris-ciliary body (ICB) were incubated under different conditions. Tissues were then frozen at -180 C. Sections (0.2-0.4 m) were cut at ~90C, freeze-dried and transferred to an SEM with an energy-dispersive x-ray spectrometer. Individual pigmented (PE) and non-pigmented (NPE) cells were analysed for their Na, K, Cl and P.

Results: This work has provided evidence (i) establishing the coupling of paired PE and NPE cells through apical membrane gap junctions; (ii) supporting the suggestion that the major pathways for Na and Cl entry into the PE cells involve the Na/H and Cl/HCO₃ antiports; (iii) suggesting that, in the presence of medium HCO₃, the bumetanide-sensitive Na, K, 2Cl symport moves these ions from the cells; (iv) indicating that there is a lower turnover of Na, K and Cl in the posterior region of the epithelium lining the ICB; (v) arguing that timolol inhibits aqueous humor secretion by a previously unrecognized cAMP-independent mechanism that may involve inhibition of Cl/HCO₃ exchange.

Conclusions: X-Ray microprobe analysis provides a unique tool for studying individual NPE and PE cells. It has provided new insights into the mechanisms of aqueous humor secretion and absorption and into regional differences in these functions.

■ 1412

Potential Role of Purines in Cross-talk between Inflow and Outflow of Aqueous Humor

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Purpose: Identify sources, targets and modes of action of purinergic regulation of aqueous humor flow and intraocular pressure (IOP).

Methods: Biophysical analyses of cells and tissues and measurements of mouse IOP.

Results: Purines are thought to modulate both inflow of aqueous humor through the ciliary epithelium and outflow through trabecular meshwork (TM) and Schlemm's Canal (SC) inner-wall cells. ATP is released by NPE and PE cells, and can be metabolized to adenosine by ectoenzymes. Thus, both ATP and adenosine can be presented downstream to TM and SC inner-wall cells. In addition to this cross-talk mechanism, ATP can be released by TM cells. Purines likely exert opposing effects on ciliary epithelial secretion. Adenosine activates Cl⁻ channels of NPE cells through A₃ adenosine receptors (AR), favoring enhanced secretion, and ATP activates Cl⁻ channels of PE cells, favoring reabsorption and thereby reduction of net secretion. The ATP appears to act on the PE cells by triggering a signal cascade culminating in cAMP production. At least in part, cAMP directly activates maxi-Cl⁻ channels. Adenosine acts on cells from the outflow pathway, as well, affecting ion channels of TM cells largely through A_{2A} and A₃ ARs, and activating K⁺ channels of SC inner-wall cells through A₁ ARs. Measurements in the living mouse indicate that the overall effect of adenosine is to increase IOP markedly, an action which is largely prevented by an A₃ AR antagonist. A₃AR-negative mice have lower IOP and display little IOP response to adenosine.

Conclusions: ATP released and metabolized to adenosine by the ciliary epithelium acts locally on inflow, and delivery of the purines downstream can contribute to purinergic regulation of aqueous humor outflow, as well.

■ 1413

Overview of cross-talk role of biologically active peptides

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Emerging evidence indicates that the mechanisms of aqueous humor secretion and maintenance of intraocular pressure (IOP) underlie autocrine and paracrine pathways. These signals are believed to be initiated, in part, by components of a novel local peptidergic system placed along the ocular ciliary epithelium. This peptidergic system consists of neuroendocrine peptides and hormones which, upon their release, target their cognate receptors by an autocrine mechanism at the peptide-producing cells (i.e., NPE and/or PE), or by a paracrine mechanism in the vascular endothelial cells, ciliary muscle cells and trabecular meshwork cells. In this overview, I will focus on the characteristic neuroendocrine properties of the ocular ciliary epithelium, including the synthesis and processing of regulatory peptides and their role in regulation of IOP. In this regard, the hypotensive effect of natriuretic peptides in lowering IOP provides an opportunity to explore the cross-talk signals between these pro-dilatory hormones and their antagonistic effects on the vasoconstrictory pressor hormones such as angiotensin II, arginine-vasopressin, and endothelin. Deciphering the signaling pathway involved in cross-talk between inflow and outflow may provide insight into new therapies to lower IOP in glaucoma.

■ 1414

Crosstalk role of endothelin

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Purpose: We determined if dexamethasone (Dex) increases the synthesis and release of ET-1 from human non-pigmented ciliary epithelial cells (HNPE) and determined if released ET-1 interacts with Dex on TM cells to alter outflow resistance.

Methods: Primary human TM cell lines and an established HNPE cell line were used. ET-1 was measured by RIA and mRNA levels of prepro-ET-1, ET receptor A (ETA) and ET receptor B (ETB) were measured by quantitative RT-PCR. ETA and ETB receptor proteins were determined using Western blot and ET-1 receptor activity was assessed by measuring calcium mobilization [Ca]²⁺ with fura-2AM and nitric oxide (NO) production using a Griess NOS assay kit.

Results: Dex increased ET-1 release and mRNA for prepro-ET-1. RU 486, a Dex antagonist, decreased Dex's action on ET-1 release and message. TM cells expressed prepro-ET-1 but it was significantly less than in HNPE. TM cells expressed mRNA for the ETA but not the ETB message. Western blot showed that both ETA and ETB receptor proteins were present and that both responded to ET-1 in increasing [Ca]²⁺ and NO production respectively. Dex had no effect on ETA receptor expression or function, however, Dex decreased ETB receptor expression and ET-1 actions on NO release.

Conclusions: The Dex induced increase in ET-1 release from HNPE cells coupled to the downstream Dex-induced suppression of ETB receptor expression and activity results in increased contraction of the TM which contributes to the decline in conventional aqueous humor outflow and increases in IOP typically seen with glucocorticoids.

The ins and outs of the aqueous humor

■ 1415

Influence of the molecular weight on dextran movement after anterior chamber perfusion in mice

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Purpose: The aim of this study was to investigate the influence of molecular weight on the intraocular distribution of various-sized dextran molecules after anterior chamber perfusion in mice.

Methods: The anterior chamber of 64 anesthetized NIH Swiss mouse eyes were perfused with various fluorescent dextran solutions for 10 minutes. The tracers included 10 kDa, 40 kDa, 70 kDa, and 500 kDa dextrans. At 10, 20, or 60 minutes after the start of the anterior chamber perfusion, the eyes were enucleated. Cryostat sections were analyzed by fluorescence microscopy.

Results: 10 kDa dextran was observed in the supraciliary space and the adjacent sclera after 10' and 20', and in the anterior sclera at 60'. 40 kDa dextran was detected in the supraciliary space and sclera after 10', in the anterior uvea after 20', and in the anterior sclera after 60'. 70 kDa dextran was observed in the supraciliary space after 10'. After 20', it was also visible in the anterior and intermediate suprachoroidal space, and in the anterior choroid. After 60', it was observed in all segments. 500 kDa dextran was observed in the supraciliary space and the anterior uvea after 10', in the anterior sclera after 20' and in the equatorial sclera after 60'.

Conclusions: The distribution of dextran molecules in the suprachoroidal space of the mouse is dependent on the molecular weight. 10 kDa, 40 kDa, and 500 kDa dextran do not reach tissues posterior to the equator. In contrast, 70 kDa dextran enters the suprachoroidal space via the uveoscleral outflow pathway and traverses to the posterior pole of the mouse eye.

■ 1441

Covert visual recognition in disease

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Some patients with visual deficits can be shown by various techniques to have residual perceptual effects, despite professed lack of awareness. The two main phenomena are "blindsight", with hemianopia or cerebral blindness from geniculostriate damage, and "covert recognition", with various agnosias from extra-striate damage. Physiological, electrophysiological, and behavioural evidence has been found for covert ability in both types of phenomena, yet whether the same mechanisms are responsible for all types of covert ability has never been clear. The anatomic basis of covert ability is of theoretical importance and continues to be debated. For blindsight the issue centers on how any perception can survive significant damage to a relatively simple neuron relay.

Arguments concern the role of projections that bypass striate cortex versus partial striate function that survives the lesion. Our results illustrate the difficulties in resolving this issue given current limitations in neuro-imaging. For visual agnosia, such as prosopagnosia, the issue centers on what generates residual signals after damage within a parallel distributed network. Arguments concern the role of dissociated alternative pathways versus continued but weak function within a network after partial damage to some modules. In prosopagnosia, several network models have reproduced covert patterns of performance. In a series of prosopagnosic patients we used imaging, perceptual and imagery tests to show that covert ability depends on the functioning of not just one processing stage, but on the extent of dysfunction across several stages and sites within a face processing network. This suggests that covert face recognition is not a marker for a specific type of damage or the recruitment of anomalous mechanisms, but an index of the degree of overall network disruption. It remains unclear what plasticity contributes to blindsight or covert recognition, in either generating covert ability or converting it into a useful guide of overt behaviour.

■ 1442

Unawareness of visual defects as a result of cortical plasticity

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Understanding of mechanisms involved in cortical plasticity has allowed for a better understanding of perception, of functional deficits and of traps in the evaluation of affected patients. It has now reached a stage allowing for the use of this knowledge to optimize rehabilitation procedures of low-vision patients, using the neuroplasticity of the visual system, which provides the neurobiologic substrate for a rational and scientifically based visual treatment strategy. Although complete restitution seems unlikely at present, significant restoration of visual function can be expected. Brain plasticity will eventually play a crucial role in reorganizing selective attentional processes in vision, altering ocular motor control, and contributing to a more efficient visual recovery.

Image processing and plasticity after lesions in visual pathways

■ 1443

Rehabilitation in Homonymous Hemianopia: an Overview

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Homonymous hemianopia causes severe disability for daily living, mainly regarding orientation and reading. The aim of rehabilitation programs is to regain or optimize these impaired functions for improving the patients' quality of life. There are 2 principal approaches: restitution and compensation. Restitutional visual field training aims to restore and enlarge the visual field by visual stimulation in the blind hemifield. One problem, however, is the insufficient fixation control during conventional perimetry. Eccentric or unstable fixation can cause a shift of the visual field border towards the hemianopic side, which can be misinterpreted as a visual field improvement and which we showed with fundus-perimetry using a Scanning Laser Ophthalmoscope. Control studies could not prove a relevant effect. Compensating methods have been successfully used: training to shift attention and to perform saccades towards the hemianopic side supports exploration and better use of the blind field by enlarging the field of gaze. The value of optical aids (mirrors, prisms) is controversial. Regarding reading it is crucial, if 2-5° of visual field are spared along the horizontal meridian in the hemianopic field (macular sparing or otherwise incomplete field defect). In macular splitting, reading is severely disturbed, but can be improved by compensating strategies, such as eccentric fixation and predictive saccades. Other approaches are vertical reading and tactile aids to keep the line. The value of a training method has to be related to its relevance to everyday life. Compensating strategies, which have to be adapted to the task, are effective to improve the utilization of the blind hemifield.

■ 1444

Adaptation of reading strategies to central scotoma

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Purpose: Macular disease is the most common cause of severe visual impairment in Europe and North America. Affected individuals develop a scotoma in the centre of the visual field that makes it difficult to read, recognise faces, or navigate independently. To accomplish these tasks, the person must learn to use peripheral vision instead of looking straight at the object of interest. The goal of this study is to track the progress of patients with newly diagnosed macular disease as they adapt to their vision loss. We hypothesize that successful adaptation requires the development of a single, stable peripheral retinal locus (PRL) for fixation, and the use of that PRL as the oculomotor reference for reading eye movements.

Methods: Patients with age related and early onset macular degeneration are being recruited into a two-year longitudinal study. Retinal scotomas are mapped with a scanning laser ophthalmoscope and fixation and reading eye movements are measured with a high speed video eye tracking system. Reading performance is assessed with standardized text presented on a computer monitor.

Results: At the outset, most patients exhibit considerable fixation instability with multiple PRLs. Reading eye movements are characterized by multiple short saccades with a high percentage of regressive eye movements. Over time, usually within six months, successful patients adopt more stable PRLs, and learn to make fewer saccades and regressions while reading.

Conclusions: Our results demonstrate that stable eccentric fixation and efficient oculomotor control are essential for successful adaptation to macular disease. Such information will help identify patient characteristics that lead to successful rehabilitation and aid in the design of more effective intervention.

■ 1451

Overview of allergic eye diseases

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There has been a recent increase in allergic diseases including eye diseases [AED]. Treatment has often been poor with much ocular morbidity resulting. Recent research work has demonstrated the underlying cellular mechanisms. Also work it has been shown that the conjunctival epithelium is abnormal, with under expression of the intra- and inter-cellular proteins responsible for cell adhesion and cell structure, raising the possibility that the atopic epithelium is more porous to allergens allowing them to gain access to the underlying cells and so initiating the allergic cascade. This opens up new treatment possibilities. Some of the genes associated with allergy have been discovered. Finally new drugs for the treatment of allergic eye disease have come onto the market, so enabling us to better treat all forms of AED, including most importantly the chronic sight threatening forms. Therefore, this symposium will discuss these advances in the context of treatment of allergic eye disease.

■ 1452

Immunopathogenesis of conjunctival remodeling in vernal keratoconjunctivitis

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Purpose: To study the expression of integrin receptors, epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), transforming growth factor- β (TGF- β), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and Ki67 antigen which is a marker for cell proliferation.

Methods: Conjunctival biopsy specimens from sixteen patients with active VKC, and nine control subjects were studied by immunohistochemical techniques.

Results: In the normal conjunctiva, very weak immunoreactivity was observed for EGFR, and VEGF in epithelial cells, and for $\alpha 3$, and $\alpha 6$ integrin subunits on basal epithelial cells, and on vascular endothelial cells in the upper substantia propria. There was no immunoreactivity for the other antibodies. In VKC specimens, strong membranous immunoreactivity for $\alpha 3$, and $\alpha 6$ integrin subunits was observed on basal and suprabasal epithelial cells, and on all vascular endothelial cells. Immunoreactivity for Ki67 antigen was observed in the nuclei of the basal and suprabasal epithelial cells. Strong immunoreactivity was observed for EGFR in the deeper layers of the epithelium, and for VEGF in all epithelial cells. Inflammatory cells expressing EGFR, VEGF, TGF- β , bFGF, and PDGF were noted in 8, 9, 11, 10, 10 specimens, respectively. The majority of inflammatory cells expressing growth factors were eosinophils ($45 \pm 4\%$), and monocytes / macrophages ($35 \pm 4\%$).

Conclusions: Chronic conjunctival inflammation in VKC is associated with upregulation of $\alpha 3$, and $\alpha 6$ integrin subunits, EGFR, VEGF, TGF- β , bFGF, and PDGF that might mediate conjunctival remodeling.

Allergic eye diseases

■ 1453

The role of ocular mucosal epithelium in allergic eye disease

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Purpose: To study the phenotype and kinetics of immune cell migration at the ocular surface after epithelial injury.

Methods: Immunohistology, electron microscopy and fluorescein activated cell sorting (FACS) analysis of lymphocytes in an ex-vivo model of human conjunctival injury.

Results: The conjunctiva possesses organised and diffuse lymphoid tissue, which is dispersed primarily in the substantia propria and as distinct subset of CD8⁺/HML-1⁺ intraepithelial lymphocytes. These include CD4⁺, CD8⁺, CD56⁺ and CD19⁺ cells. During ocular surface insult and injury, a preferential and polarised migration of these cells occurs towards the epithelial basement membrane. In the process distinct channels are formed in the stroma, which lead to pores in the basement membrane. Metalloproteases, chemokines and adhesion molecules may play a significant role in this micro-environmental or peripheral homing of cells.

Conclusions: This model provides an excellent opportunity of studying various immune mediated events at the ocular surface.

■ 1454

Treatment of acute allergic eye disease

McGILL J

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Discussion about the recent evidence outlining the rising prevalence of allergic disease throughout the world, and the possible causes. An outline of the various types of allergic eye diseases will be given. Will then deal with underlying mechanisms for the allergic cascade and recent advances in changes in the epithelial structure that could be responsible for this cascade. These include the findings that the epithelium in Seasonal Allergic Conjunctivitis has an abnormal expression of structural proteins.

■ 1455

Treatment of chronic forms of allergic eye disease

TUFT SJ

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Purpose: To describe the features and management of chronic allergic eye disease.

Methods: A review of the clinical features of the patients with vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) seen at a postgraduate tertiary referral centre in London. The management protocols and treatment options will be described.

Results: Chronic inflammation results in anatomical changes of the lids and ocular surface which account for many of the features of this disease. Techniques to control the allergic symptoms and manage the secondary complications are described. Identification of realistic outcome goals is essential.

Conclusions: Chronic allergic eye disease can result in unremitting ocular discomfort. With appropriate management secondary complication can be minimised.

Growth factor signalling in the lens

■ 1461

Roles for FGF and Wnt signalling in differentiation of lens cells

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Purpose: How the lens develops its highly ordered architecture and growth patterns is a major question in developmental biology. During embryogenesis, cells in the anterior and posterior segments of the lens vesicle, differentiate into the epithelial and fibre cells, respectively. Our studies have aimed to identify the molecules that regulate the divergent fates of lens cells.

Methods: The roles of various growth factors in regulating lens cell fates were studied in rat lens epithelial explant cultures and in transgenic and mutant mouse models.

Results: There is now compelling evidence that members of the FGF growth factor family are key initiators of fibre differentiation in mammals. Other studies support a role for various members of the TGF-beta superfamily in this process and indicate that a cascade of growth factor signalling may be required for normal fibre differentiation. Less is known about the anterior segment; however, recent studies point to an important role for the Wnt growth factor family in epithelial differentiation. Studies on expression of Wnts, their various receptors and regulatory molecules indicate a role for Wnt signaling in the formation and maintenance of the epithelial sheet. This is supported by our recent analysis of a mouse with a mutation in the Wnt co-receptor, LRP6, which showed dysmorphogenesis of the lens epithelium.

Conclusions: These studies indicate the importance of FGF and Wnt signalling in determining the polarity and ordered growth of the lens.

■ 1462

Regional Differences in Epidermal Growth Factor Receptor Signaling

Components in the Human Lens

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Purpose: The lens epithelium can be separated into two regions, the non-dividing central zone and the equator, the site of all division in the normal lens. In the present study we investigated the distribution of EGF/EGFR signalling components and related this to mitotic distribution in the lens.

Methods: Anterior and equatorial regions of the native epithelium were prepared separately from donor lenses. In vitro capsular bags were prepared from donor eyes and cultured. Receptor distribution was determined by immunocytochemistry and RT-PCR. Western blot analysis of PLCgamma and ERK (total and active) was carried out on cell lysates. Function was determined by calcium imaging of FURA-2 loaded cells and also, in the case of capsular bags, by cell growth.

Results: Immunocytochemistry and RT-PCR showed an even distribution of EGFR across the native epithelium. Whole lenses, however, in response to 10ng/ml EGF only exhibit a calcium response at the equatorial region. Western blots demonstrated significantly greater expression of PLCgamma and ERK (total and active) in the equator relative to the central region. Addition of EGF increased growth rates of cells in capsular bags and an EGFR inhibitor decreased rates. Cells on the posterior capsule also invoked a calcium response upon EGF exposure.

Conclusions: EGFR is evenly distributed across the entire epithelium, while expression of signalling proteins PLCgamma and ERK has a marked bias to the equator. In a wounded system EGF/EGFR signalling can play both an autocrine and paracrine role. Therefore, EGF/EGFR signalling function appears to be regulated by the expression of downstream signalling proteins and not receptor expression.

■ 1463

The mitogenic action of platelet derived growth factor (PDGF) is mediated through an endogenous superoxide anion-generating system in the human lens epithelial cells

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Purpose: Reactive oxygen species (ROS) has been implicated in many mammalian cells to have a physiological function in regulating and activating the mitogenic-associated signal transduction pathways in cells, stimulated by certain growth factors or cytokines for various cellular functions. Arachidonic acid (AA) is known to be the immediate activator of the membrane NADPH oxidase to generate superoxide in phagocytic and non-phagocytic cells. Past studies have been concentrated on the harmful effect of ROS but not the physiological function of ROS in the lens epithelial cells. We use PDGF as a model to examine if ROS is associated with the mechanism of mitogenic function in human lens epithelial (HLE) B3 cells and its link to AA-mediated endogenous ROS generation.

Methods: Confluent HLE cells were subjected to stimulation by AA, its metabolites and specific inhibitors to its metabolic enzymes. The generation of superoxide anion was quantified using lucigenin-amplified chemiluminescence (LUCL) in live HLE cells, recorded with a luminometer (LumiStar BMG). Cells preloaded with superoxide dismutase (SOD) or mannitol were used as negative controls. A time- and concentration-dependent AA-stimulated activation of mitogen activated protein kinases (JNK, MEK and ERK) was done using western blot analysis with respective phospho-specific antibodies. The endogenous AA release in HLE cells upon PDGF stimulation was tested by using H³-AA preloaded cells for 24 hrs, followed by monitoring the labeled AA release into the culture medium after addition of 1-20 ng/ml of PDGF.

Results: Our results showed that AA at dosage of 30-150 μ M proportionally induced luminescence generation but was ineffective in cells preloaded with SOD or mannitol. Mainly the 5-lipoxygenase pathway contributed the generation of superoxide anion. Western blot analysis of cell lysates showed that AA at dosage of 30-150 μ M progressively and transiently activated MEK, ERK and JNK during the first 30 min. PDGF stimulated the cells to release AA in vivo in a time- and concentration-dependent manner.

Conclusions: An NADPH oxidase-associated ROS generating system is present in HLE cells and can be activated specifically by AA and its metabolite of 5-HETE. This ROS-generating system participates in mediation of the mitogenic function of PDGF.

■ 1464

Mimicking the electrical signals of the lens - the effects on migration and wound healing of lens epithelial cells

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Purpose: An endogenous electrical field (EF) exists in the vertebrate lens, but the influences of this on lens epithelial cell (LEC) function are unclear. We have mimicked this electrical signal in culture and studied the effects on isolated LECs and on wounded monolayers of LECs.

Methods: Standard tissue culture techniques were used. EF application was made using specially designed culture chambers. The design features of these chambers that allow the endogenous EF of the lens to be accurately mimicked have been published fully in IOVS 44, 244-249 (2003). The effects of a physiological EF were studied on LECs from either the anterior cap, or the equatorial region of the adult bovine lens.

Results: The migration of LECs was directed by the applied EF. Directed migration was a growth factor dependent event, since it was inhibited completely in serum-free medium and restored partially by the addition of bFGF. Anterior and equatorial LECs responded differently. Anterior cells migrated anodally, but equatorial cells migrated either cathodally or anodally, depending on the EF strength. A physiological EF also regulated the closure of wounds in LEC monolayers. EF-directed migration of single LECs and of wound closure required activation of the MAP kinase signaling pathway.

Conclusions: Mimicking the endogenous electrical signals of the lens directed migration and wound healing of LECs strikingly. LEC migration during development and at the equator as differentiation into fibre cells begins may be under electrical control. In addition, an applied EF may be useful in regulating the aberrant migration of LECs that occurs during posterior capsule opacification.

■ 2101

Alterations of the corneal topographic pattern during a one minute blink interval in healthy subjects

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Purpose: To observe the change of the corneal topographic pattern in healthy subjects during a one minute pause in blinking.

Methods: Using TMS-1 corneal topograph photos were executed 5, 15, 30 and 60 seconds after a complete blink in 36 right eyes of 36 healthy subjects. The topographic pattern of each image was assessed from the color-coded topographic map, and was classified in 5 groups (in order of decreasing regularity: round, oval, symmetric bow-tie, asymmetric bow-tie and irregular).

Results: During a one-minute long interblink period, change in the topographic pattern was found in 19 normal subjects (52,78%) and it was stable in 17 subjects (47,21%). In the whole group of normal subjects, the alteration of the topographic pattern was statistically significant ($p=0.03$). The proportion of regular and irregular patterns increased at 15 seconds compared to that at 5 seconds, then decreased with time.

Conclusions: Tear film dynamics might change the topographic pattern of corneal surface during the interblink period even in healthy eyes in more than the half of the cases. Thus the standardisation of the time interval of topographic image capture after blinking is important. After blinking the surface regularity shows improvement due to the building-up of the tear film. This mechanism is followed by the distortion of the corneal surface which is the sign of the break-up phenomenon of the tear film.

■ 2102

Variability of flap thickness with the hansatome microkeratome

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Purpose: To analyze the variability and the factors influencing the flap thickness (FT) obtained with the Hansatome microkeratome (HMK) by means of intraoperative pachymetry (IP).

Methods: Retrospective analysis of 272 eyes (164 myopic LASIK patients). Preoperative parameters studied include age, keratometry, spherical equivalent (SE), corneal central thickness (CCT), corneal diameter, and A-scan axial length of the eye. IP was performed centrally and at four midperipheral positions (1) before the microkeratome cut, (2) after the HMK cut, and (3) after the laser ablation. Three groups were established according to the microkeratome heads used (nominal thickness): (A) 160 μm , (B) 180 μm , and (C) low-compression 180 μm . Statistical analysis was mostly based on linear regression.

Results: The average \pm SD FT was =134 \pm 22 μm (group A), 164 \pm 27 μm (group B) and 116 \pm 15 μm (group C). FT correlated positively with CCT in the standard-compression HMK head groups (A and B, $p<0.01$) but not in the low-compression group (C). The remaining parameters were not found significant in all groups, except for a slight correlation with preoperative SE. The reuse of the same blade for the second eye of the same patient resulted in a significant reduction of FT even in the low-compression group C (122 μm in first eye vs. 109 μm in the second).

Conclusions: The HMK creates flaps consistently thinner than the nominal value. Variability is larger in the standard-compression groups, correlating with CCT. The low-compression head creates flaps even thinner, but with lower variability. This may explain why the influence of CCT and other parameters did not reach statistical significance. IP remains a valuable method to assess the actual FT and ablation depth.

■ 2103

Corneal permeability after LASEK for myopia measured with fluorophotometry

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Purpose: To evaluate corneal permeability after surface excimer laser for myopia.

Methods: Six eyes of six patients who underwent surface excimer laser for myopia were examined with fluorophotometry after having put a fluorescein minitab in the conjunctival sulcus. Six control eyes of six different patients, matched with age, underwent the same procedure.

Results: The cornea/tearfilm complex of the treated eyes presented lower fluorescein concentrations at 3 hours measurements compared to the control group. However, anterior chamber concentrations were much higher in the treated group compared to the control group at 3 hours measurements.

Conclusions: Corneal permeability is dramatically increased after surface excimer laser procedures for myopia.

■ 2104

Effect of LASIK to correct low to moderate myopia in contrast sensitivity and accommodation in non-presbyopic patients

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Purpose: To evaluate the effect on accommodation amplitude and contrast sensitivity function before and after LASIK to correct low to moderate myopia in non-presbyopic patients

Methods: This is a prospective, single masked interventional study. Contrast sensitivity and accommodation amplitude were measured by a single examiner using the CSV-1000 and minus lens methods respectively. The mean spherical was -3.25 ± 1.35 D (range -0.25 to -6.75 D) with astigmatism lower than -3.00 D. The preoperative visual acuity was 20/20 or better. LASIK was performed using the Technolas laser, Hansatome microkeratome, the same surgeon and nomogram setting in all cases. The ANOVA and paired two tails Student-t test were performed.

Results: We analyzed 43 eyes of 43 consecutive patients who underwent LASIK for low to moderate myopia. There was no reduction in contrast sensitivity function measured in mesopic conditions at least using the CSV-1000; 3, 6, 12 and 18 cpd were the frequencies evaluated. On the other hand, we found a significant reduction in amplitude of accommodation from preoperative mean of $8.3 \text{ D} \pm 3.2 \text{ D}$ (range: 3.5-14.7) to $6.4 \text{ D} \pm 1.4 \text{ D}$ (range: 3.7-9.7), $6.3 \text{ D} \pm 1.4 \text{ D}$ (range: 3.7-9.7) and $6.6 \text{ D} \pm 1.4 \text{ D}$ (range: 4.5-10.5) at week, 1 month and 3 month respectively ($p = 0.001$ in all cases) after the procedure.

Conclusions: It appears that contrast sensitivity does not change after LASIK. We found evidence that amplitude of accommodation is reduced. The fact that this amplitude is lower than normal after LASIK may explain the frequent complains in near tasks in the early postoperative period.

Refractive surgery

■ 2105

Effect of LASIK to correct low to moderate myopia in low contrast visual acuity

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Purpose: To study the change in low contrast visual acuity in low to moderate myopic patients after LASIK.

Methods: This is a prospective, single masked, interventional case series study. The inclusion criteria were myopia lower than 7.00D and astigmatism lower than 3.00D, and visual acuity 20/20 or better. Low contrast visual acuity was measured by a single masked examiner under mesopic conditions using ETDRS 2.5 % contrast. Measures were analyzed before LASIK, 1 week, 1 month, 3 months after surgery. LASIK was performed using the Technolas laser, the Hansatome microkeratome, the same nomogram settings and surgeon in all cases. The ANOVA and paired two tails Student's T test analyses were performed.

Results: We analyzed 43 eyes of 43 consecutive patients who underwent LASIK for low to moderate myopia. The mean spherical was -3.25 ± 1.35 D (range -0.25 to -6.75 D) and astigmatism lower than 3.00D. The low contrast visual acuity before LASIK was 44.75 ± 5.1 (range: 30-56). After surgery, the means were 41.4 ± 5 (range: 30-51), 44.2 ± 5.1 (range: 32-54), and 43.1 ± 5.7 (range: 30-56), at one week, 1 month and 3 months respectively. There was no reduction in low contrast visual acuity after LASIK, at 1 month and 3 months (p: 0.6; p: 0.2, respectively).

Conclusions: Our results suggest that low contrast visual acuity measured by ETDRS 2.5 % contrast is not affected by LASIK to correct low to moderate myopia.

■ 2106

Laser in Situ Keratomileusis (LASIK) in human corneas:

Immunohistochemical findings in human donor corneas confirm in vitro LASIK model

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Purpose: To establish an in vitro model of laser in situ keratomileusis (LASIK) in human donor eyes and to test its validity in comparison with human donor corneas with prior LASIK.

Methods: LASIK was performed on 20 organ cultured human cornea discs. The excimer laser ablations performed ranged from 0 - 12 diopters. The corneas were maintained in culture for up to 6 months and were then investigated with light and transmission electron microscopy. Corneal sections of in vitro and two post mortem eyes were immunohistochemically stained for collagen type I, III, IV and VI, laminin and fibronectin.

Results: Ultrastructural investigations in the peripheral cornea revealed a disarrangement of collagen fibers indicating scar formation. These findings were not observed in the central area. Immunohistochemically fibronectin was consistently detected along Descemet's membrane and the peripheral as well as in the central part of the LASIK incision interface. In contrast, laminin could only be detected at the margin of the LASIK flap related to the ingrowth of epithelial cells. Staining for collagen type I, III, IV, VI showed a homogenous pattern with no enrichment in the area of the LASIK interface.

Conclusions: Light microscopical and immunohistochemical findings in the donor eyes with prior LASIK treatment showed conformity with the organ culture LASIK model. This study demonstrates that morphological changes following LASIK in an organ culture model simulates the in vivo situation. Therefore this model appears to be an appropriate tool for further investigations of corneal wound healing changes after LASIK.

■ 2111

Multifocal VEP using Cross-validation in Glaucoma Research

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Purpose: The diagnostic value of multifocal VEP in glaucoma research is still under debate. Several previous studies proclaim it to be a useful tool for clinical applications; according to other studies different problems still retard its clinical use. Aim of the present study was to examine, whether the mf VEP data obtained with the RETIscan system are appropriate to formulate a classification rule for glaucoma.

Methods: We examined 65 eyes of 38 advanced glaucoma patients and 27 normal subjects, using 4 occipital goldcup-electrodes for bipolar recording and a CRT Monitor (dartboard, diameter 60, chequerboard pattern reversal, 60 segments) for stimulation. In each case 8 cumulative measurements (77 s each) were made. The data of the 60 segments were cross-correlated with the aid of a RETIscan-internal VEP norm ("VEP finder"), then combined in 16 sectors and evaluated via the classification technique "double-bagging" and Wilcoxon-U-test.

Results: In three out of the 16 sectors the VEP amplitudes of the patients were significantly reduced (Wilcoxon-U-test). Applying double-bagging on the cross-correlated data (= with VEP finder) results in a sensitivity of 75 % and a specificity of 71 %, the estimated misclassification rate was 27 %. In case of the uncorrelated data (= without VEP finder) the same analysis achieved a sensitivity of about 60 % and a specificity of 40 %.

Conclusions: The achieved sensitivity and specificity suggest that - using the RETIscan system for recording - a classification of the VEP data, i.e. a separation between normal and glaucoma subjects is possible.

■ 2112

An investigation of motion perception in early glaucoma

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Purpose: To determine if motion sensitivity is preferentially impaired in early glaucoma.

Methods: 16 control and 10 glaucomatous eyes had contrast sensitivity thresholds (CST) measured with luminance modulated sinusoidal gratings at three spatial frequencies (0.5 cycles per degree (CPD), 2CPD and 8.5CPD) each at two drift velocities of 0Hz and 5Hz. A control stimulus at 4.9CPD was also used. CST thresholds were measured monocularly by a linear staircase technique analogous to the Humphrey's 24-2 threshold algorithm (HVF), which subjects also undertook. Stimuli were projected onto a computer monitor in scotopic conditions. Subjects fixated centrally and a two alternative spatial forced-choice paradigm was applied requiring subjects to choose between left or right stimuli centred at 10-degrees eccentricity on the horizontal meridian. Stimuli thus avoided the blind spot.

Results: The glaucoma subjects had reduced contrast sensitivity relative to controls both with stationary ($t=3.19, p<.002$) and with moving stimuli ($t=6.997; p<0.00000025$). The Ratio score (Moving - Stationary) / (Moving) was significantly reduced in the patients ($t=4.3; p<0.00121$), indicating a greater impairment with moving stimuli.

Conclusions: Both stationary and motion perception were reduced in early glaucoma suggesting both pathways were affected, however as there was a significantly greater impairment of motion sensitivity it is likely that the motion conducting magnocellular pathway is preferentially damaged early in the disease.

■ 2113

SITA, SWAP and FDT in early glaucoma patients, ocular hypertensives and normals

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Purpose: Purpose: The aim of the study was to compare SITA, SWAP and FDT outcomes and their skill of identifying normal people and patients suffering from Early Glaucoma or Ocular Hypertension.

Methods: Methods: 65 patients were evaluated. They were submitted to a full ocular examination and to the three perimetric tests in the same day. One eye for each subject was randomly selected. They were divided into 3 groups on the basis of the clinical data: 18 subjects were considered as normals, 17 patients were classified as early glaucoma and 30 as ocular hypertensives.

Results: Results: There is statistical association between clinical judgement and SITA (Fisher: 0.000), SWAP (Fisher 0.022) and FDT (0.020). The Kappa statistic is 0.17 for FDT vs SWAP, 0.25 for FDT vs SITA e 0.32 for SWAP vs SITA. All the tests have high specificity (respectively 94.4%, 100% and 94.4%) but low sensitivity in comparison to clinical judgement. When compared each pair of perimetric test only SITA vs SWAP is sensitive (80%) and specific (81.6%).

Conclusions: Conclusions: All tests have high specificity but low sensitivity. The only ROC curve statistically significant is the logistic model SWAP vs SITA with ROC = 0.81. There is low agreement among the three exams.

■ 2114

The effect of defect localization on the Statpac General Height estimation in SAP and SWAP

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Purpose: The general height (GH) index is used to calculate Pattern Deviation maps. We investigated the effect of visual field defect size and localization on the current estimation of the GH in SAP and SWAP.

Methods: Humphrey 30-2 FT visual fields using both SAP and SWAP in a pseudo-random order were obtained from 132 healthy subjects. All subjects were experienced with SAP and SWAP. Localized field loss (-10dB) was simulated for 5 different mean eccentricities within the 30-2 pattern. For each eccentricity increasing size of field loss was simulated using 4, 8, 12, 16, and 20 test points respectively. Thus, 25 simulated localized defects were generated. Each of these 25 defects were superimposed on each of the measured fields. GH was calculated for each field before and after superimposition of defect. The difference (dGH) between these two GH values was compared for each of the 25 defects.

Results: dGH increased with increasing size and eccentricity, being minimal (mean - 0.05, range 0 - -0.56 [SAP], mean -0.08, range 0 - -1.45 [SWAP]) for small central defects rising to mean -0.68, range -0.02 - -2.35 [SAP], mean -1.07, range 0 - -2.63 [SWAP] for large peripheral defects. dGH was significantly larger for SWAP than for SAP for 17 of the 25 defect patterns.

Conclusions: Localized field loss confounds the estimation of GH. This effect is more marked for large or peripheral defects. New methods for estimation of the GH taking these findings into account are likely to enhance the calculation of Pattern Deviation Maps and thus improve the ability to detect localized field loss and monitor localized field loss progression.

■ 2115

Evaluation and significance of acquired dyschromatopsia in diabetes

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Purpose: Computerised pseudoisochromatic (CPIC) tests, giving the opportunity of quantitative evaluation of colour discrimination, constitute a new tool for the examination of acquired colour vision disorders.

Methods: Colour vision of 60 diabetic patients (10 without retinopathy and 50 with retinopathy of different degrees of severity) was evaluated with the CPIC test. Colour difference thresholds were measured separately in the three main confusion axes (protan, deutan and tritan), and the total colour discrimination threshold scores were calculated. All patients underwent detailed ophthalmologic examination, and the retinopathy status was graded using digital fundus photography, according to ETDRS criteria. The presence and extent of clinically significant macular oedema was demonstrated by fluorescein angiography.

Results: Diabetic patients without retinopathy did not show significant difference in any axis from the values of non-diabetic controls. In patients with retinopathy, all CPIC scores were elevated with increasing severity of retinopathy, but the tritan score proved to be the most sensitive: significant correlation was found between the tritan scores and the extent of macular oedema. Considerable elevation of the tritan value was noticed as well in laser-treated eyes.

Conclusions: The tritan type colour vision disorder in diabetes may develop as a result of photoreceptor disorientation in macular oedema or as a consequence of damage of the perifoveal area, and it may precede the decrease of visual acuity. On the basis of our experiences, the CPIC test is a simple and useful method in the diagnosis of tritan type dyschromatopsia caused by diabetic retinopathy, and it may play a part in early detection and follow-up of diabetic macular oedema.

■ 2116

Colour assessment & diagnosis: the new CAD test for the measurement of congenital and acquired loss of chromatic sensitivity

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Purpose: Colour vision, in particular chromatic sensitivity (CS), is a very good indicator of the normal functioning of the retina. CS is affected most and earliest in a number of diseases of the retina and the optic nerve. Diagnosis of congenital deficiencies or detection of small changes in CS as a result of disease are therefore of great interest. Our purpose was to develop a new Colour Assessment and Diagnosis (CAD) test to measure small changes in CS and provide accurate classification of even minimal congenital deficiencies.

Methods: The method developed is based on burying a moving, colour-defined stimulus in dynamic luminance contrast (LC) noise. The LC noise provides effective masking of LC signals without affecting the thresholds for detection of colour signals (Proc.R.Soc.Lond. B Biol.Sci. 258: 327-334, 1994). Detection thresholds are measured along 16 directions in colour space and the results are plotted in the CIE-(x,y) 1931 colour system.

Results: 100 normal trichromats and 120 colour deficient observers have been studied using the CAD test and a battery of conventional colour vision tests. The data obtained in normal trichromats were used to define the "normal" trichromat's performance on the CAD test and provide a template for instant diagnosis of normal or deficient colour vision. Data from deutan and protan subjects form distinct patterns that can be used to classify even minimal congenital deficiencies.

Conclusions: The present findings show that the CAD test provides an efficient and rapid method to detect, classify and measure CS loss using a colour system that is reproducible and recognised internationally.

■ 2141

Isolation and expansion of adult human eye neural progenitors

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Purpose: Isolate and generate high amounts of adult human retinal progenitors.

Methods: After full retinal development, retinal stem cells can be retrieved in adult mice at the level of the ciliary margin. By anatomical homology, we isolated pigmented cells from the pars plana of human donors of various ages using enzymatic dissociation and growth stimulation with EGF.

Results: Pigmented cells proliferates in response to insulin only, forming big clusters of cells termed spheres. The presence of EGF and FGF-2 increased the number of proliferating progenitors. No differences in sphere growth or number were observed between the donors, including for people aged 80. The kinetics of sphere growth is comparable to that observed in mice. After cell differentiation induction, single spheres generated both neuron-like and glia cells attested by the expression of β -tubulin-III and vimentin respectively as well as by their morphology. Expansion of neural progenitors can be achieved by controlled conditions: a 10 million-fold expansion can be reached in 42 days. After expansion, cells maintain the ability to generate neurons and glia, but to a lesser extent.

Conclusions: It appears that adult human retinal progenitors provide a new tool to study human neurogenesis and may have the potential to be a source of photoreceptors that can be used for transplantation studies in animal models of retinal degeneration.

■ 2142

Stem Cells in Adult Human Retina and in Epiretinal Membranes

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Purpose: Nestin is an intermediate filament marker for neural progenitor/stem cells. We aimed to identify nestin-positive cells in adult human retina and within surgically-removed epiretinal membranes.

Methods: Adult human retina and epiretinal membranes were studied. Tissue was fixed and processed for immunohistochemical detection of nestin and glial fibrillary acidic protein (GFAP) expression.

Results: Nestin-positive cells were found in both adult human retina and in epiretinal membranes. In retina these presumed progenitor cells are most prominent at the ora serrata where they possess fibrillary processes, and are arranged radially within or superficially on the retina and in the peripapillary retina where they are rounded, with speckled cytoplasmic nestin staining. In the peripapillary retina many of the cells in the retinal ganglion cell layer are nestin-positive. These cells appear to represent nestin-positive neurones. In epiretinal membranes we found patches of nestin-positive cells that co-expressed glial fibrillary acidic protein.

Conclusions: These findings suggest that some neurones and glia in the adult human retina are nestin-positive and thus have regenerative capacity. The role of these cells in pathological responses to retinal disease is inferred by the presence of large numbers of ectopic nestin-positive cells in epiretinal membranes. We hypothesise that nestin-positive cells represent a population of progenitor cells that differentiate into the cells that make up retinal scar tissue in response to injury.

■ 2143

Comparative Proteome Analysis of Retinal Pigment Epithelial Cell**Dedifferentiation in vitro**

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Purpose: To better understand the process of retinal pigment epithelial (RPE) dedifferentiation in vitro and to determine its potential significance in the pathophysiology of proliferative vitreoretinopathy (PVR).

Methods: Total cellular proteins derived from differentiated, native and monolayers of dedifferentiated, cultured human RPE cells were processed and analyzed by 2-D electrophoresis. Silver stained spots of interest were excised, digested in situ and analyzed using MALDI-TOF mass spectroscopy and database research.

Results: Proteins, which are required for interaction with photoreceptor cells including RPE 65, CRALBP, and CRBP were absent in dedifferentiated RPE cells. Proteins involved in other specialized functions such as phagocytosis and exocytosis, including Cathepsin D and Clathrin were still present. Dedifferentiation led to a strong shift towards increased expression of proteins associated with cell shape, cell adhesion and stress fiber formation (cytokeratin 19, vinculin etc.), and also acquired increased expression of factors involved in translation and tumorigenic signal transduction (annexin I, translation initiation factor (eIF)-5A etc).

Conclusions: Dedifferentiation of human RPE cells in vitro results in downregulation of proteins associated with highly specialized functions of the RPE and induces the differential expression of proteins related to cytoskeleton organization, cell shape, migration and mediation of proliferative signal transduction. Further investigation of candidate proteins may identify additional targets for treatment or prevention of diseases associated with RPE dedifferentiation.

■ 2144

Serum-free cultivation of human adult choroidal melanocytes

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Purpose: To cultivate human adult choroidal melanocytes without serum, toxic or tumor-promoting supplements.

Methods: Choroids of human donor eyes were incubated in 0.6 U/ml dispase II / 0.5 ug/ml collagenase I+IV for 18h as sole enzyme treatment or after preincubation in 1 mg/ml collagenase I+IV for 4h to release RPE cells first. Enzyme reaction was stopped by protease inhibitor, cells were washed and seeded in serum-free Melanocyte Growth Medium M2 (PromoCell) in gelatin coated or uncoated T25 cell culture flasks. Medium was changed 2-3/week. Contaminating RPE cells were eliminated by geneticin for 7-10d before passaging with trypsin. Trypsinized cells were seeded at 2,000 cells/cm² on gelatin-coated or uncoated culture dishes. Eventually, cells were cryopreserved in liquid N₂ using medium M2 + 10% DMSO.

Results: Under serum-free conditions, cultures underwent more than 10 cumulative population doublings. Coating of culture dishes did not significantly influence attachment or proliferation. Freshly isolated cells showed a bipolar morphology, whereas subcultured cells became smaller with a bipolar to stellate morphology. Confluent cultures consisted of bipolar cells in a whorling pattern and triangular cells in a cobblestone-like pattern. Triangular cells seemed not to be senescent but quiescent, and started to proliferate after trypsinisation, showing again a bipolar morphology. Cultured cells lost pigment after initial seeding, but displayed melanogenesis throughout the entire cultivation period.

Conclusions: Melanocyte Growth Medium M2 is a commercially available, serum-free medium that contains no phorbol esters or supplements like cholera toxin. Using this medium, cultures of human adult choroidal melanocytes could successfully be established and maintained for several months.

■ 2145

Decreased production of SPARC contributes to enhanced invasion of lens epithelial cells in vitro

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Purpose: A structurally abnormal lens capsule, damaged by the underlying lens cells, is believed to be partly responsible for the cataracts in SPARC-null mice. Lens epithelial cell (LEC) cultures from wt and SPARC-null mice were produced to study the role of SPARC in the regulation of LEC function.

Methods: LECs were cultured from lens explants. Proliferation was measured by an MTT (tetrazolium salt) assay. In vitro adhesive and invasive capacities of LECs were evaluated on matrigel-coated plates and transwells. SPARC, matrix metalloproteinases (MMPs), and adhesive molecules were assessed by RT-PCR, immunocytochemistry, and immunoblotting. A Giemsa-banded karyotype was performed for chromosome analysis.

Results: Two long-term cultures were studied, WP10 cells produced abundant SPARC whereas KP11.12 cells did not. KP11.12 LECs exhibited a significantly increased invasion through matrigel, relative to wt cells. The addition of exogenous SPARC to the KP11.12 cells significantly reduced their invasion. WP10 cells displayed a 3-fold increase in SPARC protein, compared with primary wt LECs, and did not invade the matrigel. WP10 cells were also less adhesive on matrigel in comparison to SPARC-null cells. MMP2 was inactive in WP10 cells, whereas the KP11.12 cells showed increased activity. Although WP10 and KP11.12 cells exhibited abnormal karyotypes, certain marker chromosomes were retained.

Conclusions: Decreased expression of SPARC in LECs is associated with enhanced invasion through matrigel in vitro, a finding indicating that LECs become invasive when SPARC is reduced or deleted. LECs could invade and damage the lens capsule and thereby contribute to the cataracts observed in SPARC-null mice.

■ 2146

Pro-angiogenic N-cadherin fragments

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Purpose: Ectodomain shedding releases soluble fragments (sN-CAD) from the extracellular domain of N-cadherin. We found previously that sE-CAD stimulated the invasion of epithelial cancer cells. Here, we investigated whether sN-CAD stimulated the invasion of endothelial cells and so exert a pro-angiogenic activity.

Methods: ARM cells are mouse sarcoma cells transfected with cDNA encoding chicken N-cadherin; when treated with plasmin their conditioned medium is a source of sN-CAD, that can be removed by immunodepletion. Angiogenesis was assessed in the chick chorioallantoic membrane and the rabbit cornea. The presence of sN-CAD was tested in conditioned medium of the retinoblastoma cell lines Y79 and WERI-Rb-1.

Results: ARM-conditioned medium stimulates angiogenesis in the chick chorioallantoic membrane and the the rabbit cornea, and this pro-angiogenic activity is abolished by immunodepletion of the conditioned medium. A 10-mer HAV-comprising peptide, identical to amino acids 235-244 from human N-cadherin, revealed the HAV-sequence as crucial for the pro-angiogenic effect of sN-CAD. In the rabbit cornea, high doses of the HAV-comprising peptide stimulated angiogenesis. sN-CAD was present in the conditioned media of retinoblastoma cell lines.

Conclusions: The present study provides evidence that sN-CAD acts as a proangiogenic molecule in the chorioallantoic membrane and in the rabbit cornea. The HAV-motif of N-cadherin is crucial since it is responsible for the pro-angiogenic effect of sN-CAD. sN-CAD might be important for inducing angiogenesis in retinoblastoma.

■ 2151

Pathogenesis of Diabetic Retinopathy: the vascular component versus the retinal cellular component

LUND ANDERSEN H

■ 2152

Assessment and follow-up of Diabetic Macular Edema by OCT

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Purpose: To discuss the role of optical coherence tomography (OCT) in the assessment and follow-up of diabetic macular edema (DME).

Methods: Data from the literature regarding the characteristics, value and reliability of morphologic, numeric and topographic information provided by OCT in patients with DME have been reviewed. Prospectively collected data on the response to laser photocoagulation of patients with clinically significant DME were also analysed.

Results: Reports on morphologic assessment of eyes with DME have demonstrated that the high axial resolution of OCT allows for accurate visualisation of epiretinal, intraretinal and subretinal changes. A retina mapping protocol of the macula enables to exactly locate and objectively quantify areas of thickening with good reproducibility. A significant decrease of foveal thickness after laser treatment for DME was almost always associated with a visual improvement in our series of patients.

Conclusions: These data suggest that OCT can be used to objectively demonstrate and quantify vitreoretinal, intraretinal and subretinal changes associated with DME and to determine a positive functional response to laser treatment.

Update on diabetic retinopathy

■ 2153

Factors influencing management of diabetic macular oedema

DOWLER J

■ 2154

New treatment strategies for Diabetic Macular Edema

MASSIN P

■ 2155

Screening for sight-threatening Diabetic Retinopathy

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Purpose: To discuss the clinical and socioeconomic implications of screening for sight-threatening Diabetic Retinopathy.

Methods: Current international screening protocols, data from established and new screening tests, experiences from north European countries and cost-effectiveness of screening were reviewed.

Results: Evidence based medicine data have shown that vision loss can be effectively prevented or delayed by early detection of retinopathy and laser treatment. Moreover it has been shown that screening for sight-threatening diabetic retinopathy and its treatment is the most cost-effective medical procedure known today. Among the screening tests, the gold standard for assessing and grading Diabetic Retinopathy remains the ETDRS slide color photography. However, new imaging systems allowing for easier and more efficient image acquisition and archiving and potentially useful for telemedicine purposes are currently being developed.

Conclusions: These data suggest that by implementing screening programs for sight-threatening Diabetic Retinopathy, substantial savings, both monetary and in sight years, may be achieved.

■ 2161

Modern eye biomechanics as an effective tool for evaluation of advanced directions in clinical and experimental research

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Purpose: The human eye is a unique biological and physiological system, with an essential number of structural elements functioning in accordance with the laws of biomechanics. The application of biomechanics, its ideas and methods in practical tasks of ophthalmology has proven to be very promising, since this synergetic science is based on analytical and experimental results of physics, mechanics, hydrodynamics, biology, ophthalmology, physiology.

Methods: We present an overview of applications of biomechanical principles that have been successfully implemented in ophthalmology. Possible future utilisations are outlined.

Results: Ocular biomechanical principles are able to validate known and to introduce new concepts and ideas of ocular function. They help to understand yet unanswered questions in this field. New biomechanically supported conceptions such as: dynamic theory of aqueous outflow, "breathing" of eye shells, optical corrections of IOP disorders are presented here and in the papers of the symposium. Other main topics to be mentioned include: biomechanical properties of eye tissues and eye structures; biomechanics of intraocular cells and cell-to-cell interactions; biomechanics of the ocular regulatory systems and interactions between those regulatory systems; biomechanics of eye trauma and eye diseases; biomechanics in ophthalmic surgery and postoperative situations; biomechanics of eye implants and surgical instruments.

Conclusions: Modern eye biomechanics represents a powerful tool for the basic scientist, biomedical engineer and practical ophthalmologist to establish new methods for diagnosis and treatment of eye disorders, as well as to better predict results of treatments.

■ 2162

Theoretical aspects of aqueous outflow and pressure distribution after glaucoma surgery

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Purpose: Applying hydraulic principles to compare the postoperative situation in non-penetrating deep sclerectomy (NPDS) and trabeculectomy (TE) as well as to attempt to explain unanswered aspects of the postoperative outflow.

Methods: A primary linear mathematical model based on fluid mechanics and clinical data was used to evaluate possible postoperative outflow pathways after filtration surgery.

Results: Based on mathematical modeling and using preoperative and postoperative IOP values the amount of postoperative outflow through the fistula (membrane) and the outflow through the residual natural drainage pathway could be estimated. Postoperative outflow after non-penetrating deep sclerectomy and trabeculectomy was compared taking into account changes in hydraulic resistance of natural aqueous outflow pathways pre- versus postoperatively. The difference in filtration blebs after TE and NPDS, as well as pointlessness to prolong the scleral lake into the suprachoroidal space in modifications of NPDS in order to increase the outflow from the scleral lake is shown with the model.

Conclusions: According to theoretical considerations on the base of biomechanics and hydrodynamics residual postoperative aqueous outflow through the conventional and uveoscleral pathways exists. NPDS provides more aqueous outflow along the natural outflow pathways than TE. Generally, the higher the postoperative IOP and / or the lower the preoperative IOP the higher the amount of aqueous utilizing natural outflow pathways postoperatively. From our calculations it seems that the reestablishment of aqueous production postoperatively in addition to factors such as wound healing may be a reason for postoperative IOP increase in some patients.

■ 2163

New dynamic theory of the aqueous outflow. A result of biomechanical analysis of non-explained clinical data

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Purpose: We have analyzed the contemporary outflow theory, using methods of morphology, physiology, ophthalmology, biochemistry, thermal physics, mechanics and biological systems regulation theory.

Methods: A new "Dynamic" theory has been issued, which is based on new mechanism of sclera "breathing" The dynamic theory corresponds better to the laws of mechanics, and it also helped to explain the results of using holinomimetics, cycloplegics and prostaglandins.

Results: The main outcome of this analysis is that the eye is fine tuned in order to pump a certain amount of AH which helps to maintain the metabolism on appropriate level, but not for keeping the IOP on the constant level. The key passive mechanism of stabilizing the IOP is ability of the elastic sclera to stretch. But, as the eye is ageing its sclera is becoming more rigid, causing the IOP to increase so the mechanism of sclera "breathing" can remain efficient, which is noticed in almost all healthy eyes. In the eye with healthy elastic sclera the effect of IOP lowering medication is hardly noticeable or even absent. But as the sclera becomes more rigid its ability to stretch decreases, the effect of IOP lowering medication gets significant.

Conclusions: Ageing of the sclera is the basic link in open angle glaucoma pathogenesis. Providing the perfect metabolism by means of AH flow through the eye can be described in terms of newly developed dynamic theory, which is based on the mechanism of "sclera breathing"

■ 2164

Biomechanical criteria and methods of assessment of sclera reinforcement procedures in progressive myopia

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Purpose: Biomechanical studies of progressive myopia pathogenesis revealed serious derangements in viscoelastic properties of highly myopic scleras. To stabilize the myopic process and prevent the associated dystrophic changes of the fundus, several types of sclera-strengthening operations (scleroplasty) are practiced in clinic. The paper is aimed at developing biomechanical criteria of assessing the real effect of these procedures on the biomechanical status of the sclera in the experiment.

Methods: Complex biomechanical studies of 88 eyes of Chinchilla rabbits at different times after scleroplasty using biological and synthetic transplants included 1) life-time assessment of the radial elasticity modulus Erad and the residual deformation d of the corneoscleral capsule by ophthalmic mechanography, 2) mechanical testing (determination of tensile strength T; meridional elasticity modulus Em and ultimate strain D) of isolated scleral tissue using Instron-1029.

Results: After all types of interventions T, Erad and Em of operated eyes demonstrate a credible, if of varied degree, growth as compared to intact eyes ($p < 0.05$). This correlates well with the clinical results, which evidence a halt or inhibition of myopia progression in 75-95% of cases. The dynamics of d and D is closely connected with the course of the post-operative period and is determined by the extent of the local edema and the stage of new connective tissue formation within the operation field.

Conclusions: The comparison of experimental data obtained in vivo and ex vivo testifies to the fact that the degree of improvement reached in biomechanical parameters of the scleral capsule of the eye can serve as an efficiency criterion of sclera reinforcing treatment of progressive myopia.

■ 2165

Contemporary conceptions of the Helmholtz's accommodation theory

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Purpose: An extensive own research in biomechanics of accommodation in animal and human eyes as well as results obtained by other researchers made it possible for us to work out key principles of interconnected regulation mechanisms of accommodation using methods of morphology, physiology, ophthalmology, optics, mechanics and regulation theory of biosystems.

Methods: Application of all these principles allowed us to offer a new interpretation of Helmholtz theory so it is in complete correspondence with laws of mechanics in all phases of accommodation.

Results: In human eye there are several extraocular and intraocular accommodation mechanisms, but the basic one is deformation of the lens and change of its refraction power (the only mechanism acknowledged by Helmholtz). This general mechanism of accommodation has two opposite states ?maximal accommodation for close look and no accommodation (complete non active relaxation of MC). So during the phase of no accommodation the lens appears to be constrained between mighty anterior portion of fibers and extra tightened VC whose pressure is elevated by the front vitreous membrane.

Conclusions: In the eye there are several accommodation mechanisms. There are only two phases of accommodation (by Helmholtz) ?active accommodation (complete or partial) for close look and no accommodation for look at the distance. There are no physiological or morphological clues to suppose active nature of disaccommodation. In our rethought Helmholtz mechanism of accommodation we consider the lens and choroid to be directly connected with each other by zonular fibers and play its part in accommodation along with VC and MC which is attached to the choroid in the area of ora serrata. The role of celioequatorial portion of fibers is secondary, besides, their design does not allow to consider them to play the key role in the executive mechanism of accommodation by R. Schachar.

■ 2166

Ex-vivo Lens Stretching Experiments on Primate Eyes

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Purpose: To measure forces and optical power in natural and refilled ex-vivo primate lenses during simulated accommodation.

Methods: Circumferential loads were applied to fresh rhesus and cynomolgus monkey lens immersed in DMEM media thru 8 PMMA shoes glued to the sclera posterior to the limbus. Each 5-6.3mm thick shoe is connected with 6-0 Prolene suture line to a single load cell by a T-shaped bar via a system of hooks and pulleys. A computer controlled stepper motor radially stretches the lens sample up to 2mm in 0.5mm steps. A Scheiner principle dual spot projection system measures the changes in lens focal length during stretching. Actual lens diameter changes are measured from video recordings made during the experimental runs. These values are used for calculating the applied strain. Load vs. strain and optical power vs. loads were evaluated, and loads vs. stepper motor displacement is used to demonstrate the repeatability of results.

Results: Using our original protocol of 1.5mm radial stretch the monkey lenses showed an accommodative change in fluid of 7 to 14D with the unstretched lens power ranging from 28 to 54D and the stretched lens ranging from 19 to 46D, with 3-12g stretching loads. With the current protocol of 2mm radial stretch the monkey lenses showed an accommodative change in fluid of 10 to 22D with the unstretched lens power ranging from 34 to 54 D and the stretched lens ranging from 22 to 43D, with 4-12g stretching loads. The single human specimen tested this year was a 19 year old with 6D of accommodation, 32.5D unstretched and 26.5D stretched with a 3.4g load.

Conclusions: An ex-vivo simulated accommodation system can be used to measure forces and optical power in natural and refilled lenses.

■ 2167

On the deformation of the lens under accommodation

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Purpose: A correct biomechanical model of an eye accommodation may help for better understanding of this visual mechanism. For the last years a number of works by R. Schachar dealt with the theory of accommodation were published. In these papers the classical theory of accommodation has been questioned. R. Schachar supposed that the increasing the zonular tension increases rather than decreases the power of the lens. One of the work by R. Schachar devoted to mathematical proofs of his hypothesis on the base of the linear theory of shells. It is clear that with this theory only small displacements of the lens can be described. The other assumption of the Schachar model is that the resultant force is parallel to the equatorial plane. So the role of the vitreous in the mechanical behaviour of the lens was ignored.

Methods: We consider the mathematical model of accommodation, which takes into account the real structure of the zonular fiber system and interaction of lens and the anterior hyaloid membrane. Nonlinear Chernykh equations are used for determination of the deformation of the lens under accommodation.

Results: The shape of the deformed lens and the change of the internal pressure in the lens are found. It is shown that the increasing the zonular tension decreases the power of the lens. The proposed model well agrees with the practical data.

Conclusions: The proposed mathematical model is consistent with the classical Helmholtz theory of accommodation.

■ 2168

Numerical studies of the importance of changes in lens geometry and stiffness in causing presbyopia

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Purpose: To improve understanding of the factors that cause presbyopia, so that the effectiveness of proposed surgical procedures to increase accommodation amplitude in presbyopes (e.g. lens re-filling of the kind described by Koopmans et al. (2003) *Invest Ophthalmol Vis Sci.* 44: 250-257.) can be assessed.

Methods: A finite element model (based on Burd et al. (2002) *Vision Research* 18: 2235-251), is used to investigate the influence of (a) lens stiffness (b) capsule stiffness (c) lens geometry and (d) contraction of the ciliary muscle on accommodation amplitude and the development of presbyopia. This model incorporates data from a number of sources, in particular Fisher's (1971) values of Young's moduli of lens nucleus and cortex as functions of age. Calculations are described for lenses of age 29 years and 45 years. A preliminary finite element model of a lens-refilling procedure, applied to an 84 year lens, is described.

Results: Using Fisher's (1971) lens stiffness values, although stiffening with age of the lens reduces the amplitude of accommodation, stiffness changes alone do not account for the computed reduction of accommodation amplitude with age. Changes with age of lens geometry are seen as being particularly influential in the development of presbyopia. The computed results of the lens-refilling analysis are compared with the in-vitro studies of Koopmans et al. (2003)

Conclusions: This study indicates that if Fisher's (1971) lens stiffness values are correct, presbyopia is only partly linked to the increasing stiffness with age of the lens, and the consequence of this would be that lens-refilling procedures should, in principle, be able to restore some, but not all, of the ability of elderly eyes to accommodate.

■ 2169

Mechanics of lens refilling

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Purpose: An insight into the mechanics of accommodation and presbyopia has been obtained by several methods, including stretching tests and calculations using the finite elements method. As an extension to this, calculations can be performed on lens refilling, the purpose being to predict possible outcomes of lens refilling procedures, expressed in terms of quality of vision.

Methods: A mechanical model of the lens was built using in vivo measurements and literature data. Changes of the lens shape as a result of zonular stretch were calculated using the finite elements method. Lens refilling was simulated, using a refill volume of 100%. In a separate simulation, the impact of an off-axis mini-capsulorhexis was calculated. The calculated shape of the lens was used as input for an optical eye model. Optical calculations were performed for three aspects of visual quality: accommodative amplitude, refraction and visual acuity.

Results: The shape of the lens after refilling differs from the natural shape of the lens. Differences in surface curvatures would change the refraction, but this can be accounted for by the refractive index of the refill material. Also the asphericity of a refilled lens differs from asphericity of the natural lens. Nevertheless, this change in asphericity does not lead to a significant change in visual acuity. With a capsulorhexis in the anterior capsular surface, the rotational symmetry is lost. This results in a small amount of astigmatism (less than 0.05D).

Conclusions: Lens refilling has many mechanical aspects. The numerical simulations suggest that lens refilling can restore accommodation and that the refilled lens can have sufficient optical quality.

■ 216a

Biomechanical aspects of the correction of accommodative disorders in myopia using functional methods

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Purpose: To study how a biomechanical procedure, infrasound pneumatic massage (PM) and low-energy trans-scleral laser stimulation (LS) of the ciliary zone can affect the state of accommodation and visual functions in young patients with myopia and other refraction errors and no eye fundus pathologies.

Methods: 286 patients aged 7-23 (18 with low hyperopia, 116 with low myopia, 115 with moderate myopia, and 33 with high myopia) have been examined using biomicroscopy, ophthalmoscopy, and refractometry. In addition, relative accommodation volume and reserve (resp. RAV and RAR) were determined. All patients received a 5-day PM treatment course. 60 of the patients who had RARE-1.5D also received LS.

Results: All patients showed an increase in both uncorrected and corrected (with previously used glasses) visual acuity by 1 to 2 lines. Asthenopic phenomena vanished after the third procedure. In 73% of the patients, RAR increased by 0.92 D. In myopic cases, the total RAV grew by 1.3 D, whilst muscular capacity for strain and relaxation estimated by the location of the nearest (pp) and furthest (pr) points of clear vision changed, too: pr "retreated" by 1.79 D ($p < 0.05$) and pp moved somewhat (by 0.56 D) closer ($p > 0.05$). In more than half of the patients the location of pp and pr of both eyes became symmetric, which is a favorable factor for the normalization of binocular visual work capacity.

Conclusions: After the treatment administered, an improvement of biomechanical parameters of ciliary muscle operation was noted in low and moderate myopia as well as in patients with visual fatigue in emmetropia and low hyperopia.

■ 2171

Running a Genetic Eye Clinic: How to get started

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Purpose: Running a Genetic Eye Clinic can be a very efficient way of managing patients with inherited eye disease. The combination of complementary Clinical skills means that diagnoses can often be reached at an earlier stage and many families require fewer attendances at the hospital.

Methods: In the first part of this special interest symposium we shall be discussing how to set up a Genetic Eye Clinic giving examples of both good and bad practice. We shall draw from our own experience and suggest ways of organising the Clinic that we have found successful including essential personnel and equipment. Pre / Post - Clinic contact with patients, the role of training and research and useful sources of genetic information will be discussed.

Results: The second part of the symposium will concentrate on the range of conditions referred to the Clinic. Modes of inheritance and the ways in which one might investigate an inherited basis for disease will be explained. Examples will be given to illustrate the relevance of chromosomal analysis and the assortment of genetic investigations that are currently available on both a diagnostic and research basis across Europe. Case studies will be drawn from a Genetic Eye Clinic in the UK and from Belgium.

Conclusions: Finally, we shall discuss the role of the Genetic Counsellor. How long should we give patients, where should counselling take place, who should be present and what do patients remember? We shall round up the session with a discussion about the ethical considerations of access to medical records, examining children, the role of predictive testing and protocols.

■ 2172

Referrals and investigations

BLACK GCM

Running a genetic Eye Clinic

■ 2173

Counselling and the future

NEWBURYR

■ 2174

Experiences from other Units

LEROYBP

■ 2191

Corneo-Conjunctival CIN: Ten-Years Retrospective Study

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Purpose: To report the clinicopathologic characteristics of patients with conjunctival squamous cell carcinoma at the Ocular Oncology Units in University Hospital and IOBA, Valladolid between 1992 and 2003.

Methods: Retrospective study of clinical records and histopathologic specimens of patients with conjunctival squamous cell carcinoma.

Results: A total of 34 patients were included in the study, 58.8% were male. The mean age at diagnosis was 60 years old (range 25-84 years). Five patients (14.7%) had history of malignant skin tumors and 5 patients (14.7%) of visceral malignancies. Eleven patients (32.3%) had a previous outdoor occupation, 23 patients (67.6%) had other eye findings related to extensive solar exposure, and 9 patients (26.4%) had previous ocular surgery or trauma. Growth of the lesion (38.2%) and inflammatory symptoms (32.3%) were the most common presenting complaints. The tumor was unilateral in 31 patients (91.1%) (16 RE/15 LE), and bilateral in two cases (5.8%). The neoplasia affected the limbus in 25 cases (73.5%), the cornea in 16 (47%), and the bulbar conjunctiva in 19 (55.8%). The clinical appearance was gelatinous in 13 patients (38.2%), leucoplakic in 11 (32.4%), and papilliform in 4 (11.8%). The initial clinical diagnosis was accurate in 89.3% of cases. Twenty two patients (64.7%) were primarily managed whereas 12 patients (35.2%) had received previous treatment elsewhere. Initial treatment was excision surgery in 24 patients (70.5%), and surgery combined with adjuvant therapies in 5 patients (14.7%). After a mean follow up of 33.6 months (range 5-124), 5 patients (14.7%) developed recurrences (mean 1.6 recurrences, range 1-3). One patient developed orbital invasion. No patient developed regional lymph node invasion, distant metastases, or tumor related death. Tumors that present recurrences showed on histopathologic study of the primary lesions less subconjunctival actinic degeneration (chi square $P < 0.05$) than non-recurrent tumors. In those tumors more severe atypia (80% vs. 30%) and dysplasia (100% vs. 51%) was also observed, although non-statistically significant.

Conclusions: Conjunctival squamous cell carcinoma is an ocular surface malignancy related to extensive sun exposure. This tumor usually presents as a unilateral gelatinous mass involving the limbus in elderly man. Less actinic degeneration and more severe degree of atypia and dysplasia may represent histopathologic risk factors for local recurrences.

■ 2192

Conjunctival Melanoma: Ten-Years Retrospective Study

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Purpose: To review the conjunctival melanoma cases at the Ocular Oncology units of the University Hospital and IOBA of Valladolid between 1992 and 2003

Methods: Retrospective study of patients with conjunctival melanoma clinically and histopathologically confirmed.

Results: A total of 13 cases (5 males/8 females) were reviewed, the mean age of the patients was 58 (range 35-79) years. Conjunctival melanoma originated from nevus (7 cases), primary acquired melanosis PAM (5 cases), or de novo (1 case). Six patients had received previous surgical treatment elsewhere. In 9 patients with primary conjunctival melanoma, four cases were handled with local excision alone, two cases with excision combined with adjuvant topical Mitomycin C, one case with excision plus cryotherapy, and one case with excision, cryotherapy and adjuvant Mitomycin C. One case was enucleated due to unrelated intraocular problems. Three recurrences previously treated elsewhere were surgically excised combined with cryotherapy (1) or cryotherapy plus adjuvant Mitomycin C (2). The mean follow up was 56 months (range 4-112). Seven patients are free from local, regional and systemic illness, 5 patients presented local recurrences (mean 2 recurrences, range 1-5), with a mean time of presentation of 16 months (range 3-59 months). Regional lymph node extension occurred in two patients, distant metastasis in two patients and one patient died from the disease.

Conclusions: Conjunctival melanoma is an uncommon tumor in the periocular region, with recurrence and dissemination capability. In the management of this neoplasia it seems essential an early clinical and histopathologic diagnosis and an aggressive initial treatment, usually combining extensive surgery with adjuvant therapies.

■ 2193

Age related pseudotumors peripheral detachment of the retinal pigment epithelium

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Purpose: Study of the spectrum of clinical presentation of a rare degenerative disorder frequently imitating an intraocular malignant tumor.

Methods: Retrospective analysis of 37 cases referred in the ocular oncology unit of the Jules Gonin Eye hospital, with the clinical diagnosis of melanoma in 18 cases, the diagnosis of undetermined intraocular tumor in 13 cases and the diagnosis of uveal metastasis in 2 cases.

Results: Eleven patients were male and 26 female with a mean age of 78 years.

Pseudotumors were unilateral in 26 cases and bilateral in 11 cases, unique in 40 eyes and multiple in 8 eyes. A neovascular membrane was present in 36 eyes, and the rupture of the retinal pigmented epithelium occurred in 10 eyes. The lesion was localized most frequently in the temporal and inferior quadrant. Visual acuity was = 0.1 in 17 eyes, 0.2 to 0.4 in 12 eyes, 0.5 to 0.8 in 10 eyes and > 0.8 in 9 eyes. In cases with visual acuity of = 0.1, the decrease of the visual acuity was due to the peripheral pseudotumor in 9 eyes, to degenerative lesions of the macula in 3 eyes and to both of them in 5 eyes.

Conclusions: The exact knowledge of the spectrum of clinical presentation of age related pseudotumoral peripheral detachment of the retinal pigment epithelium avoids errors in clinical diagnosis

■ 2194

Circumscribed choroidal hemangioma treated with photodynamic therapy: an indocyanine-green angiographic study

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Purpose: To analyze early and late indocyanine green angiography (ICGA) behaviour of circumscribed choroidal hemangioma (CCH) treated with photodynamic therapy (PDT).

Methods: Seven consecutive patients affected by CCH underwent PDT treatment of their lesion. PDT was randomly performed with two different treatment modalities: standard PDT treatment (verteporfin 6 mg/sqm; 30 ml IV in 10 min; treatment at 15 min; 692 nm; 50 J/sqcm; 83 sec) and bolus PDT treatment (verteporfin 6 mg/sqm; IV bolus in 1 min; treatment at 5 min; 692 nm; 166sec). ICGA angiography was performed pre-op, and: 10 minutes, 1 day, 1 and 4 weeks, and every 3 months post-op. Best-corrected visual acuity and fundus photography were performed at each angiographic examination.

Results: Three patients were treated with standard PDT and four with bolus regimen. One single treatment was performed in each patient. Best-corrected visual acuity improved with both PDT treatment regimens. CCH flattened in both treatment groups, faster in bolus treated eyes. ICGA documented a massive involvement of choroidal circulation with PDT bolus, and progressive disappearance of pre-op characteristics in both treatment groups. Reactive pigment-epithelium hyperplasia was more evident in the bolus PDT treated eyes.

Conclusions: The management of CCH should be tailored to the tumor size, location, and related ocular symptoms, moreover it should critically consider long term side effects of any proposed modality. PDT seems to be a promising and safe treatment modality for the treatment of symptomatic CCH. ICGA may represent an invaluable diagnostic tool not only to diagnose but also to follow CCHs treated with PDT.

■ 2195

Ultrasonographic tissue characteristics of mushroom-shaped versus dome-shaped uveal melanomas

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*Ophthalmology, Hadassah Univ. Hospital, Jerusalem***Purpose:** To evaluate the ultrasonographic tissue characteristics of mushroom-shaped versus dome-shaped uveal melanomas.**Methods:** Twenty-nine eyes of 29 consecutive patients with uveal melanoma, having a mean age of 57 years (range 25 - 89 years), who presented with either dome-shaped (18 eyes) or mushroom-shaped (11 eyes) tumors were studied. Patients were examined clinically and ultrasonically using A- and B-mode standardized echography. The echographic parameters included tumor base size, height, internal reflectivity, regularity, vascularity and extra-scleral extension. The A-scans of the two groups were normalized and the average A-scan patterns of the groups were compared.**Results:** A-scans of dome-shaped tumors showed homogenous low reflectivity. A-scans of mushroom-shaped tumors showed high and irregular reflectivity at the tumor apex, up to 60% of its height, while the neck (lower 40% of the height) had significantly lower reflectivity. The difference between the two groups was statistically significant (paired student's t-test, $p=0.0002$).**Conclusions:** The ultrasonographic pattern of uveal melanoma can reflect its microscopic structure and can suggest the existence of vascular congestion in the apex of the mushroom-shaped tumors, as is seen in histological examination, probably due to compression of blood vessels in the tumor neck.

■ 2196

Neuroinvasive malignant melanoma of the uvea

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*(1) Eye Pathology Institute, University of Copenhagen, Copenhagen, (2) Department of Ophthalmology, Rigshospitalet, Copenhagen, (3) Centre for Cancer Documentation, Department of Prevention and Documentation, Danish Cancer Society, Copenhagen***Purpose:** To characterize a neuroinvasive phenotype of uveal melanoma (NUM), based on clinical ophthalmological findings.**Methods:** Sixty-six consecutive patients with NUM between 1940 and 1999 were ascertained from the register of the Eye Pathology Institute, Univ. of Copenhagen, Denmark. The definition of NUM is a uveal melanoma with invasion of tumor cells into the optic nerve or its sheaths. Hospital reports were collected and in each case the following factors were analysed: gender, age at diagnosis, intraocular pressure, surgery or radiotherapy prior to enucleation, metastases and mortality. Data were compared with melanomas (n=66) of the optic nerve head (ONHM) and with melanomas (n=66) without neuroinvasion and without invasion of the optic nerve head (UM).**Results:** The mean age (68 yrs. vs. 63 yrs), the percentage of men (59% vs. 44%) and mean intraocular pressure (36mmHg vs. 20mmHg) were all significantly higher values in NUM than in UM ($p<0.05$). Half of the patients with NUM had a blind eye at the time of enucleation, whereas only 10% of the patients with UM had a blind eye at the time of enucleation. No differences in treatment with ocular surgery or radiotherapy prior to the enucleation were seen between the three groups. Death, due to metastatic spread, occurred significantly earlier in patients with NUM compared to patients with UM (2-year mortality: NUM 50%, ONHM 30% and UM 15%).**Conclusions:** A neuroinvasive uveal melanoma most often presents in a blind eye with increased intraocular pressure. Furthermore, neuroinvasion is a factor of poor prognosis.

■ 2197

How the Tumor, Node, Metastasis (TNM) classification of malignant uveal melanoma has evolved

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Purpose: To evaluate how the new 6th edition of TNM (TNM6) improves upon the 5th edition (TNM5).

Methods: Survival status of 289 consecutive patients with a choroidal and ciliary body melanoma in the Helsinki University Central Hospital district in 1962-1981 was verified by chart, registry, death certificate and immunohistochemistry review. Tumour dimensions, ciliary body involvement and extraocular extension were recorded.

Results: Of 283 uveal melanomas of known dimensions, 5% were classified as T1, 67% as T2, 23% as T3 and 6% as T4 according to TNM6. The corresponding percentages based on TNM5 were 8%, 17%, 66% and 10%, respectively. Of T2/TNM6 tumours, 64% came from the T3, 24% from T2 and 5% from T1 group of TNM5. In TNM6, tumours with extraocular extension fell rather evenly into groups T2 (41%) and T4 (55%), whereas in TNM5 all were classified as T4. Of ciliochoroidal tumours, 54% were classified as T2 and 46% as T3 in TNM6 based on their size, instead of being T3 in TNM5 (based on choroidal extension). The 5-year survival estimates of 86% for T1, 71% for T2, 61% for T3 and 15% for T4 suggest some improvement in separating patients according to prognosis (difference in chi-square, 2.59; $p=0.11$) as compared to TNM5 (corresponding figures, 91%, 71%, 69% and 25%, respectively)

Conclusions: TNM6 tends to classify most patients in group T2. The classification has scope for further improvement.

■ 2198

Conservation of eyes with choroidal melanoma by a multimodality approach to treatment: an audit of 1632 patients

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Purpose: To report on conservation of eyes with choroidal melanoma with a multimodality approach to treatment.

Methods: A prospective, non-comparative, interventional case series of 1632 patients with choroidal melanoma treated at a single center was reviewed. Predictive factors for primary and secondary enucleation were identified.

Results: The patients had a mean age of 59.9 years. The mean tumor diameter was 12.2 mm and the mean tumor thickness was 5.5 mm. Multiple logistic regression showed the predictive factors for primary enucleation to be: age > 60 years ($P<0.001$); reduced visual acuity ($P<0.001$); posterior extension close to or involving optic disc and fovea ($P<0.001$); retinal invasion ($P=0.004$); optic disc involvement ($P<0.001$); circumferential spread around ciliary body, iris or angle ($P<0.001$); basal tumor diameter ($P<0.001$); and tumor thickness ($P<0.001$). After conservative treatment the median follow-up was 2.86 years, exceeding 5 years in 254 patients. The actuarial rate of secondary enucleation was 11.1% at 5 years. Cox multivariate analysis indicated the factors independently predicting secondary enucleation as: nasal/midline tumor location ($P<0.001$); disc involvement ($P=0.012$); tumor diameter ($P=0.013$); and tumor thickness ($P<0.001$). Ocular conservation ranged from 99% to under 50% according to a predictive score.

Conclusions: Ocular conservation was attempted in 65% of patients and achieved in 89% of these, with success depending on tumor diameter, tumor thickness, disc involvement, and coronal location.

■ 2201

Reliability of the manual assessment of endothelial quality in eyebanks

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Purpose: To assess the reliability of the manual endothelial cell (EC) count performed during cornea organ culture by all the technicians of the 22 French eye banks.

Methods: A questionnaire asked for details of the technical features of the EC count. Counting exercise: 4 flat mounted alizarin stained human corneal endothelia were sent successively to all eye banks. Technicians had to determine the four EC densities using their usual method.

Results: Questionnaire: 20/22 of the eye banks used only manual counting methods, directly through a microscope, and 62 technicians were involved. Counting of EC within the borders of a grid was the most common method (17/22). Of the eight banks (36%) that did not calibrate their microscopes, six reported the highest ECD. Of the 14 others (64%), six applied a "magnification correcting factor" to the counts. Most of the banks (12/22) counted 100 EC or less. Counting exercise: overall inter-technician variability ranged from an under estimation of 40% to an overestimation of 82% of the real ECD. More than 50% of the technicians provided a result far for 50% and more from the exact ECD. The intra bank variability was up to 47%.

Conclusions: These studies raised an unacceptable variability in ECD between banks. This gives the threshold of 2000 EC/mm² for graft delivery a very poor value. Lack of microscope calibration appears to be the leading cause of variance. Other factors such as differences in counting strategy, the evaluation of too small numbers of EC, and the "human factor" may also contribute to this variability. Modern computerised endothelial analyser could help to dramatically reduce the inter and intra bank variability.

■ 2202

Long term progression of astigmatism after penetrating keratoplasty for keratoconus

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Purpose: To study the changes in astigmatism throughout a 20-year period using keratometry and refraction in patients who underwent penetrating keratoplasty (PK) for keratoconus.

Methods: Retrospective study of patients who underwent PKP for KC from 1975-1979. We reviewed refraction and keratometry results at 1,3,5,7,10,15, 20 and 25 years after suture removal, and slit lamp findings.

Results: Eighty eyes with mean follow-up of 20 years (range: 15-25 years) were included in the study. Graft size, suture technique, and time of suture removal had no significant influence on astigmatism at last follow-up. We observed a stabilization of keratometric astigmatism in the first 7 years followed by a progressive increase from 10 years after suture removal to the last follow-up. The mean absolute value of the difference vector (DV) calculated by vector analysis was 7.17 +/- 4.35 D. In 70% of cases, progression of the astigmatism was evident with mean absolute DV of 9.10 +/- 3.65 D. There was a significant correlation between the preoperative and final axis of astigmatism (Pearson r =0.39, p=0.0008). The major slit lamp finding was a peripheral crescent-shaped thinning at the graft-host junction with absence of Bowman's layer on histopathology.

Conclusions: In spite of refractive stability obtained during the first years after PKP for KC, increasing astigmatism thereafter suggests that there is a progression of the disease in the host cornea.

■ 2203

Highly up-regulated proteins in aqueous humour from patients with endothelial rejection of corneal transplants : a proteomic analysis

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Purpose: To compare the proteomic composition of aqueous humour from patients with rejection of corneal transplants (patients) and from patients with cataract (controls).

Methods: 50 microliter of aqueous humour from five patients and seven controls were used to make two-dimensional gel electrophoresis. Computerized image analysis was done to find protein spots, that were more than a factor two up-regulated in patients as compared with controls. Up-regulated spots were identified by immunoblotting and mass spectrometry.

Results: 950 spots were detected on two-dimensional gels. 31 of the 950 spots were significantly up-regulated in aqueous humour from patients as compared with controls. The 31 up-regulated spots were derived from 6 different proteins : albumin, alpha-1-antitrypsin, apolipoprotein J, cytokeratin type II, serin proteinase inhibitor, and transthyretin.

Conclusions: The proteomic composition of aqueous humour differs significantly between patients with rejection of corneal transplants and patients with cataract. The identification of the up-regulated proteins suggests, that the changes in aqueous humor are due to three different mechanisms : breakdown of the aqueous-blood barrier, enzymatic degradation, and liberation of locally synthesized proteins.

■ 2204

Endothelial cell death during acute corneal graft rejection

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Purpose: The exact mechanism involved in endothelial cell death during corneal graft rejection remains unclear. In a rat model of corneal rejection, we have evaluated the time course of cell infiltration.

Methods: Corneal graft rejection was induced by grafting corneas from Brown-Norway rats to Lewis recipient. Graft rejection was observed in 100% of the cases at day 14. At day 2, 4, 10 and 14 after the graft, animals (4 per time point), were sacrificed and the eyes were fixed in 4% paraformaldehyde and cryo protected in sucrose. Cryo-sections (5µm) and flat-mounted cornea were used for immunohistochemistry with antibodies. At day 11, another group of 4 grafted rats were sacrificed and the corneas were flat mounted, labeled with a nuclear fluorescent dye and observed under a confocal fluorescent microscope. TUNEL assay was used to detect apoptotic cells.

Results: Endothelial cells density dramatically decreased during the graft rejection process in the button and at a lesser extend in the recipient cornea. Both macrophage and lymphocytes activation and migration were observed first mainly located at the trephination bed, then migrating inside and invading the button. TUNEL assay was positive for the superficial epithelial cells as expected, and in some infiltrating cells at late stage of the rejection process, but no staining was detected in the endothelial cell layer at any time.

Conclusions: From these observations, we hypothesize that endothelial cells loss during the acute corneal graft rejection could not result from an apoptotic caspase-dependent mechanism.

■ 2205

Corneal endothelial transplantation using femtosecond laser technology

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*Dept. of Ophthalmology, Faculty of Clinical Medicine Mannheim of the University Heidelberg, Mannheim***Purpose:** To describe a technique of corneal endothelial transplantation using femtosecond laser technology. Design. Experimental case report.**Methods:** Using a femtosecond laser, a thin deep corneal layer including Descemet's membrane and corneal endothelium was prepared with a diameter of 6 mm on its endothelial side, and a diameter of 8 mm on its stromal side. On top of the deep corneal lamella, a thick corneal flap with three positional pikes and a hinge was created in 5 freshly enucleated porcine eye. After opening the flap, the deep corneal layer with Descemet's membrane and corneal endothelium was removed and inserted into the bed of recipient eyes which had been prepared in the same way.**Results:** For all eyes included in the study, the deep corneal grafts and the flaps could be prepared without major difficulties. The deep lamellar allografts could easily be transplanted into the recipient beds of other eyes. They were eventually covered by the autologous corneal flap of the recipient eye. The time taken for the preparation of the deep corneal grafts and the flaps was less than 5 minutes in all cases.**Conclusions:** Femtosecond laser technology may allow non-mechanical posterior lamellar keratoplasty with transplantation of Descemet's membrane and corneal endothelium, leaving most of the corneal stroma and corneal surface untouched.

■ 2206

Femtosecond laser penetrating keratoplasty with conical incisions and positional pikes

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*Dept. of Ophthalmology, Faculty of Clinical Medicine Mannheim of the University Heidelberg, Mannheim***Purpose:** Penetrating keratoplasty has usually been performed taking a trephine for excision of the corneal donor button and for preparation of the recipient corneal bed. In some centers, an Excimer laser has been used to create almost perfect sagittal incisions into the donor cornea and recipient cornea in an effort to improve the adaptation of donor button and recipient bed and to decrease postoperative corneal astigmatism (1). Using new laser technology, the strength of non-mechanical trephination in corneal surgery may be further increased by creating conical incisions instead of sagittal incisions. Conical incisions with a wider inner (endothelial) diameter and a smaller outer (epithelial) diameter may increase wound stability since they allow the intraocular pressure to press the donor button into the recipient bed and because they additionally increase the wound surface. With none of the techniques available so far, conical incisions have been possible. It was the purpose of the present study to describe the possibility to create congruent conical incisions for the donor button and the recipient bed using the new femtosecond laser technology (2-4).**Methods:** The study included 10 eyes of slaughter house pigs. Using a corneal contact lens and a femtosecond laser (20/10 Perfect Vision, Am Taubenfeld 21/1, D-69123 Heidelberg, Germany) with a wavelength of 1060 nm, a spot size of 10 micrometers and a laser pulse duration of about 600 femtoseconds, various corneal buttons were prepared with an outer (epithelial) diameter ranging between 5.0 mm and 7.5 mm, an inner (endothelial) diameter ranging between 6.0 mm and 9.0 mm, and a conical angle between the direction of the incision and the sagittal axis varying between 0° and 60°. To further increase the rotational stability of the donor button in the recipient bed, 4 positional pikes were carved into the donor button, and 4 corresponding notches were created into the recipient bed. The angle at the top of the positional pikes measured 20° or 30°. The height of the peaks varied between 0.25 mm and 0.40 mm.**Results:** For all eyes included in the study, the corneal buttons could be prepared without major difficulties. The corneal buttons could easily be repositioned into their original beds as well as into the recipient beds of other eyes in which the recipient beds were created with the same diameter and the same conical angle as for the donor button. The time taken for the preparation of the corneal buttons including positioning of the corneal contact lens, the laser application itself, and the removal of the corneal button was less than 5 minutes in all cases.**Conclusions:** In corneal surgery, femtosecond laser technology allows to create new types of corneal incisions such as conical incisions with positional edges in the graft and congruent conical incisions with corresponding notches in the recipient bed. In view of the positive force of the intraocular pressure pressing a conical graft into the corneal bed of the recipient, in view of the increased wound surface of conical incisions versus sagittal incisions, and considering that positional pikes may increase postoperative rotational stability of the graft and decrease corneal astigmatism, femtosecond laser surgery warrants further investigation for penetrating keratoplasty.

■ 2207

Dephosphorylation of regulatory light chain of Myosin II by adenosine in the corneal endothelium

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Purpose: Activation of adenosine-sensitive receptors is associated with enhanced barrier integrity in the corneal endothelium. This study has examined phosphorylation status of regulatory light chain of Myosin II (MLC) in response to A₂B agonists in cultured bovine corneal endothelial cells (BCEC).

Methods: Phosphorylated status of MLC was determined by urea-glycerol gel and western blotting. Phosphorylation status of CREB, by western blotting, was used to confirm activation of PKA in response to A₂B agonists. Activation of ERK1/2 was assessed by western blotting to examine its influence on MLC phosphorylation.

Results: Exposure to adenosine and NECA, A₂B-sensitive P₁ agonists, induced phosphorylation of CREB in a manner similar to forskolin. Exposure to adenosine, NECA and forskolin, at similar concentrations and durations of exposure, led to dephosphorylation of MLC and also induced ERK1/2 phosphorylation. The latter could be prevented by pre-exposure to U0126, a MAPK inhibitor. However, prior treatment with U0126 was without any significant effect on MLC dephosphorylation induced by adenosine.

Conclusions: Adenosine response in BCEC is mediated through A₂B receptors. Activation of cAMP-PKA axis in BCEC induces MLC dephosphorylation; opposing effect of activated ERK1/2 by promoting MLC phosphorylation is insignificant. Enhanced barrier integrity upon stimulation of cAMP-PKA axis could be attributed to relaxation of the cortical actin cytoskeleton by MLC dephosphorylation.

■ 2241

cAMP-induced short-circuit currents increase in pig ciliary processes: effect of drugs modulating intracellular calcium and anion channel transport

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Purpose: To investigate whether drugs known to decrease intracellular calcium concentration or anion channel transport can inhibit cAMP-induced transepithelial short-circuit (Isc) increase in isolated porcine ciliary processes.

Methods: Isolated porcine ciliary processes were mounted in a Ussing-type chamber and bathed with physiologic or calcium-free Krebs-Ringer's solution. The changes of short-circuit current induced by cAMP (10 nM-30 μ M) were studied. In physiologic Krebs-Ringer's solution Isc changes induced by cAMP (10 nM-30 μ M) were also investigated in the presence or the absence of nimodipine (L-type calcium channel blocker, 30 μ M), mibefradil (T-type calcium blocker, 30 μ M), thapsigargin (intracellular calcium releaser, 1 μ M), or niflumic acid (presumed calcium-activated Cl⁻ channel antagonist, 0.1 mM). Drugs were applied on the non-pigment epithelium side.

Results: In a concentration-dependent manner, cAMP increased transepithelial Isc (10 μ M: 17.07 \pm 0.75 μ A/cm²) and potential difference (PD) changes (943.7 \pm 66.4 μ V) that became more negative on the NPE side. The effect of cAMP on Isc increase could be prevented by bathing the tissue in calcium-free Krebs-Ringer's solution (10 μ M: 3.76 \pm 0.80 μ A/cm², P<0.001). The increase in Isc induced by cAMP was significantly reduced by nimodipine (10 μ M: 14.78 \pm 1.26 μ A/cm², P<0.05), mibefradil (10 μ M: 10.81 \pm 2.04 μ A/cm², P<0.01), thapsigargin (10 μ M: 8.48 \pm 1.06 μ A/cm², P<0.01), or niflumic acid (10 μ M: 3.71 \pm 1.02 μ A/cm², P<0.001).

Conclusions: In isolated porcine ciliary processes cAMP appears to increase stroma-to-aqueous anion transport, an effect that also can apparently be prevented by drugs or situations decreasing intracellular calcium concentration.

■ 2242

Effect of acetazolamide on short-circuit current increase induced by the NO-donor sodium nitroprusside in pig ciliary processes

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Purpose: To assess whether the carbonic anhydrase inhibitor acetazolamide can modulate short-circuit current (Isc) increase induced by the nitric oxide (NO) donor sodium nitroprusside (SNP) and the cGMP analog 8-pCPT-cGMP in isolated porcine ciliary processes.

Methods: In an Ussing-type chamber, Isc increase induced by either SNP (0.1 μ M-0.1 mM) or 8-pCPT-cGMP (0.01 μ M-0.1 mM) were measured in the presence or in the absence of acetazolamide (0.1 mM). Experiments were also conducted with SNP and 8-pCPT-cGMP in HCO₃⁻-free (isomolar replacement of HCO₃⁻ by Cl⁻) and physiologic Krebs-Ringer's solution. Drugs were applied on the non-pigmented epithelium (NPE) side.

Results: In a dose-dependent manner, SNP (0.1 mM: 10.58 \pm 0.85 μ A/cm²) or 8-pCPT-cGMP (0.1 mM: 15.36 \pm 0.90 μ A/cm²) increased transepithelial Isc. In experiments conducted in HCO₃⁻-free Krebs-Ringer's solution, the effect of SNP (0.1 mM: 2.50 \pm 0.35 μ A/cm², P<0.001) or 8-pCPT-cGMP (0.1 mM: 5.92 \pm 0.60 μ A/cm², P<0.001) were strongly inhibited. In the presence of acetazolamide, the effect of SNP (0.1 mM: 6.30 \pm 1.00 μ A/cm², P<0.05) or 8-pCPT-cGMP (0.1 mM: 11.68 \pm 0.99 μ A/cm², P<0.05) were moderately reduced.

Conclusions: Conditions known to decrease HCO₃⁻ concentrations appear to also inhibit transepithelial ionic transports induced by the NO donor SNP.

■ 2243

Liposomal lipid composition modulates cyclosporine A cytotoxicity on retinal pigment epithelium cells in culture

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Purpose: To examine the cytotoxicity of cyclosporine A (CsA) formulated as sterically stabilized (SSL) and conventional liposomes (CL) on human RPE cells in culture.

Methods: The composition of formulations was PC/CHOL/PG/CsA (CL1);

PC/CHOL/PEG-PE/CsA (SSL1) and PC/CHOL/PEG-PE/CsA (SSL2). RPE cells (D 407) grown to confluence in DMEM supplemented with antibiotics and 5% fetal bovine serum in a 5% CO₂ incubator at 37°C were exposed to liposomes for 24 - 96 h. The viability of RPE cells was assessed using premix WST-1 assay and cell morphology was evaluated by phase contrast microscopy.

Results: CL1 did not demonstrate any cytotoxic effect on RPE cells for 24 and 48 h, but SSL2 was found to be highly toxic at all concentrations tested. SSL1 dramatically reversed the high cytotoxic potential that observed with SSL2 by controlling CsA release. After exposure of SSL1 to RPE cells for 24 and 48 h, EC₅₀ could not be calculated, while 80-90% of cells were alive. When the RPE cells were treated for 96 h, the viability was found to be 80-85 % with CL1 and 25-30% with SSL1.

Conclusions: The liposomal lipid composition had critical roles for CsA solubilization and had the potential to modulate CsA cytotoxicity on RPE cells. Acknowledgment: This study was supported by grants from the National Science and Technology Research Council of Turkey (TUBITAK) (Project Nr:SBAG-2119). The lipids were kindly provided by Genzyme Corp. (Cambridge, MA, USA).

■ 2244

Alkylphosphocholines act as antiproliferative and anticontractile agents in human Tenon fibroblasts in vitro

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Purpose: To investigate the effect of Alkylphosphocholines (APCs) on human Tenon fibroblast (HTF) proliferation and cell-mediated collagen lattice contraction.

Methods: HTFs were isolated from tissue samples of five consented patients which were obtained during surgery and cultured in DMEM, 10% FCS under standard cell culture conditions. After splitting, HTFs were treated with one of four APCs, respectively, in concentrations spanning the 50% inhibitory concentration (IC₅₀) as determined previously. Cell proliferation inhibition was assessed by the MTT assay. For contraction inhibition, three-dimensional collagen gels were seeded with HTFs and the gel size was measured. Cell viability was determined by the trypan blue exclusion assay.

Results: For all four APCs, dose-response curves were measured. The IC₅₀ concentration varied between 10 µM (Erucyl-PC, C22:1-PC) and 30 µM (Oleyl-PC, C18:1-PC). At their IC₅₀ concentration, all four APCs were equally effective in proliferation and contraction inhibition of HTFs at non-toxic concentrations. These effects were observed 72 hours after a single application of APCs. All experiments were performed in the presence of serum.

Conclusions: APCs are effective inhibitors of HTF proliferation and cell-mediated contraction of collagen lattices at non-toxic concentrations. This might open a new perspective on anticarring therapy after glaucoma filtration surgery.

■ 2245

Does maillard reaction intermediates influence intracellular transport in retinal cells

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Purpose: The accumulation of Maillard reaction products and intermediates (leading also to advanced glycation endproducts, AGEs) are known to be associated with the risk of diabetic neuropathy and other diseases. Effects of AGEs on the cellular function are still unclear. Possible neuroprotective effects by the alpha 2 agonist brimonidine were tested.

Methods: In cell cultures (neuronal PC 12, retinal E1A, glial N11) we investigated the effects of AGE induced metabolic stress by incubation (0-24h) with glyoxal the intermediate product of AGE formation (0-800µM). Microvesicle movement was documented by video enhanced contrast microscopy and averaged velocities were calculated subsequently. The effect of co-incubation with the alpha 2 agonist brimonidine was documented.

Results: Glyoxal produces an altered distribution of vesicles and a statistically significant time and dose dependent reduction of microvesicle velocity (30-60% of control value) in all three cell lines. The co-incubation with brimonidine (0.0005 %) led to a significant reduction of the decrease in vesicle velocity ($p < 0.001$) to 133.9 nm/s in comparison to 90 nm/s (glyoxal) (control: 156 nm/s).

Conclusions: We were able to show significant effects of AGEs and its intermediates on the microvesicle velocity of the cells. Unknown metabolic disturbances or dysfunction of tubulin, microtubule associated proteins or other motorproteins might be causal factors. In the concentrations tested brimonidine was able to block the AGE mediated velocity decrease by for few hours.

■ 2246

Neuronal NO-synthase does not contribute to choroidal blood flow regulation

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Purpose: It is known that nitric oxide (NO) plays an important role in choroidal blood flow homeostasis. Kiel et al. discovered NO as a major player in choroidal autoregulation, however, the source of NO has not been defined yet and it is not clear to which extent the two known constitutive NO-synthases (endothelial- and neuronal NOS) contribute to net production of NO in the choroidal vasculature under physiological conditions.

Methods: Anesthetized rabbits (n=7) were instrumented with an ear artery cannula to measure mean arterial pressure (MAP), and a vitreous cannula to measure IOP; hydraulic occluders were placed around the descending aorta and the inferior caval vein to manipulate choroidal perfusion pressure over a wide range. Choroidal blood flow was measured by laser Doppler flowmetry. A protocol with three subsequent measurements was performed in each animal. Pressure flow relationships and baseline data were recorded under control conditions, in response to TRIM (20 mg/kg, i.v. infusion) and in response to L-NAME (15 mg/kg, i.v. bolus injection).

Results: Neuronal NOS inhibition with TRIM had no statistically significant effect on baseline parameters and pressure flow relationships of the choroid. However, inhibition of both neuronal and endothelial NOS with L-NAME injection after TRIM application showed a substantial reduction of choroidal blood flow and IOP and an increase in MAP, perfusion pressure and vascular resistance. L-NAME also shifted the pressure flow relationship significantly downwards.

Conclusions: Choroidal nitric oxide vasodilator tone in the anesthetized rabbit seems largely to be due to the activity of endothelial nitric oxide synthases.

■ 2247

Inhibition of the Na, K-ATPase Alpha 2 isoform alters capacitative calcium entry in optic nerve astrocytes

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Purpose: In astrocytes, the Na,K-ATPase a2 isoform is localized in a pattern suggesting a possible link to endoplasmic reticulum (ER) function. Here we tested whether selective inhibition of Na,K-ATPase a2 alters capacitative calcium entry (CCE), the mechanism by which ER calcium stores refill following depletion.

Methods: Studies were conducted on cultured rat optic nerve astrocytes. Na,K-ATPase a2 was selectively inhibited using a low concentration of ouabain. Cytosolic calcium and sodium concentrations were measured using Fura-2 and SBFI respectively.

Results: The magnitude of the increase in cytosolic calcium concentration during CCE was almost doubled in astrocytes exposed to 1mM ouabain. The same concentration of ouabain was not sufficient to cause a cell-wide increase of cytosolic sodium concentration, nor to inhibit Na,K-ATPase a1 isoform activity in rat kidney. To examine the effect of Na,K-ATPase a2 isoform inhibition on ER calcium stores, astrocytes were exposed to the agonist ATP (50mM) in the absence of extracellular calcium. In astrocytes exposed to 1mM ouabain, the calcium response to ATP was increased by ~80%.

Conclusions: Taken together, the results suggest that selective inhibition of the Na,K-ATPase a2 isoform has the potential to change CCE, alter calcium signaling responses and possibly increase calcium ER store size.

■ 2251

Therapeutic consequences from investigations of inherited macular degenerations

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"Macular degeneration" is a catch-all term for a number of different disorders characterized by progressive central visual loss and degeneration of the macula and underlying retinal pigment epithelium. Genetically determined macular degenerations include age-related macular degenerations (ARMD) which are by far the most common form of the disease and represent the leading cause of legal blindness among those aged over 65 in most developed countries worldwide. Determining the genetic component of the age-related macular degeneration complex trait has been the primary goal of ophthalmic genetics research for almost a decade. However and despite intensive researches, the etiology and pathogenesis of the disease are poorly understood and effective treatment options are limited for most patients. Conversely, over the past few years significant progress has been made in the discovery of genes causing Mendelian macular forms which share many important features with ARMD but are more readily studied by molecular genetic approaches. Molecular defects in the peripherin/RDS, VMD2, EFEMP1, TIMP3, ELOVL4 and ABCA4 genes have been related to the most common forms of inherited macular degenerations ie adult vitelliform macular dystrophy, Best macular dystrophy, Doyme honeycomb retinal dystrophy/Malattia leventinese, Sorsby' fundus dystrophy, dominant Stargardt-like disease, recessive Stargardt disease/Fundus flavimaculatus, respectively. These discoveries have not only brought to light the wide genetic and allelic heterogeneity of macular degenerations. Above all, they have pointed out the diversity of mechanisms involved in the occurrence of the disease and emphasized that specific therapeutic approaches may be required to treat macular degenerations. But at the same time, for some of these genes, the identification of "specific" mutations in other diseases including some age-related macular degenerations and retinitis pigmentosa (RP) suggest that these treatments could apply to other retinal dystrophies. Gene identification in these macular dystrophies has represent the first step towards the understanding of their pathophysiology and thus the first step towards the development of therapeutic tools. Additionally, it has offer the possibility to create animal models by knocking "out or in" the normal genes, these models being of precious help to assist the development of therapeutic interventions. As an example to illustrates the therapeutic consequences from the identification of macular dystrophies genes, we will consider more particularly Stargardt/Fundus flavimaculatus phenotypes. Indeed, the knowledge of the disease gene, of its function in the retina and the existence of mouse models has allow to imagine several strinckingly different therapeutic approaches not only to treat this condition but also some ARMD and RP. These approaches will be reviewed in detail. The other macular dystrophies will also be considered especially dominant forms of the disease fo

■ 2252

Transpupillary thermotherapy and induction of heat shock proteins

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Purpose: To compare the biophysical basics of transpupillary thermotherapy with conventional laser coagulation as a treatment of CNV due to ARMD.

Methods: By calculation of the tissue temperature rise following TTT and laser coagulation, molecular biological alterations were compared. The results of these studies will be reviewed. In addition, complications of TTT treatment will be discussed.

Results: The characteristics of TTT to treat CNV as a result of ARMD are: large spot size, long duration and low retinal irradiance leading to a subthreshold laser effect with a photothermal laser-tissue interaction. This means a low grade hyperthermia. In contrast to tissue temperatures >43°C, temperatures of <41°C do not lead to photocoagulation, but cause metabolic activation. The production of heat shock proteins is induced in order to protect the cells against potentially lethal noxes by acting as molecular chaperones and by anti-apoptotic effects. Complications of TTT are presented. They are likely the consequences of over- or under-treatment.

Conclusions: The aim must be to measure the temperature rise induced by TTT in order to achieve a controlled effect and to prevent these complications.

■ 2253

Stem cell transplantation

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Purpose: Stem cells are multipotential cells with the capacity of self-renewal. They can be expanded in vitro and are amenable to genetic manipulations. Because of these properties, transplantation of stem cells might be a strategy to replace degenerated retinal cell types or to target therapeutic gene products to the diseased retina. Here, we will summarize experiments that were performed to analyze the therapeutic potential of stem cells for retinal disorders.

Methods: Cell culture experiments were carried out to study the ability of embryonic, bone marrow-derived, neural and retinal stem cells to differentiate into cell types with a retinal phenotype. Transplantation experiments were performed to investigate the integration and differentiation of stem cells in developing, adult and diseased retinas in vivo.

Results: In vitro studies revealed the potential of different types of stem cells to differentiate into cells with a retinal phenotype. Grafted stem cells integrated into developing and diseased adult retinas, survived for extended periods of time and differentiated into glial and/or neuronal cell types. Differentiation of donor cells into cell types expressing retina-specific antigens has also been reported.

Conclusions: Different types of stem cells are capable to integrate into pathologically altered adult retinas and display the potential to differentiate into cells with a retinal phenotype. They are thus candidate cells for the development of cell-based therapies of retinal disorders. To establish protocols that efficiently direct the differentiation of multipotent stem cells into authentic retinal cell types and to functionally characterize donor cells in recipient retinas are among the challenging tasks of future work. Supported by the Bundesministerium für Bildung und Forschung (01GN0126).

■ 2254

Surgical treatment of venous occlusion

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Purpose: To assess the visual outcome, the incidence of chorioretinal anastomosis (CRA) and the decrease in foveal thickness measured by Optical Coherence Tomography (OCT) of Radial Optic Neurotomy (RON) with one compared to three nasal neurotomies in patients with Central Retinal Vein Occlusion (CRVO).

Methods: Prospective study including 37 patients (37 eyes) with CRVO of less than 12 months duration, and visual acuity (VA) < 20/125. Pars Plana Vitrectomy, Posterior Hyaloid Dissection and nasal RON using a microvitrectomy blade, was done. Three RON were performed in an attempt to increase neural ring decompression in 23 patients, while 14 underwent single RON. VA and OCT were registered preoperatively and at 1, 3 and 6 months.

Results: Clearing of hemorrhages and decrease in macular edema as measured by OCT was observed in all patients. 43% of patients in the single-RON group gained > 2 lines versus 10.5% after triple-RON, p=0.04. Mean decrease of macular thickness on OCT was 39% with one neurotomy versus 48% with three. New CRA appeared in 43% of cases after single-RON versus 48% after triple-RON.

Conclusions: Surgical decompression of CRVO with RON seems to improve or at least stabilize the prognosis of patients with severe CRVO. Improvement presumably occurs due to the optic nerve head decompression, the vitrectomy itself, as well as by the newly formed CRA that drain the retinal circulation to the choroid and accelerate the resolution of the retinal edema. Single-RON appears to be more effective, despite lower decrease in macular thickness on OCT and fewer CRA, compared to triple-RON. A randomized trial is needed to prove the efficacy and safety of this procedure and definitively demonstrate the optimal number of neurotomies.

■ 2255

Techniques of autologous RPE transplantation

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Purpose: Transplantation of the RPE is a promising therapy for retinal degenerations. Our technique of autologous RPE suspension transplantation for fCNV in AMD is described. Other clinical approaches and future directions are included.

Methods: After vitrectomy, the retina is bleb detached with BSS+ pt.1. The RPE is aspirated from 2-4 disc diameters (DD) with a custom instrument. While the CNV is extracted, the aspirate is counted. Then it is reinjected in BSS under perfluorocarbon under the macula. A gas tamponade is performed at the end. Membrane excision only serves as control. The aspirate was investigated for viability, with cell culture and immunostaining. We studied the harvesting efficacy for the aspiration instrument. An in vitro model for the lower limit of transplanted cells, minimal plating densities required for confluence, was established.

Results: 1500-6000 viable RPE cells are transplanted. Visual acuity improvement is statistically significant from control. Further, prevention of retinal and choriocapillaries atrophy and low CNV recurrence rate (3.4%/ 2years) is observed. Complication rates are comparable with vitrectomy and subretinal surgery. Aspirates were cultured for up to 4 months and positive for cytokeratin. The harvesting efficacy with the aspiration instrument was at 19 976 cells (std=6016)/ 2-4 DD with 82% viability in cadaver eyes. 1400- 2000 adult human RPE-s were required for confluence in a 96 well dish (0.35cm²).

Conclusions: Autologous RPE transplantation combined with subretinal surgery might be a treatment option for fCNV due to AMD. Future directions aim for autologous RPE ex vivo expansion on Bruch's membrane equivalents and growth factor stimulation of in situ RPE wound healing.

■ 2256

Two years experience of translocation of autologous pigment epithelium and choroids in patients with age-related macular degeneration

VAN MEURS JC

■ 2257

Retinal Prosthesis: Subretinal versus preretinal approach

WALTER P

■ 2261

Structural imaging I. Simultaneous en-face OCT and confocal imaging in the anterior segment

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Purpose: To report studies of high resolution imaging in the anterior chamber by collecting OCT images oriented in rectangular directions: B-scan images (which contain the optic axis) and C-scan images (constant depth).

Methods: We researched two versatile en-face OCT systems capable of collecting B-scan and C-scan images from the anterior chamber. The two systems operate on different wavelength, at 820 and 1300 nm. Both systems can collect B-scan and C-scan images. The system at 820 nm is a dual channel system which delivers pairs of en-face OCT and confocal images.

Results: Using different aperture optics, images with a high magnification of the cornea, or of the entire anterior segment are generated. The dual channel system offers: (i) versatility, being capable of displaying both C-scan OCT images as well as B-scan OCT images; (ii) overall eye guidance, using the confocal image and (iii) correction for the en-face movement in the images generated by en-face imaging using the confocal image.

Conclusions: En-face OCT imaging opens the way for accurate high resolution imaging in rectangular, multiplanar directions in the anterior segment. Stacks of en-face OCT images can be acquired and used for 3D reconstructions. Animations of pairs of OCT/confocal images demonstrate the utility of the dual imaging system for in vivo imaging of the anterior segment of the eye.

■ 2262

Ultrahigh resolution ophthalmic optical coherence tomography: Recent developments

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Purpose: To present recent developments in posterior segment OCT.

Methods: A compact, new generation ultrahigh resolution ophthalmic optical coherence tomography (OCT) system has been developed and used in a clinical setting for the first time. In vivo ultrahigh resolution ophthalmic OCT has been performed in more than 300 eyes of 200 patients.

Results: OCT examinations demonstrated unprecedented visualization of intraretinal morphology, especially the photoreceptor layer in several retinal pathologies, as well as the potential to enhance sensitivity and specificity for early ophthalmic diagnosis, to monitor the efficacy of therapy as well as to contribute to a better understanding of ocular pathogenesis.

Conclusions: Using state of the art laser technology, including ultra broad bandwidth femtosecond solid state lasers, fiber lasers and photonic crystal fibers based light sources, ultrahigh resolution ophthalmic optical coherence tomography imaging with unprecedented axial resolution and enhanced penetration in the 800nm, 1050 nm as well as 1350 nm wavelength region was made possible. With an extension of this technique, extraction of spatially resolved spectroscopic information is enabled to improve image contrast and to obtain functional or biochemical properties of the investigated tissue.

■ 2263

Functional imaging 1. A new tool for noninvasive intraocular pressure measurements

STORK W

■ 2264

Functional imaging 2. Actual and future developments for ocular blood flow measurements

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Purpose: A variety of eye diseases including diabetic retinopathy and glaucoma is associated with ocular perfusion abnormalities. There is, however, currently no generally accepted technique for the quantitative measurement of ocular blood flow available.

Methods: In the present talk a number of new innovative techniques for the assessment of ocular blood flow are presented. These techniques include methods that are specifically developed for animal research, but also methods with potential clinical applications.

Results: Preliminary results with each of the techniques are presented.

Conclusions: A number of innovative techniques for the measurement of ocular blood flow parameters are currently in development. Hopefully this may be a step towards clinical applications of ocular blood flow measurements.

■ 2265

Wavefront aberrations: recent developments

MROCHEN M

■ 2266

Ultrashort Lasers for Corneal Surgery

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Purpose: Femtosecond (fs) laser pulses may offer new possibilities in the field of refractive surgery, especially when using the laser as a microkeratome. By induction of nonlinear absorption processes the laser can be used to perform intrastromal cuts. The conventional microkeratome, associated with numerous potential side effects, can possibly be replaced. Furthermore refractive lenticules can be prepared within the stroma and removed in a single step operation.

Methods: Animals studies on 10 rabbits were conducted, in which cuts were order to create both a lamellar flap and an intrastromal refractive lenticule. The flap was lifted, the lenticule extracted and, finally, the flap was repositioned (intrastromal laser Keratomileusis, ILK). The corneal samples were collected up to 120 days after treatment and processed for histopathological analysis. Moreover, keratoplasty studies on ex-vivo pig eyes were conducted

Results: All flaps could be opened and prepared lenticules could be extracted in one piece by the surgeon. The treated corneas developed mild wound healing reaction, comparable to what is known from excimer laser in situ keratomileusis (LASIK) studies. The woundhealing was restricted to the flap-stroma interface, most pronounced at the periphery of the flaps.

Conclusions: The use of the fs-laser offers new possibilities in preparation of corneal flaps, possibly providing advantages in comparison to conventional microkeratomes and, furthermore, the fs-laser has the potential to create intrastromal refractive lenticules for complete refractive procedures (ILK). Due to the high precision of the prepared cuts, applications like keratoplasty or new ways in the treatment of prebyopia are possible.

Novel Ophthalmic Technologies

■ 2267

Selective RPE-therapy (SRT) by repetitive μ s-laser pulse exposure

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Purpose: Selective RPE-therapy (SRT) is a new laser procedure targeting on retinal diseases, which are thought to be associated with a degradation of the retinal pigment epithelium (RPE).

Methods: In SRT a train of 30 green μ s-laser pulses from a Nd:YLF-Laser (527 nm) is applied to the fundus with a repetition rate of 100 Hz. After applying radiant exposures around 650 mJ/cm² per pulse, angiographically observed fluorescein leakage demonstrates RPE-defects. However, the lesions are ophthalmoscopically invisible. Neither bleeding nor scotoma, proved by microperimetry, were observed, thus demonstrating no adverse effects to the choroidea or the photoreceptors, respectively.

Conclusions: As introduction of the technique, in vitro investigations leading to the development of the method will be presented. The mechanisms behind selective RPE-effects will be discussed basing on in vitro results and including temperature calculations. As a result of the principal investigations, a non-invasive on-line dosimetry control system was developed and will be described, which is useful in order to avoid angiography post treatment. Further, the technique also allows, to the first time, an on-line temperature determination at the laser treatment spots on the retina. First results will be presented and discussed.

■ 2271

Search for antioxidative defense (AOD) genes that will prevent maturity onset cataract

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Purpose: This laboratory is attempting to prevent the process by which the transparent aging human lens develops a cataract.

Methods: Both human (HLE-B3) and murine (alphaTN4-1) immortal lens epithelial cells were conditioned to survive peroxide stress (either hydrogen peroxide or tertiary butyl hydroperoxide (TBOOH)) that will cause cataract in in vitro experiments. This was accomplished by gradually increasing peroxide concentrations over a period of six to eight months. Gene expression in these cell lines was then compared to control cells utilizing Affymetrix gene expression microchips capable of detecting either 12,422 murine genes or 22,000 human genes. Utilizing either expression or adenoviral vectors, cells were enriched in one or two AOD genes shown by the chip analysis to have a large increase in expression.

Results: Examination of cell lines resistant to TBOOH or hydrogen peroxide indicated that a small number of AOD genes were upregulated. These genes will be discussed and gene expression differences in the cell lines reported. Two of the major categories of AOD genes revealed by this work are peroxidases and GSH transferases. A major representative of each category of AOD genes, namely catalase and GSH-S-transferase alpha 2 (GST-alpha2) were co-transfected into the murine and human cell lines. Preliminary experiments indicate that enrichment of these AOD genes protect the cells from oxidative stress.

Conclusions: The results suggest that enriching or increasing the expression of catalase and GST-alpha2 may protect lens epithelial cells and possibly the lens from peroxide and other types of oxidative stress.

■ 2272

Galactokinase gene mutations and age-related cataract. Lack of association in an Italian population

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Purpose: To investigate possible associations between sequence changes in the galactokinase gene (GALK1) and age-related cataract in a European population.

Methods: Cataractous and unaffected individuals were selected from those participating in the Collaborative Italian-American Clinical Trial of Nutritional Supplements and Age-Related Cataract or attending the Section of Ophthalmology of the University of Parma for cataract surgery. Opacities were assessed by slit-lamp and retro-illumination lens photographs. Mutations were identified by PCR amplification of individual exons and sequencing using an ABI 377 Prism or 3100 automated DNA sequencer.

Results: DNA samples were obtained from 115 individuals with clear lenses and from 185 individuals with cataract (106 with any nuclear, 88 with any cortical, and 25 with any posterior sub capsular cataract). 157 of the 185 patients with cataract (85%) were age-matched with a control within an age range of + 1 yr. Although SNPs causing amino acid changes in the galactokinase protein were identified in: Exon 4: 1184M; Exon 6: G274D; and Exon 7: V338A; there were no significant differences between control and cataractous individuals. Similarly although 78 samples showed a C to T transition 43 bases into intron 7, there was no difference in C/T distribution between cases and controls (p=0.87).

Conclusions: In this northern Italian population age-related cataract does not appear to be associated with GALK1 alleles. Since this is due to a lack of sequence changes in both affected and control individuals, this study cannot rule out the possibility of an association in other populations.

Genes and age-related cataract

■ 2273

Microarray Analysis Of The Mouse Lens During Maturation And Cataractogenesis

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Purpose: Defects in the physiology of the lens during ageing commonly result in cataracts. Susceptibility to cataract may also depend on mutations and/or polymorphisms in genes with roles in lens maturation and physiology. Here, microarrays have been used for gene expression profiling on mouse lenses during maturation and cataractogenesis.

Methods: For studies on normal mouse lenses, 33P labelled target lens RNA was hybridised to mouse GeneFilters microarrays (ResGen[™]). Microarrays were also carried out in our facility to identify genes differentially expressed in lenses from wild-type mice versus lenses from the Secreted protein acidic and rich in cysteine (Sparc) ^{-/-} knockout mouse (in which age-onset cortical cataract is the major feature). Data were analysed to select for differentially regulated genes. Selected gene expression was confirmed using semi-quantitative RT-PCR.

Results: In the ResGen[™] array experiments, 1668 genes were expressed in the lens at levels significantly above background. In the Sparc experiments, differential expression of 20 genes was confirmed. Genes included collagen sub-types, glypican-3 (when mutated causes Simpson-Golabi-Behmel syndrome, accompanied by age-onset cataracts), and metallothionein 2. The most intriguing observation was that haemoglobin was expressed in the lens and its expression was significantly downregulated in the lenses of 9-month old Sparc ^{-/-} mice compared to controls. A chaperone (eraf) involved in the correct folding of haemoglobin was also down-regulated in Sparc ^{-/-} mice.

Conclusions: Since oxidative damage is a major cause of cataract during ageing, we hypothesise that haemoglobin may have a role in apoptosis, oxygen transport and oxygen buffering in the lens.

■ 2274

Identification and functional clustering of global gene expression differences between age-related cataract and clear human lenses and aging clear human lenses

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Purpose: To identify the global gene expression differences between lens epithelia isolated from age-related cataract and clear human lenses and aging clear lenses.

Methods: Gene expression changes were detected by DNA microarray hybridization chips containing about 20,000 genes. Genes were clustered according to their known functions. Secondary confirmations of identified gene expression differences were conducted on separately prepared RNA samples.

Results: Analysis of over 20,000 genes revealed that 143 transcripts were increased and 363 transcripts were decreased at levels of 4-fold or greater between epithelia isolated from cataract versus age-matched clear lenses. Of the genes increased in cataract, the majority have known functions involving transcription, nucleic acid binding and processing, protein processing, ion transport and cell growth/maintenance. Of the genes decreased in cataract, the majority have known functions involving heat-shock/chaperone activity, protein synthesis/degradation, oxidative stress and metal binding. Far fewer gene expression differences were detected in aging clear human lenses and lens epithelia.

Conclusions: These data provide evidence that cataract is associated with multiple previously identified and novel changes in lens epithelial gene expression. The identified pathways provide insight into those mechanisms likely to be important for cataract. In contrast to the cataract to clear lens comparison, far fewer gene expression differences were detected in aging lenses. Individual genes, their associated pathways and ongoing functional analysis of these will be described.

■ 2275

Comparative microarray analysis of the mRNA expression profiles of human age-related nuclear cataracts and age-matched transparent lenses

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Purpose: To identify genes differentially expressed in age-related nuclear cataracts.

Methods: Total RNA was extracted from pools of 5 mm capsulorrhexis epithelial samples microdissected at surgery from eyes with nuclear cataract or, post-mortem, from transparent lenses. mRNA levels in the two samples were comparatively analyzed by hybridization to DNA microarrays containing 4132 known human genes. Only spots consistently modulated over 4 independent comparisons were retained for analysis. A subset of the mRNA expression differences were further confirmed by real-time PCR.

Results: 288 genes were down-regulated and 7 up-regulated by a factor of 2 or more in cataractous lenses. Gelsolin and glucosidase I were the most up and the most down-regulated mRNAs, respectively. The mRNAs decreased in cataract included those coding for CRYBA4, for some chaperone proteins, for ribosomal proteins. Presenilin 1, presenilin 2 and amyloid beta (A4) precursor protein binding were all significantly down-regulated. Among the down-regulated mRNAs of the "cellular stress" class, we only found glutathione S-transferase A4.

Conclusions: Our results confirm some transcript level changes previously documented and provide novel information on the global modification of the gene expression profile in cataract. They also highlight a close similarity, both in terms of the type of response (mainly downregulation) and of the genes it entails between cataract and other age-related diseases, most notably Alzheimer. The present data contribute to a better understanding of the molecular mechanisms underlying senile opacification of the human lens.

■ 2291

Episcleral Brachytherapy Vs Combined Therapy (Brachytherapy with Transpupillary Thermotherapy) in Choroidal Melanoma

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Purpose: To compare tumor control and complications between episcleral brachytherapy and combined therapy (episcleral brachytherapy plus adjuvant transpupillary thermotherapy -TTT) for uveal melanoma.

Methods: Sixty four (64) patients were evaluated. Fifty-six patients were treated with episcleral brachytherapy alone (University Hospital) and 8 were treated with combined treatment (brachytherapy, University Hospital, TTT, Instituto Universitario de Valladolid). SPSS statistical package was used to analyse the data.

Results: The mean follow-up time for episcleral brachytherapy was 27.19 months and 16.65 months for those patients with combined therapy. Tumor size prior to treatment with episcleral brachytherapy was 10.30 mm (SD 0.75 mm) for median base diameter and 4.62 mm (SD 0.43 mm) for median height. Those treated with combined therapy showed median base diameter of 10.05 mm (SD 0.77 mm) and median height of 4.35 mm (SD 0.92mm). On the last exam after treatment, largest diameter and height for episcleral brachytherapy was 7.70mm (SD 0.58 mm) and 2.85 mm (SD 0.48 mm) respectively, and for combined therapy 8.11 mm (SD 1.28 mm) and 2.52 mm (SD 0.64 mm). Median visual acuity before episcleral brachytherapy was 0.6 and 0.5 at the last follow-up, whereas for combined therapy initial acuity was 1.3 and 1.5 at last visit. These differences were not statistically significant. Regarding treatment complications radiation retinopathy was present in 25.9% of patients with episcleral brachytherapy vs. 12.5% in patients with combined therapy. Other complications only appeared after episcleral brachytherapy alone, including radiation neuropathy (3.7%), cataract (24.1%), ptosis (1.9%), scleral necrosis (3.7%) and vitreous hemorrhage (13%).

Conclusions: Combined therapy seems to have less radiation related complications and better visual acuity outcomes with similar tumor control in uveal melanoma. However these differences are not statistically significant probably due to small sample size. New studies with more patients and longer follow-up are needed to confirm these preliminary results.

■ 2292

Ocular complications after Iodine Brachytherapy (IBT) for large ciliochoroidal melanomas

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Purpose: To report the cumulative incidence of ocular complications of IBT in a population-based group of patients with a uveal melanoma, large by COMS criteria.

Methods: Of 121 patients with a large ciliochoroidal melanoma, seen in a national referral center in 1991-2001, 96 were managed with IBT (median apical dose, 87 Gy). Median follow-up time was 3.5 years (range, 0.3-10.4). Time to cataract, iris neovascularization, secondary glaucoma, maculopathy, optic neuropathy, persistent exudative retinal detachment (RD), and vitreous hemorrhage were assessed with cumulative incidence analysis and competing risks regression. Death and enucleation were modeled as competing risks.

Results: The 5-year cumulative incidence of cataract was 70% (95% CI, 58-79), of iris neovascularization 62% (95% CI, 50-71), of secondary glaucoma 60% (95% CI, 48-70), and of maculopathy and optic neuropathy, 52% (95% CI, 35-65) and 46% (95% CI, 30-61), respectively. Corresponding incidence of persistent RD was 25% (95% CI, 15-36) and of vitreous hemorrhage 36% (95% CI, 23-48). Four fifths of complications appeared within 3 years of treatment. Tumor height (which correlates to scleral dose) was associated with time to cataract ($P=0.017$), iris neovascularization ($P=0.087$) and persistent RD ($P=0.046$). Maculopathy and optic neuropathy were associated with distance to fovea ($P=0.015$) and with distance to disc ($P=0.015$), respectively. A posterior location did not protect from cataract after IBT, but increased the risk for maculopathy and optic neuropathy.

Conclusions: Cumulative incidence analysis provides least biased estimates of incidence in a setting with frequent competing risks such as metastatic death from a large melanoma.

■ 2293

Ciliary body melanomas. Survival and prognostic aspects post brachytherapy or protontherapy

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Purpose: Our main goals are, at 5 and 10 years after treatment, to study survival of uveal melanomas which involve the ciliary body and to review prognostic aspects.

Methods: 106 tumors (27 ciliary body melanomas and 79 choroidociliary melanomas) were included in this study concerning patients treated between June 1983 and April 1998. 72 patients were treated with 106-ruthenium applicators and 34 were treated with protontherapy. Some large tumors or recurrences needed a second treatment. The mean follow up period was 91 months.

Results: The mean tumor sizes before treatment were 6.6 mm for melanoma thickness and 10.3 mm for mean largest basal melanoma diameters (LTD). 71 % of the patients were still alive at 5 years, 35 % had metastasis and 27.7 % developed recurrences.

Multivariate analysis showed that the risk factors for melanoma-related death were: LTD superior to 13 mm, presence of exsudative retinal detachment, macroscopic iris root involvement at the time of diagnosis, and choroidociliary location.

Conclusions: With 71 % of patients still alive at 5 years, melanomas involving the ciliary body do not seem through this review, to appear as less severe tumors than more posterior uveal tumors. Due to sizes differences between ciliary body melanomas and choroido-ciliary melanomas, we cannot come to the conclusion that choroido-ciliary melanomas have a poorer prognosis than ciliary body melanomas. Largest tumor diameter superior to 13 mm, presence of exsudative retinal detachment and macroscopic iris root involvement at the time of diagnosis are important risk factors for melanoma-related death, as shown by the multivariate analysis.

■ 2294

Iris Color in a Spanish Cohort of Patients with Uveal Melanoma

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Purpose: To report the distribution of iris color in patients with uveal melanoma and evaluate its prognostic significance.

Methods: Retrospective study of 160 patients from the Ocular Oncology Unit at University Hospital of Valladolid. Iris color was classified in three groups: brown, hazel-green and blue-grey. Classical prognostic factors of uveal melanoma were correlated with iris color. SPSS statistical package was used to analyse the data.

Results: Thirty eight percent (38.1%) of patients had brown iris, 40% hazel-green and 16.9% blue-grey. No patient with brown iris showed extraocular extension, whereas 11.5 % of hazel-green and 20% of blue-grey colored iris did show on CT scan this finding. Differences were statistically significant (chi square $p=0,004$). The mean survival was 11.04 years for brown color patients, 8.25 years for hazel-green, and 5.18 years for blue-grey, however these differences were not statistically significant. No differences were found on cellular type, largest diameter or other prognostic factors.

Conclusions: In comparison with other studies this cohort shows a greater proportion of darker iris in patients harvesting uveal melanoma. This finding is probably related to a major proportion of pigmented iris in our population. Extraocular extension has been more common in patients with lighter iris in this cohort, however no other unfavourable prognostic factor has been associated with lighter iris. Although a trend is present towards a better survival in dark iris patients, differences are not statistically significant. Prospective studies with more patients and longer follow-up are necessary in order to demonstrate a better prognostic in patients with darker iris suffering of uveal melanoma.

■ 2295

Nucleolar diameter and cell proliferation as independent predictors of mortality from malignant ciliochoroidal melanoma

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Purpose: To study to which extent nucleolar diameter and cell proliferation indices predict mortality from malignant melanoma of the choroid and ciliary body.

Methods: A population-based cohort study of melanoma-specific mortality of 167 consecutive patients who had an eye enucleated because of ciliochoroidal melanoma in 1972-1981 was conducted. Largest nucleoli were measured from digital photographs of silver-stained tumors along a central 5-mm-wide linear field parallel to tumor base. The mean of the ten largest nucleoli (MLN) was calculated. Cell proliferation was determined with antibodies to proliferative cell nuclear antigen (PCNA). Microvascular loops and networks and microvascular density (MVD) were also assessed.

Results: Both MLN and PCNA could be determined from 115 melanomas. MLN ranged from 2.60 to 6.18 μm (median, 4.05) and PCNA from 6 to 131 (median, 29) pr HPF. The associations of large MLN with presence of epithelioid cells and MVD were statistically significant. The 10-year melanoma-specific survival decreased most strongly with MLN (0.74, 0.60 and 0.42 according to tertiles; $P = 0.0060$), presence of loops and networks ($P = 0.0001$), and increasing MVD ($P = 0.0001$); the effect of PCNA was smaller in this data set. By Cox regression, MLN was an independent predictor of survival, adjusting for presence of epithelioid cells, loops and networks, and MVD.

Conclusions: MLN and microvascular loops and networks are independent predictors of survival. MLN and MVD are partially interrelated.

■ 2296

Detection of circulating malignant cells in peripheral blood of uveal melanoma patients

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Purpose: Metastasis is the major cause of treatment failure and death in human malignancies such as uveal melanoma (UM). Only about 1% of UM patients will show evidence of metastatic disease during initial systemic work-up upon diagnosis. However, approximately 50% of patients will subsequently develop metastases and die, at interval of approximately 6.5 years following treatment such as enucleation or radiation therapy. These findings suggest that UM cells may have already disseminated by the time of initial diagnosis and that the patient may have subclinical metastatic disease in the form of circulating malignant cells (CMC's) or micrometastasis. At the present time, we are not able to completely understand the natural history of the disease. The kinetics of events that culminate with metastatic disease to the liver following treatment of the primary lesion have not yet been elucidated. Therefore, the purpose of the proposed study is to characterize the latent period of metastatic UM by detecting CMC's in blood collected from uveal melanoma patients at various stages of the disease (ie. time of diagnosis, treatment, follow-up and detection of metastasis). Correlation between the presence of CMC's and the final patient outcome will then be established.

Methods: The first phase of the study consist of validating RT-PCR as an accurate method to detect CMC using double markers (tyrosinase and MART-1/Melan A). Blood from healthy volunteers is spiked with increased concentration of cultured UM cells in order to determine the specificity, sensitivity, and lower detection level of the technique. The second phase consist on a prospective clinical study, which enroll UM patients currently attending the Oncology Clinic in the Department of Ophthalmology at the Royal Victoria Hospital. The initial enrollment visit includes a complete ocular examination with ultrasound measurements of the tumor and fundus photos. Blood samples are collected every 3 months and immediately process to detect CMC. Patients are grouped according to treatment: local radiation therapy, enucleation, and follow-up only. RT-PCR results will be correlated with clinical prognostic factors such as tumor location and size at the time of diagnosis, and histopathological prognostic factors such as cell type, mitotic activity, vascular closed loops, tumor infiltrating lymphocytes, extrascleral extension, vortex vein involvement and cytomorphometry. Finally the RT-PCR results will be correlated with the final outcome of the patient.

Results: RT-PCR proved to be a very sensitive and specific method capable of detecting 1000 cells in 2ml of spiked peripheral blood. The use of nested RT-PCR further decreased the lower detection level to 10 cells in 2ml of spiked blood. Both tyrosinase and Melan A are equally sensitive as markers for melanoma cells. The Clinical Trial started in February 3, 2003 and enrolled up to date 20 UM patients. Four patients were surgically treated, 12 were irradiated and 3 are being follow-up. Results of their blood analysis will be presented.

Conclusions: RT-PCR is a sensitive method for the detection of UM cells.

Identification of CMCs by RT-PCR in UM patients may enable new approaches in monitoring high-risk patients. Blood is an attractive easily available source of genetic information that may be analyze in the clinical setting to detect subclinical spread of disease so that adjuvant therapies can be directed at early stages of dissemination.

■ 2297

Necrosis of a choroidal melanoma after brachytherapy

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Purpose: Presenting a clinical case of choroidal melanoma with necrosis after brachytherapy and the difficulties in diagnosing this problem.

Methods: Presentation of a clinical case: a 73 year old lady was referred for choroidal melanoma of the eye. Fundus examination and fluo-angiographic examination showed a pigmented mass with retinal detachment. Ultrasound measured a tumour thickness of 9 mm and a diameter of 13 mm, a retinal detachment was present. Brachy-therapy with a Strontium-90 applicator was performed. Follow-up showed a reduction of the retinal detachment and decrease of the post-radiation oedema. 8 weeks later she presented with a painful and red eye because of elevated eye pressure of 70 mmHg; acute glaucoma was diagnosed and treatment was conservative; meanwhile a new ultrasound was performed which gave indication for tumour growth with scleral infiltration and extra-ocular extension for which an enucleation was performed.

Results: Pathologic examination showed that the eye was filled with a semi-fluid mass; microscopy showed a necrotic pigmented mass without active melanocytes, no scleral infiltration, but signs of scleritis.

Conclusions: Brachytherapy can cause necrosis of the tumour with an inflammatory reaction and acute glaucoma; the inflammation can mimic growth of the tumour.

■ 2298

Chemothermotherapy for retinoblastoma :analysis of relapse and failure

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Purpose: Chemothermotherapy (CTT) has shown very good results in the treatment of small to medium size retinoblastomas , without vitreous involment .risk factors for failure and relapses are analysed in a retrospective study.

Methods: All the retinoblastomas tumours treated by CTT alone in our institution from 1995 to 2001 , have been reviewed. Tumours requiring several cycles or that relapsed and required another treatment are compared to the lesions that responded to CTT.Risk factors for relapse and failures were analysed.LL

Results: 266 tumours in 102 children were treated (184 after an initial chemoreduction phase)After a median follow up of 44 months , 7,1%of tumours relapsed or did not respond after CTT .All relapses occured during the first two years of follow up.94,25%during the first year. 43,8% of relapses were local and 38% involved the vitreous. enucleation was necessary for 3 children and external beam radiation for 4 of them . In a multivariate analysis , the main risk factor for relapse is the tumour diameter at diagnosis of 3,5 mm ore more .

Conclusions: These findings confirm that CTT is an effective treatment for retinoblastomas of 15 mm or less , the main rik factor for failure is the initial diameter of the tumour at diagnosis .

OOTF: Ocular Oncology Task Force 2

■ 2299

Epidemiology and presenting signs of retinoblastoma: Experience of a single institution (1952-2002)

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Purpose: The aim of this paper is to report the epidemiological characteristics of children with retinoblastoma observed at the retinoblastoma referral center-University of Siena during the last 50 years.

Methods: The medical records of all patients with RB diagnosed and treated at the Retinoblastoma Referral Center-University of Siena between 1952 and 2002 were reviewed. A total of 307 cases (513 affected eyes) were identified. The following characteristics were evaluated: sex, laterality, age at diagnosis, family history of RB, place of residence at diagnosis and presenting signs. The patients were divided into two groups: an historical one (1952-1979) and a recent one (1980-2002) considering the different therapeutic choices with the introduction of chemotherapy in the treatment of intraocular RB.

Results: During the 50-year study period 307 RB cases were examined. In our study 158 (51.5%) patients were males and 149 (48.5%) females (M:F ratio of 1.06:1); 201 (65.4%) were unilateral and 106 (34.5%) bilateral. Age at diagnosis ranged from birth to 15 years. Overall, the median age at diagnosis was 13 months. In 41 (13.3%) cases, family history of RB was positive. Of 307 cases studied, 47 (15.3%) were resident in Tuscany.

Conclusions: Our data on incidence of RB by sex, laterality, age at diagnosis, family history, place of residence and presenting signs presented here offer a good, even if not yet complete, epidemiological analysis of RB in Italy.

■ 229a

Pleomorphic adenoma of the lacrimal gland in irradiated bilateral retinoblastoma

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Purpose: The authors report a case of pleomorphic adenoma of the lacrimal gland, that developed in the field of irradiation for bilateral retinoblastoma.

Methods: Case report.

Results: A 4 month old girl, with bilateral retinoblastoma, underwent enucleation of the right eye, systemic chemotherapy and bilateral irradiation. 18 years later, she developed a mass at the supero-temporal quadrant of the left orbit. The mass has been completely excised.

Conclusions: Pleomorphic adenoma is rare in children and teenagers; it usually presents as a painless, slow growing mass in healthy adults. In this case developed as a secondary tumor after irradiation for retinoblastoma. Even if by the term "second non ocular tumors" only malignant tumors are meant, the onset of a pleomorphic adenoma with high mitotic index in a 18 years old girl irradiated bilaterally for retinoblastoma is quite interesting.

■ 229b

Uveal melanoma in a 18 years old black male

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Uveal melanoma is rather an exceptional disease in both young adult before 20 years and black African population. We report the case of a choroidal melanoma in an 18 years old black young male. The tumor was treated by proton beam therapy in April 2000. Despite the apparent tumor control, the affected eye had to be enucleated 36 months after the initial treatment due to the development of neovascular glaucoma. Pathological examination reported a tumor mainly necrotic and did not find any living cells. Nevertheless, a cultured tumor fragment revealed the presence of melanocytes, being still able to divide. In spite of recognized efficiency of protontherapy in uveal melanomas, this observation underlines the possibility for long-term cells survival.

Modern clinical strategies for consistent IOP management

■ 2301

What did we learn from the big treatment trials?

MIGLIORIS

■ 2302

The importance of continued diurnal IOP control

CASTILLO A

■ 2303

Medical treatment of Glaucoma in 2003

HOLLÓGH

1st department of Ophthalmology, Semmelweis Univ., Budapest

■ 2304

Perforating or non-perforating filtering surgery?

GANDOLFI

Modern clinical strategies for consistent IOP management

■ 2305

Pearls to improve trabeculectomy

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Purpose: To present a modified technique of trabeculectomy preventing early postoperative complications.

Methods: A fornix based conjunctival flap and a wide scleral flap dissected centrally into clear cornea will favor the posterior drainage of aqueous humor promoting the formation of a diffuse bleb. The anterior chamber maintainer prevents the anterior chamber to collapse during the operation and washes out inflammatory cells, cytokines, and debris. Releasable/adjustable sutures will keep the anterior chamber formed in the early postoperative period and allow tailoring the pressure to the desired postoperative level.

Results: This modified technique of trabeculectomy will be presented step by step.

Conclusions: It is now possible to prevent early post-operative complications like flat anterior chambers and hypotony after trabeculectomy using relatively simple measures.

■ 2311

Changes in refraction with accommodation

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Wavefront technology can produce iso-dioptic lines showing that there is a topographic variation in power over the ocular surface bound by the pupil. Both changes in pupil size and accommodation can alter the pattern of these iso-dioptic lines. Standard routine refraction produces a net spherocylindrical result which is a subjective integration of ocular surface power over the pupil along 2 mutually perpendicular axes. Applying gaussian optics to a model eye to calculate the expected changes in spherocylindrical refraction for a) a range of ametropia, b) viewing distances and c) theoretical effects of asymmetric accommodation. We conclude, accommodation can adjust the magnitude of astigmatism. Using Landolt C targets we tested our model by performing distance and near refraction on various eyes (normals, keratoconus, post-graft, post-LASIK). The results indicate why, in certain select cases, near spherocylindrical refraction should be performed to maximize near vision performance.

■ 2312

Real quantitative effects on subjective quality of vision with accommodation

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Ray tracing proves that, the spherical aberration of the eye increases with accommodation when viewing near objects. Accommodation reduces the optical performance of the eye, is the subjective quality of the eye also reduced? Using a new test device based on Moire patterns and a Badal lens system we examined vision at infinity, 1m, 0.5m and 0.3m at relatively high and low contrast levels. The optical arrangement allows direct comparison of vision at all test distances. We report on our results on normal pre- and post-presbyopes with insignificant distance corrections. Vision performance was greater at infinity, and reduced at 0.3m. within the pre-presbyopic group. We conclude, accommodation benefits the eye in terms of gaussian optics and refraction but, the overall optical quality of the eye is reduced during near vision.

■ 2313

The retinal image: with normal accommodation and with multifocal IOLs

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The optical quality of the retinal image and how it changes with accommodation in normal eyes will be presented and compared with the images generated from eyes implanted with multifocal intraocular lenses (mIOL). Distance and near objects, under different illumination conditions will be considered to understand the new imagery created on the retina. From the new imagery created we will be able to understand the visual quality of patients implanted with multifocal IOLs and how this quality compares in normal phakic eyes. Examples of Contrast Sensitivity Functions (performance index that most usefully documents human spatial vision) of patients implanted with mIOLs at distance and near will be shown to illustrate the relationship between optical and visual function with mIOLs.

■ 2314

Basic principles of accommodation and presbyopia

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Presbyopia is a condition of age rather than ageing and, as such, is developed from the situation where the normal age-related reduction in amplitude of accommodation reaches a point when the clarity of vision at near cannot be sustained for long enough to satisfy an individual's requirements. Most of our facility to accommodate has been lost by 55 years-of-age and subsequent deterioration in visual performance at near is attributable to characteristics of senescent vision. Our understanding of the cause of presbyopia has then to be derived principally from our understanding of the mechanism of accommodation in young eyes. Hermann von Helmholtz did much to clarify these mechanisms, but despite research in the 100 last years, there is still no consensus on their precise nature. This communication presents a summary of issues, past and present, which have figured in the literature on the physiology of accommodation and presbyopia, and confirms that the pathophysiology of presbyopia is likely to result from deterioration in structure and function of a number of inter-related tissues. Changes in crystalline lens dimensions with age, the associated change in geometry of zonular attachments, and changes in viscoelastic properties of the lens capsule and lens matrix would, however, appear to be the principal correlates for the onset of presbyopia. Recent models of the biomechanics of accommodation have drawn attention to the feasibility of extralenticular contributions to presbyopia and have examined properties of the elasticity and leverage provided by posterior, anterior and tensile fibre systems.

■ 2341

Adrenomedullin relaxes isolated bovine retinal arteries

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Purpose: Adrenomedullin (AM), a peptide noted in a broad variety of tissues and cells, is known to induce relaxations in different vascular beds and isolated blood vessels, including the (extra-ocular) central retinal artery. The present study aimed to assess and to characterise the vasorelaxing effect of AM on (intra-ocular) isolated bovine retinal arteries (BRA).

Methods: Retinal arteries were isolated from bovine eyes and mounted in a wire-myograph for isometric tension recording. Concentration-response curves were constructed by cumulative addition of AM (1pM to 0.1M) in the organ bath.

Results: AM caused a concentration-dependent relaxation of the BRA. The estimated pEC₅₀ is 7.90±/0.03, and the estimated maximal relaxation is 59.20±/1.00% (n=21). The AM-response was reduced after removal of the endothelium of the BRA (30nM: from 38.14±/4.90% to 25.71±/4.32% relaxation, n=7, p<0.05). Also treatment of the BRA with the NOS-inhibitor L-NA or the soluble guanylyl cyclase inhibitor ODQ reduced the AM-response. Cyclo-oxygenase inhibition resulted in an increase in the AM-response. The adenosine receptor antagonist 8-SPT had no influence on the AM-response. The AM-receptor antagonist AM 22-52 caused a small, but significant reduction in the AM-response (30nM: from 55.33±/9.65% to 47.50±/8.26% relaxation, n=6, p<0.05). The CGRP-receptor antagonist CGRP 8-37 caused a more pronounced decrease in AM-response (30nM: from 55.43±/10.53% to 17.77±/6.57% relaxation, n=6, p<0.05).

Conclusions: The present study demonstrates that AM relaxes BRA. Endothelium-derived NO and stimulation of CGRP-receptors seem to be involved. Stimulation of AM-receptors seem to play a minor role. Prostanoids and activation of adenosine receptors do not appear to be involved in the AM-response.

■ 2342

Vasoactive response of travoprost versus latanoprost, prostaglandin F2 alpha, and U46619 in isolated porcine ciliary arteries

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Purpose: To assess the vasoconstrictive properties of different prostanoids or drugs sharing structural pharmacological similarities with prostanoids in isolated porcine ciliary arteries.

Methods: In a myograph system for measurements of isometric forces, quiescent vessels were exposed to increasing concentrations (0.1 nM - 0.1 mM) of U46619 (thromboxane A₂ analog), prostaglandin F₂alpha (PGF₂alpha), latanoprost, or travoprost, in the presence or in the absence of 10 µM of SQ29548, a thromboxane (TP)-receptor antagonist. Results are expressed in percent of the maximal contraction evoked by 100 mM potassium chloride.

Results: At the highest concentration tested (0.1 mM), contractions evoked by PGF₂alpha (87.86 ± 3.5%), U46619 (66.63 ± 4.1%), and latanoprost (60.31 ± 5.1%), were significantly (P < 0.001) more pronounced than those observed with travoprost (28.56 ± 4.6%). The contraction induced by PGF₂alpha, U46619, and latanoprost were very significantly (P < 0.001) blunted by SQ29548. The mild contraction evoked by travoprost was also reduced by SQ29548 but not in a significant manner in the present experiments.

Conclusions: Apparently, in contrast to travoprost, PGF₂alpha, U46619, and latanoprost are able to induce marked contractions that can be strongly inhibited by the thromboxane (TP)-receptor antagonist SQ29548 in isolated porcine ciliary arteries.

■ 2343

Nitric oxide plays a role in choroidal blood flow regulation during light/dark transitions in healthy subjects

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Purpose: It has recently been reported that light/dark transitions lead to changes in choroidal blood flow. A variety of studies indicate that nitric oxide is a key regulator of choroidal blood flow. In the present study we hypothesised that the choroidal blood flow response to changes in retinal illumination may be modified by nitric oxide synthase inhibition.

Methods: In 15 healthy subjects the response of choroidal perfusion to light/dark transitions was studied in a randomised placebo-controlled three way cross-over study using laser Doppler flowmetry and laser interferometric measurement of fundus pulsation amplitude. The responses were studied during placebo infusion, during infusion of the nitric oxide synthase inhibitor L-NMMA and during infusion of phenylephrine, which was selected as a control for L-NMMA.

Results: Before drug administration a transition from light to dark reduced both choroidal hemodynamic parameters by 11 - 20% L-NMMA ($p < 0.001$), but not phenylephrine or placebo significantly reduced this response. In addition, L-NMMA was the only drug to reduce basal choroidal blood flow.

Conclusions: Nitric oxide appears to be involved in the change in choroidal blood flow during light/dark transitions in healthy humans. Whether this nitric oxide is of neural or endothelial sources remains to be established, because L-NMMA is a non-specific inhibitor of nitric oxide synthase.

■ 2344

Effect of intravenous histamine on retinal and choroidal blood flow in humans

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Purpose: Histamine is an important regulator of vascular tone in the brain. Here, we report on the effect of intravenously administered histamine on retinal and choroidal blood flow.

Methods: A randomized, double masked, two way cross over study was performed in 14 healthy volunteers. Placebo or histamine was administered intravenously in stepwise increasing doses (0.08 μ g/kg/min, 0.16 μ g/kg/min and 0.32 μ g/kg/min). Retinal vessel diameters were measured using the Zeiss retinal vessel analyzer, retinal venous blood speed was assessed using bi-directional laser Doppler flowmetry. Retinal blood flow was calculated using retinal vessel diameters and blood speed. Subfoveal and pulsatile choroidal blood flow was measured with laser Doppler flowmetry and laser interferometry, respectively.

Results: Pulsatile choroidal blood flow increased by $+4.7\pm 3.3\%$, $+9.0\pm 8.0\%$ and $+14\pm 7.0\%$ ($p=0.001$, ANOVA), subfoveal choroidal blood flow by $+8\pm 11\%$, $+13\pm 11\%$ and $+13\pm 12\%$ ($p=0.003$, ANOVA) after infusion of histamine. Calculated retinal blood flow decreased by $-6.5\pm 14\%$, $-3.6\pm 20\%$ and $-7.5\pm 12\%$ but this effect did not reach level of significance.

Conclusions: Histamine in the selected doses increases choroidal blood flow, but has no significant effect on retinal blood flow. If this increase in choroidal blood flow is associated with pathologic conditions remains yet to be clarified. Financial support of the "Fonds zur Förderung der wissenschaftlichen Forschung" grant No. FWF-P16514 is gratefully acknowledged.

■ 2345

Contribution of biomechanical and hemodynamical factors into the genesis of myopic chorioretinal dystrophiesTARUTTA EP, KUSHNAREVICH NYU, IOMDINA EN
*Helmholtz Research Institute for Eye Diseases, Moscow***Purpose:** To find biomechanical and hemodynamic criteria of myopia changing to a degenerative form that could be applied at the earliest stage possible.**Methods:** Biomechanical parameters, such as length of the antero-posterior axis (APA), transversal diameter (TD), acoustic density of the sclera at the equator (ADS1) and the posterior pole (ADS2), as well as hemodynamic parameters, including rheographic coefficient (RC), linear blood flow rate and blood flow volume in the ophthalmic artery, the ciliary body and the posterior ciliary arteries were measured for 270 eyes of 135 patients aged 7-17 with myopia of 0.5-16 D. Of these, 136 eyes had uncomplicated myopia and 134 eyes had differing forms of peripheral vitreochorioretinal dystrophies (PVCRD). Coefficients r of correlation between the parameters measured and the condition of the eye fundus were determined.**Results:** Figures for ADS showed the closest correlation with PVCRD onset. The parameter was considerably reduced in PVCRD-affected eyes in the posterior pole (ADS2, $r = -0.42$) and especially in the equatorial zone (ADS1, $r = -0.56$). In contrast to APA, which showed no credible differentiation between the groups, the TD of the eyes with PVCRD was averagely 0.8 mm longer than that of the eyes with unchanged eye fundus ($p < 0.001$). Despite the fact that the RC proved to be averagely 24% lower in degenerative myopia, a weak correlation between the hemodynamic parameters and PVCRD onset was only revealed for linear blood flow rate in the ciliary body ($r = -0.19$).**Conclusions:** In all probability, the leading role in the genesis of myopically induced PVCRDs belongs to the biomechanical factor of sclera distension, primarily in the equatorial area of the eye.

■ 2346

Diabetic retinopathy is accompanied with changes in the frequency of retinal vasomotion as studied with the retinal vessel analyzer

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(1) *Department of Ophthalmology, Århus University Hospital, DENMARK, (2) Department of Ophthalmology, Århus University Hospital, Århus***Purpose:** Early diabetic retinopathy is characterized by retinal hyperperfusion due to a disturbance in the tone regulation of retinal arterioles. This disturbance affects the pressure autoregulation, but changes in vasomotion has also been suggested to be involved. Vasomotion is intermittent contraction of arterioles which is important for fluid homeostasis and tissue oxygenation. A disturbance in retinal vasomotion can explain that retinopathy lesions are dynamic, localized, and are generated in the microcirculation. Therefore, a further characterization of vasomotion in diabetic patients is pertinent.**Methods:** The Retinal Vessel Analyzer was used to measure diameter changes in first order retinal vessels. Video recordings of 3 min duration were digitized, a box of interest overlapping a large arteriole was defined, and the diameter was by computer software determined dynamically (25 times per second). The precision of the measurements was 1% for a 100 microns vessel.**Results:** Studies on five diabetic patients with CSMO and eight age-matched normal persons showed a significant ($p=0.029$) lower frequency of vasomotion in diabetic patients (mean=2.42/min & #61617; 1.08) than in normals (mean=3.89/min & #61617; 0.87). There was no difference in mean vessel diameter and the vasomotion amplitude in the two groups.**Conclusions:** The frequency of vasomotion is decreased in patients with CSMO as compared to normal persons. This may contribute to the pathogenesis of diabetic maculopathy.

■ 2347

Vasomotion in retinal arterioles is inhibited by cyclic GMP

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Purpose: Disturbances in the regulation of retinal blood flow with hyperperfusion are a key element in the pathogenesis of diabetic retinopathy (DR). This hyperperfusion may be facilitated by disturbances in vasomotion, which is periodic oscillations in the tone of retinal arterioles. Previous in vitro studies suggest that disturbances in vasomotion may be influenced by changes in the metabolism of Nitric Oxide (NO), but evidence from retinal vessels is lacking.

Methods: Retinal porcine arterioles (n=61) (inner dia 150 microns) were mounted in a small-vessel myograph for isometric measurements. When spontaneous and stable vasomotion had occurred cGMP levels were decreased with NG-Nitroarginine Methyl Ester (L-NAME) (n=6). cGMP levels were increased either with 8-Br-cyclicGMP (n=6), or by inhibition of phosphodiesterase 5 using Zaprinast (n=6). The frequency and amplitude of vasomotion were evaluated by Fast Fourier Analysis. During experiments intracellular free calcium was measured continuously using the fluorescent dye Fura2-AM.

Results: Vasomotion was observed in 52% of the mounted vessels and was found to be stable in 32%. L-NAME increased the frequency of the vasomotion significantly (p=0,016). 8-Br-cGMP and Zaprinast both decreased the frequency significantly (p=0,016). The force pattern was found to follow intracellular calcium levels, with a delay of 6 sec.

Conclusions: A L-NAME induced decrease in cGMP increased the frequency of retinal vasomotion. Addition of 8-Br-cGMP or inhibition of cGMP degradation lowered this frequency. This demonstrates the inhibitory role of the cGMP in the regulation of vasomotion in the retinal arterioles. This may be of importance for pharmacological intervention in retinal disease characterized by hyperperfusion, such as DR.

■ 2348

EDHF in bradykinin induced relaxation of the porcine posterior ciliary artery

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Purpose: This study examined the role of EDHF in porcine posterior ciliary arteries using small vessel myography. The relaxation response of these vessels to bradykinin was studied with the aim of demonstrating that in the presence of NO and COX inhibition, there is a substantial residual relaxation which is abolished by using inhibitors of EDHF.

Methods: Porcine eyes were obtained and the posterior ciliary vessels were dissected out microscopically and mounted in wire myograph for isometric force measurement. Vessels were precontracted using PGF-2a ($1 \times 10^{-7} \text{M}$ to $3 \times 10^{-7} \text{M}$) and relaxed using Bradykinin ($1 \times 10^{-10} \text{M}$ to $3 \times 10^{-6} \text{M}$). CCRCs were obtained to bradykinin before and after addition of inhibitors. L-NAME $1 \times 10^{-3} \text{M}$ was used to block NO synthesis. Meclofenamic acid $1 \times 10^{-5} \text{M}$ was used to block COX. A combination of $1 \times 10^{-7} \text{M}$ Charybdotoxin and $1 \times 10^{-7} \text{M}$ Apamin was used to block EDHF.

Results: Posterior ciliary artery relaxation produced by bradykinin was reduced in the presence of $1 \times 10^{-3} \text{M}$ L-NAME. (Mean maximum relaxation 53.58% vs 82.56% in controls). The addition of the $1 \times 10^{-5} \text{M}$ Meclofenamic acid to $1 \times 10^{-3} \text{M}$ L-NAME caused no further effect on arterial relaxation. Relaxation was abolished when L-NAME and Meclofenamic acid were combined with both the small conductance K^+ channel blocker Apamin $1 \times 10^{-7} \text{M}$ and the intermediate conductance K^+ channel blocker Charybdotoxin $1 \times 10^{-7} \text{M}$. (Mean maximum relaxation 15.2% vs controls with L-NAME and Meclo only 54.28%, p=0.028).

Conclusions: These results suggest that in the porcine posterior ciliary arteries relaxation in response to bradykinin is mediated by two mechanisms, NO and EDHF. Cyclooxygenase products have no significant effect.

■ 2351

Macular Imaging. Available techniques for human macular mapping

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Multimodal macula mapping is presented as a combination of a variety of diagnostic tools and techniques to examine the macular region in order to obtain information on its structure and function in a clinical environment. New methodologies for multimodal macula mapping based on a combination of scanning laser angiography, retinal leakage analysis, retinal thickness analysis, and visual field testing demonstrates the potential of macula mapping. Multimodal macula mapping appears to offer unique perspectives and insights that are expected to contribute to improved diagnosis and better understanding of macular diseases.

■ 2352

Non-invasive SLO Imaging - Autofluorescence

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■ 2353

Retinal imaging with RTA (Retinal Thickness Analyzing) and OCT Optical Coherence Tomography

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Purpose: To evaluate retinal thickness in diabetic and non-diabetic patients with non-invasive RTA (Talia, Israel) and OCT (Zeiss).

Methods: RTA and OCT retinal maps were made in healthy persons and in diabetic patients with and without diabetic retinopathy. Stereoscopic fundus photographs were made in all investigated persons, fluorography was performed in most diabetic patients with retinopathy. In diabetic patients retinal imaging was carried out before and after photocoagulation to evaluate the results of the treatment.

Results: Retinal thickness was increased in diabetics without retinopathy in comparison to healthy controls and retinal thickness decreased after photocoagulation as could be measured by means of RTA. Maximal mydriasis and clear media are required to perform retinal mapping with RTA as well as OCT.

Conclusions: Our studies suggest that retinal mapping with RTA may be considered to be an useful non-invasive, objective, adjunctive method in diagnosing diabetic retinopathy, RTA as well as OCT may give additional clinical information in patients with diabetic retinopathy and other retinal diseases, e.g. macular holes and may be of help in the follow-up of various retinal conditions. OCT mapping may give additional information about the vitreoretinal interface and intraretinal changes. Prospective, longitudinal cohort studies are now carried out to sustain the outcome of our recent clinical studies.

■ 2354

Retinal Thickness mapping: problems and solutions

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Several techniques now exist that allow for measuring and mapping human retinal thickness. There is a wealth of papers in the literature on the better and more reliable technique for different patient groups and discussion on the different numbers for retinal thickness presented by different groups. Among the different techniques, we will discuss what is being measured and some of the problems and eventual solutions of the Optical Coherence Tomograph (OCT), the Heidelberg Retinal Tomograph (HRT) and the Retinal Thickness Analyzer (RTA).

■ 2355

Characterisation of diabetic clinically significant macular edema. Mapping vs Measurement

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Purpose: To compare in a prospective, non-randomized, comparative trial, the extent and outlines of areas of the macular edema identified by stereofundus photography (SFP), Retinal Thickness Analyzer (RTA, Talia Technology, Israel) and Optical Coherence Tomography (OCT, Zeiss Humphrey Instruments, USA) in eyes of diabetic patients diagnosed with clinically significant macular edema (CSME).

Methods: Twenty-five eyes from 17 patients with type 2 diabetes and with a diagnosis of CSME based on SFP using ETDRS guidelines were re-examined within two weeks with the RTA and OCT, in the same session. The maps of the areas of edema obtained from the SFP, RTA, and OCT, were superimposed and compared in a central area of 3 mm in diameter and between SFP and RTA in a larger 4.2 mm square area centered on the fovea. Areas of increased retinal thickness with the RTA and OCT were identified by using reference maps from healthy control populations within the same age range and considering only values over mean +2SD.

Results: Areas of abnormally increased retinal thickness outlined by the RTA showed good correlation with the areas of retinal edema outlined by SFP in 21 of the 25 eyes. The areas outlined by the RTA were more frequently larger than the areas outlined using SFP. The 4 eyes that showed RTA values within normal limits, showed, characteristically, hard exudates on SFP. Areas of increased retinal thickness were detected by the OCT in only 12 of the 25 eyes.

Conclusions: The RTA identifies well the areas of retinal edema outlined by SFP. Disagreements between RTA measurements and SFP are apparently due to the presence of isolated hard exudates. OCT is less reliable for identifying localized zones of mild retinal edema.

■ 2361

Effect of residual sevoflurane on retinal function following day case surgery

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Purpose: To characterise the postoperative electroretinogram changes and resulting retinal function following sevoflurane anaesthesia

Methods: Electroretinograms were performed on 10 asa I or II patients who did not receive preanaesthetic medication and underwent sevoflurane anaesthesia.

Electroretinograms were recorded preoperatively, immediately after discharge from recovery room at 2 and 4 hours postoperatively. Contrast sensitivity and Snellen visual acuity tests were performed at the same time points. Patients completed visual analogue scales for sedation, anxiety and pain immediately before each erg recording. The time at which criteria for home readiness were first met was also noted.

Results: The latencies of the b-wave on the photopic erg and oscillatory potentials (op) were greater (31.7 ± 1.2 and 31 ± 0.9 vs 30.6 ± 1.4 ms, $p=0.02$ and $p=0.03$) and (23.1 ± 3.1 and 22.6 ± 4.0 ms vs 22.4 ± 3.3 ms, $p=0.01$) at each of the postoperative time points compared to preoperatively. The b-wave and op amplitudes diminished (157.7 ± 29.5 and 155.1 ± 34.9 vs 158.6 ± 38.8 uv) and (17.8 ± 9.0 and 16.2 ± 6.1 vs 18.2 ± 7.8 uv) compared to preoperatively. The amplitude of the n95 on pERG testing was decreased postoperatively (3.02 ± 0.6 and 2.6 ± 0.5 vs 3.27 ± 0.4 $p=0.003$). Snellen visual acuity was unchanged. Contrast sensitivity was diminished postoperatively (376 and 349 vs 404 $p=0.04$) compared to preoperative scores.

Conclusions: We have demonstrated that postoperative erg abnormalities are present in patients who have undergone sevoflurane anaesthesia. These abnormalities are associated with altered retinal function in the form of decreased contrast sensitivity and persisted beyond the time at which standard clinical discharge criteria had been met.

■ 2362

Subthreshold photopic (cone) electroretinography: normative values and clinical application.

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Purpose: Determine if the typical, biphasic, short-wavelength cone ERG waveform can be obtained with other wavelengths, including white light.

Methods: Photopic ERG (background of 50 cd. m⁻²) luminance-response functions were obtained from normal subjects to flashes of blue (intensity: > -2.40 log cd.sec.m⁻²), red (> -2.23 log cd.sec.m⁻²) and white (> -2.23 log cd.sec.m⁻²) light. Waveforms were compared to those obtained from patients affected with severe retinal disorders (e.g. ISCEV standard photopic ERGs $<5\%$ of normal amplitude).

Results: b-waves of similar amplitudes (e.g. 5.5 ± 1.9 μV) were evoked to flashes of -1.6 log cd.sec.m⁻² (blue) and -1.2 log cd.sec.m⁻² (red and white). These b-waves peaked at 26.8 ± 1.4 msec. In blue ERGs, b-waves were followed (at 43.7 ± 4.3 msec) by a positive (post-b-wave) component resulting in the typical biphasic waveforms characteristic of short-wavelength ERGs. This typical waveform could also be obtained with red and white flashes provided that they were 0.5-0.8 log unit dimmer than the blue flashes.

Conclusions: Our results suggest that the typical biphasic short-wavelength ERG, claimed to result from the summation of the L-M and S-cones, can also be generated with any light stimuli provided that they are dim enough. Comparing the resulting waveforms with clinical (pathological) data clearly helped in the interpretation of the very low voltage (white light) ERG signals which characterizes severe retinal disorders in that it allowed us to further categorize classes of pathophysiological processes (e.g. conditions with enhanced or abolished post-b-wave positivity). Funded by CIHR, GRENE and Réseau-Vision.

■ 2363

Quantification of relative damage of chromatic pathways in diseases involving the central or peripheral retina

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Purpose: We aim to quantify damage in the koniocellular and parvocellular pathways using psychophysical methods, and to provide a framework for testing physiological theories regarding early damage and disease progression in optic neuropathies and retinal disorders.

Methods: We used a psychophysical multiple interleaved staircase method, taken from an adapted Cambridge Colour Test, which allowed for determination of non-biased measures (protan, deutan, tritan and discrimination ellipses' axis lengths, and ellipses' angle and axis ratio). Size and luminance noise were especially adequate to ensure good co-activation of both pathways. All control (n = 95) and patient (n=159) eyes underwent full eye examination (including perimetry).

Results: Involvement of the red-green (parvocellular) axis was significantly more specific if the central retina was affected, as assessed by the measurement of ellipses' axis ratios. In disorders with predominant involvement of the peripheral retina there was on the contrary a significant bias for elongation of the tritan axis. Another marker of differential involvement of central vs peripheral retina was ellipse angle, which departed from 80 in CIE 1976 u'v' space in macular disorders. Length measures provided statistically robust indications regarding disease progression.

Conclusions: Testing simultaneously the konio and parvocellular pathways has allowed us to find a tool that can predict relative involvement of central and peripheral retina in several disorders, such as optical neuropathies and inherited retinal diseases. It can also provide quantitative markers to assess early functional damage and disease progression.

■ 2364

Visual development of the M and P pathways in healthy preterm infants

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Purpose: The aim of this study was to investigate the visual development of healthy preterm infants over the first year of life using pattern visual evoked potentials.

Methods: VEP were tested in 55 preterm infants (gestation : 24 to 30 weeks) and 52 fullterm infants aged between 3 and 52 weeks. All were free of any metabolic, ocular or neurological disease. We presented phase reversing sine wave gratings that were varied in spatial frequency (0.5 and 2.5 c deg-1) and contrast (4, 12, 28, 95%) for a total of eight conditions. VEPs were recorded at Oz (reference and ground to earlobes). Peak-to-baseline amplitudes and latencies were calculated for the N1 and P1 components of the VEPs.

Results: In the three lowest contrast conditions at 2.5 c deg-1, the N1 had a greater amplitude throughout the first year for the fullterm compared to the preterm infants. The difference was even more pronounced for the P1 component which displayed a greater amplitude for fullterms than for preterms in six of our eight testing conditions. No differences between preterms and fullterms were found concerning latency development of those two components during the first year of life.

Conclusions: Our previous study have shown that N1 component can be linked to the underlying parvocellular activity, while P1 to the magnocellular pathway. The present results suggest that normal preterm infants would present a developmental delay of the magnocellular pathway at least during the first year of life.

■ 2365

Comparison of pattern reversal and concentric motion evoked potentials in amblyopia

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Purpose: Some recent studies suggest that the motion pathway remains unimpaired in amblyopia while others continue to show deficits. We here compared pattern reversal and concentric motion evoked potentials in amblyopia.

Methods: Pattern were produced by the "Freiburg Evoked Potential" system.

Checkerboard patterns had 0.4° checksize, 98% contrast and reversed at 2 rps. The dartboard-like motion stimuli either contracted or expanded with a speed of $13^\circ/s$. They had 20% contrast, a luminance of 45 cd/m², covered $40^\circ \times 30^\circ$. The EEG was derived from OZ, O1, and O2 and was referenced to averaged ears.

Results: Pattern-reversal VEPs from the amblyopic eye showed reduced amplitudes compared to the fellow eye (amplitude ratio: 0.61 ± 0.09), and a prolonged latency with a difference of 12 ± 4.9 ms. These findings were more marked for patients with lower acuity. The interocular N2-amplitude ratio was 0.74 ± 0.09 , the interocular latency difference was 4.3 ± 3.7 ms, mainly caused by cases of deep amblyopia.

Conclusions: Hess et al. (1997) found a reduced motion aftereffect in amblyopic eyes, suggesting some deficit in motion perception. Kubova et al. (1996) introduced that motion VEPs are completely normal in amblyopia, concluding that amblyopia affects the magnocellular pathway less than the parvocellular pathway. The present study suggests that the degree amblyopia plays a role, as we found reduced motion-related responses in deep amblyopia, but normal motion responses in intermediate amblyopia. Fanni Molnar thankfully acknowledges at DOG and the exchange stipend for visiting the Albert-Ludwigs University in Freiburg in 2002.

■ 2366

Visual screening in a rural population from Romania

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Purpose: To evaluate the particularities of eye screening in a rural children population from Iasi region, Romania.

Methods: During a period of 2 years (February 2001 - March 2003), in a voluntary ophthalmological screening organized by World Vision Romania in 4 rural communities, 348 children were included in a prospective eye study. All children were examined for: refraction, motility and ocular fixation. Selected children were examined and treated in Eye Clinic from Iasi.

Results: Children were divided in 3 groups: 19% preschool, 56% were between 6 and 10 years and 25% - over 10 years of age. Ocular and visual disorders founded were: 29% refractive errors, 7.9% strabismus, 11.5% amblyopia and 2.8% ocular inflammations.

Conclusions: Early detection and early treatment of ocular disorders identified with visual screening can reduce amblyopia in children population.

■ 2367

Design of an optical system for an intraocular vision aid

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Purpose: The work presented here describes new optical concepts for the use as a part of an intraocular vision aid.

Methods: In the past, an approach to implant a compact vision aid where a opaque cornea is given, was discussed. The approach yields to an improved visual acuity, giving back a certain amount of vision. The main aspect of the vision aid is to transfer information to the retina which is representing a captured image of the patients field of view.

Results: The limited display resolution available inside the eye and the limited dimensions of the eye build the constrains of the optical system.

Conclusions: A combination of a spatial light modulator together with an imaging lens system build the main part of the optical concept. Intelligent spatial information distribution schemes for the retina ensure a high outcome for the patient. Various retinal acuities are realized. The employment of integrated zoom capabilities with an unconventional approach is discussed, as well as in-vivo adjustment mechanism of the focal plane.

■ 2371

Aey12, a new mutation on mouse chromosome 10 shows a phenotype similar to Maf mutants

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Purpose: Aim of the study is the morphological and genetical characterization of a new mouse mutant, Aey12.

Methods: A genome-wide linkage analysis was performed using microsatellite markers. Histological analysis and in-situ hybridisation were done between E12.5 and E17.5.

Results: Histological analysis demonstrated a stop of eye development at the lens vesicle stage. The lens vesicle is not filled by primary fibers, which is similar to observations in mouse Maf mutants. The spatial expression pattern of Pax6 is limited to a more central area; Pax2 expression at the posterior part of the eye is not affected. Surprisingly, g-crystallin transcripts can be detected in the lens vesicle. The mutants are fertile, and homozygotes are viable. Genome-wide linkage analysis mapped the mutation on the top of mouse chromosome 10 between the markers D10Mit123 and D10Mit206, which is different from the genetic position of Maf on mouse chromosome 8. Several candidate genes in the critical region have been excluded by sequencing (Rab32, Syntaxin11, Zac1, Nucleolin-homolog, Aig1). Because of the similarity to Maf mutants, also the expression of Maf was investigated. At early stages of development only a smaller Maf transcript is present. It corresponds to an alternative splice product representing mainly the 5'- and the 3'- end without the bipartite nuclear targeting sequence, the leucine zipper or the basic-leucine zipper domain. This transcript was overrepresented in the Aey12 mutants.

Conclusions: A new mouse mutant (Aey12) was characterized by a phenotype similar to Maf mutants. However, the different genetic localization indicates that in the Aey12 mutants another gene of the maf pathway is affected.

■ 2372

Microarray analysis of Sparc null mouse lenses reveals a role for haemoglobin in lens biology?

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Purpose: Sparc is a calcium, hydroxyapatite and collagen binding protein which is implicated in cell proliferation, tissue morphogenesis and repair. Sparc null mice show sub-cortical posterior cataract formation and eventual rupture of the lens capsule after six months of age. We have carried out microarrays on embryonic eyes and adult lenses from these mice at various ages, with the aim of identifying genes involved in the progression of disease.

Methods: Array studies were carried out using E14.5 eyes, 4 month and 9 month old lenses from Sparc null mice and age, strain and sex matched controls, using the 15k mouse NIA clone set. Differentially regulated genes were selected from multiple repetitions; these were checked for differential gene expression using semi-quantitative RT-PCR.

Results: Results: Using primers specific to different haemoglobin genes, we have confirmed the downregulation of multiple murine haemoglobin mRNAs in the lenses of 9-month old Sparc null mice as compared with controls. Another downregulated gene, EraF, is involved in the correct folding of haemoglobin proteins. Other genes confirmed to date include Sparc itself, collagen sub-types, metallothionein 2 and glypican-3. Glypican-3 causes Simpson-Golabi-Behmel syndrome; when mutated, symptoms of this disorder include age-onset cataracts.

Conclusions: Conclusions: We have confirmed the expression of haemoglobins in the murine lens. Haemoglobins may have a role in apoptosis, the maintenance of lens structure, oxygen transport and/or oxygen buffering in the lens; their downregulation as a result of Sparc loss may play a role in the pathogenesis of age-onset cataract in these mice.

■ 2373

New insights in eye development in the aphakia mouse

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Purpose: Aim of the work is a functional analysis of the mouse mutant aphakia, which is characterized by missing eye lenses caused by 2 deletions in the Pitx3 promoter.

Methods: Expression profiles from aphakia and wild-type embryos during lens development (embryonic stages E 9.5 till E 13.5) were compared using the DNA Microarray Technology. The results were confirmed by Real Time PCR, and in cases of special interest by promoter assays.

Results: 23 genes showed significant differences in their expression patterns between aphakia and wild type, from which 15 are upregulated in the mutant. The most interesting results include the Clock gene which is upregulated in aphakia at E 11.5. It is involved in circadian regulation by acting as a transcription factor. We could corroborate the expression results with Real Time PCR. Another promising gene is the transcription factor AP2. Its downregulation in the mutant at E 11.5 is supported by Real Time PCR results. As a binding site for AP2 is predicted in one of the deletions in the Pitx3 promoter in the mutant, we cotransfected the transcription factor with the Pitx3 promoter in the HEK 293 cell line, which is negative for AP2 expression. The transfection was successful as shown by RT-PCR, but no changes in promoter activity could be detected.

Conclusions: The loss of Pitx3 activity in the homozygous ak mutants lead to a stop of lens development at embryonic stage 10.5. We detected the first differences in gene expression in the microarrays at E 11.5. Further analysis by real-time PCR and in situ hybridization will reveal details of the correlations.

■ 2374

Promoters as a tool to target gene expression into the mouse retina using subretinal injection of self-inactivating lentiviral vector

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Purpose: Gene transfer appears to be a promising approach to prevent photoreceptor loss. However specific cell type targeting using particular promoters has not yet been extensively described for lentiviral vector delivery in the retina. To complete our previous study on intravitreal lentiviral delivery into the mouse retina (Kostic et al, 2003), we described activities of different promoters (Ubiquitin (Ubi), phosphoglycerate kinase (PGK), elongation factor-1 (EFS) and rhodopsin (Rho) promoters) using subretinal injection of self-inactivating lentiviral vectors in DBA/2 mice eyes.

Methods: Self-inactivated lentiviral vector preparations were subretinally injected into adult DBA/2 mice eyes (n=6 to 18). Cell transductions were observed 7 days post-injection.

Results: Subretinal injections in adult mice improve Rho promoter activity in photoreceptors (3% of the photoreceptors around the injection site) and restrain Ubi, PGK and EFS expression pattern to RPE cells only.

Conclusions: Thus although RPE cells seem to be easily transduced by self-inactivating lentiviral vectors, subretinal injections greatly improved photoreceptor transduction by such viral vector. Additionally, using subretinal injections, both EFS and PGK promoters should be appropriate for delivery of a secreted survival factor to target adjacent degenerating photoreceptors, or for gene replacement in RPE cells. However further experiments have to be performed to improve photoreceptor transduction efficiency.

Accommodative and pseudoaccommodative cataract surgery

■ 2411

Introduction: Surgical Alternatives for the Correction of Presbyopia

ALIO y SANZ JL

■ 2412

Pseudoaccommodation with Pseudophakic lens surgery

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■ 2413

Accommodative Power of a New Foldable Intraocular Lens

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Purpose: To assess the accommodative power of a new foldable monofocal intraocular lens.

Methods: The prospective randomized clinical interventional study included 40 patients attending the hospital for cataract surgery and who were randomly distributed into a study group receiving a new foldable monofocal intraocular lens with flexible haptics, and a control group receiving a standard foldable intraocular lens. Mean follow-up period was 8.51 ± 1.34 months (range 4 to 11 month). The method as standard cataract surgery consisting of clear cornea incision, capsulorrhexis, phacoemulsification, and intraocular lens implantation, with topical anaesthesia. Main outcome measures were preoperative and postoperative visual acuity for near and distance, range of accommodation, and change in anterior chamber depth.

Results: In the study group compared with the control group, range of accommodation was significantly ($p=0.01$) higher (1.01 ± 0.4 diopters versus 0.50 ± 0.11 diopters) and change in anterior chamber depth was significantly more pronounced (0.82 ± 0.30 versus 0.40 ± 0.32 , $p=0.01$). Both groups did not vary significantly in best corrected vision (0.94 ± 0.12 versus 0.93 ± 0.18 ; $p=0.74$).

Conclusions: During a mean follow-up period of 8 months after implantation, the new foldable monofocal intraocular lens with flexible haptics showed an accommodative power of about one diopter what was significantly higher than the accommodative power of a conventional monofocal flexible intraocular lens. The difference in the accommodative power between the two intraocular lenses was paralleled by a difference in the change of the anterior chamber depth.

■ 2414

Comparison between pseudoaccommodative and multifocal lens replacement

GALALA

Accommodative and pseudoaccommodative cataract surgery

■ 2415

Restoration of accommodation by real accommodative IOL: Is it feasible?

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■ 2416

Principals of Pseudo-Phakic-Accommodation Mechanisms

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■ 2441

Applanation resonator sensors - a new technique for measuring intraocular pressure

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Purpose: Traditionally, IOP is determined by applanation tonometry, a method where the force needed to flatten a certain area of the cornea is measured. In a recently implemented applanation method for IOP measurement, the contact area was measured with a resonator sensor device. The basic principle of the sensor is a piezoelectric element oscillating in its resonance frequency. The sensor produces a frequency shift proportional to the contact area. A force transducer mounted in the same device as the piezoelectric element measured simultaneously the contact force. This applanation resonator sensor (ARS) was evaluated in an in vitro pig-eye set-up with promising results. The aim of the current study was to evaluate the ARS-system in a clinical setting.

Methods: The study included 24 healthy volunteers with normal IOP and 24 patients with elevated IOP. The ARS was mounted on a biomicroscope in a similar position as Goldmann applanation tonometry (GAT) and the ARS tonometry was compared with mean (n=6) GAT readings. For each measurement force was plotted against frequency and the slope was determined and interpreted as proportional to IOP.

Results: The correlation between IOP(ARS) and IOP(GAT) was $R=0.92$ ($p<0.01$, $n=102$). The standard deviation was 3.2 mm Hg.

Conclusions: The resonator sensor technology is introducing a new methodology for measuring intraocular pressure. The method is, to our knowledge, the first to combine simultaneous, continuous sampling of both force and area. The strong correlation between IOP(ARS) and IOP(GAT) together with moderate standard deviation indicates that with further development of the ARS method there is a potential for a new user-friendly, clinical tonometer.

■ 2442

On the mechanical meaning of the measuring value of the intraocular pressure

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Purpose: At present time the methods of measuring of the intraocular pressure (IOP) based on the measuring of the diameter of a contact zone when the plane load acts on the cornea are widely used. It is known that the tables for calculation of the IOP are made with assumption that the eye shell is modelled as a thin shell with averaged parameters. It is also assumed that the change of the IOP after loading the cornea with a flat weight is equal to the change of the IOP when the volume of the liquid in the eye is increased on the value, which is equal to the volume of the spherical segment based on the circle, the area of which is equal to the contact area of the cornea and load. We are interested in evaluating the possible error due to the dispersion of the elastic properties of the sclera and cornea and due to the method for calculation of the additional IOP.

Methods: The stress-strain state and the change of the inner pressure in the sphere, filled with incompressible liquid after applying of the plane load are obtained from solution of equations of the general theory of elasticity. The wide range of values of the elastic constants were considered.

Results: The results show that the actual value of IOP may considerably differ from the measured IOP.

Conclusions: For correct diagnosis of the disease it is important to realise, that the measured value of IOP could considerably differ from actual IOP.

■ 2443

Pachymetry before or after applanation tonometry: does it matter?

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Purpose: Pachymetry has become a standard assessment in patients with ocular hypertension and normal tension glaucoma. We wanted to evaluate if the accuracy of central corneal thickness (CCT) measurement was influenced by applanation tonometry (AT) and vice versa.

Methods: We examined prospectively 60 healthy volunteers. Contact lens wearers or patients with previous surgery were excluded. The sequence of the examinations in the right eye was: CCT - AT - CCT. The sequence of the examinations in the left eye was: AT - CCT - AT. The measurements were done consecutively. One clinician examined all the patients with the same pachymeter (DGH-500 Pachette TM) and Goldmann applanation tonometer. Two tonometry and 5 pachymetry measurements were averaged each time. Pearson correlation coefficient was used to calculate the correlations and a paired t-test to calculate the differences between the measurements.

Results: 36 female and 24 males were included. The mean age was 62 ± 13 years (27 - 90 years). The average refractive error for both eyes was +0.3 D (-6 to +6 D). In the right eye the mean CCT before and after AT was respectively $565 \pm 33 \mu$ and $566 \pm 34 \mu$ ($p=0.34$, $r=0.98$). In the left eye the mean AT before and after pachymetry was respectively 19.4 ± 4.7 mmHg and 19.2 ± 4.3 mmHg ($p=0.43$, $r=0.86$).

Conclusions: There was no difference in the CCT measured before or just after AT, nor was there any difference between AT taken before or just after pachymetry. The sequence of the two examinations does not seem to matter in normal individuals.

■ 2444

Early and late damage of parvo and koniocellular function in ocular hypertension and glaucoma: correlation with clinical markers of disease progression

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Purpose: To evaluate quantitative parameters of chromatic processing within the parvo and koniocellular systems, in order to determine whether they are affected in early stages of the natural history of glaucoma, and to correlate them with other clinical markers of disease progression.

Methods: A psychophysical procedure modified from the Cambridge Colour Test was used to assess color discrimination in three different groups: primary open angle glaucoma patients ($n=51$ eyes), ocular hypertensive patients ($n=95$ eyes) and controls ($n=46$ eyes). Psychophysical data were correlated with perimetric and clinical data obtained from the same populations.

Results: Both the lengths of the major axis of chromatic discrimination ellipses and confusion vectors were very significantly different between the three groups. These measures actually followed a significant gradient of worsening performance from the control to hypertensive and glaucoma groups, which was steeper for the tritan axis. There was a highly significant positive correlation between test parameters and cup/disc ratio and a significant negative correlation with the perimetric mean deviation index. Interestingly, ellipse length in ocular hypertensive patients correlated very significantly with the time of duration of their hypertensive state.

Conclusions: Early dysfunction of both parvo and koniocellular systems suggests that both functional redundancy and specific pathway involvement hypotheses in the pathophysiology of glaucoma must be refined. It is remarkable that this psychophysical test can detect visual dysfunction in a significant subset of long-run hypertensive subjects with still unchanged perimetry parameters.

■ 2445

Elevated Atrial Natriuretic Peptide (ANP) expression in Primary Open Angle Glaucoma (POAG).

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Purpose: Atrial Natriuretic Peptide (ANP) is involved in the modulation of intraocular pressure (IOP). The aim of this study was to investigate in human eyes with and without Primary Open Angle Glaucoma (POAG), the expression of ANP in the anterior segment as well as the ANP levels in aqueous humour (AH).

Methods: Immunohistochemistry was done in 4µm paraffin sections of formalin-fixed eyes using a monoclonal antibody against ANP (Cimbus CBL66) and developed with a supersensitive kit (LSAB 2, Dako). ANP was extracted from plasma and AH samples using Sep Pak Cartridge C18 (Waters) and measured by Radioimmunoassay. AH was obtained at the time of trabeculectomy from 40 patients with POAG, and during cataract surgery in 20 patients without POAG. To compare concentrations in AH, the Mann-Whitney U-test was used.

Results: Immunoreactive ANP was localized to the ciliary body (non pigmented epithelial cells and stroma) and ciliary muscle, with its expression prominent in POAG. ANP levels in AH were significantly higher ($p < 0.05$) in POAG patients (median 61.46 pg/ml, range 1.00-963.68 pg/ml) than in cataract patients (median 10.13 pg/ml, range 1.51-85.97 pg/ml). We have not found any significant differences in plasma ANP levels between both groups.

Conclusions: Our data indicate that the enhanced ANP expression in the anterior segment and AH during human POAG may play an important physiological role in modulating IOP in POAG.

■ 2446

The intraocular pressure lowering efficacy of travoprost monotherapy in patients formerly on brimonidine monotherapy

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Purpose: Assess the intraocular pressure (IOP) lowering effect of travoprost 0.004% in patients previously treated with brimonidine monotherapy.

Methods: Two open label, prospective trials were conducted under the S.T.A.R.T. and S.T.A.R.T. II protocols. These were 4-week, open-label studies, conducted at 956 sites, enrolling a total of 9,386 patients. Exclusion criteria were in accordance with product labeling. Demographics and baseline IOPs were collected and study medication was dispensed at the initial visit. Patients were instructed to discontinue brimonidine, instill one drop of travoprost q.d. in each affected eye at approximately 8 PM hours, and return for a 4-week follow-up visit. Efficacy was assessed by the mean IOP change from baseline to follow-up.

Results: Of the 9,386 patients in the two studies a total of 331 patients, 227 and 104 respectively, were treated with brimonidine. Average baseline IOP with brimonidine was 21.9 mmHg (SD=4.8) in S.T.A.R.T. and 22.0 mmHg (SD=4.6) in S.T.A.R.T. II. Baseline IOPs decreased to 17.2 (SD=3.7) in S.T.A.R.T. and 17.1 (SD=4.1) in S.T.A.R.T. II after 1 month of travoprost monotherapy. The differences of 4.7 mmHg, a reduction of 22% for S.T.A.R.T. and 4.9 mmHg a reduction of 22% for S.T.A.R.T. II were statistically significant ($p < 0.0001$).

Conclusions: Results show that travoprost 0.004% significantly reduced IOP on average by at least 4.7 mmHg, which is a reduction of 22%, in patients for which brimonidine was replaced. Results from S.T.A.R.T. II are in accord with the efficacy data reported for TRAVATAN® in the S.T.A.R.T. Study.

■ 2447

An analysis of the intraocular pressure lowering efficacy of travoprost monotherapy in patients formerly on beta-blocker monotherapy

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Purpose: Assess the intraocular pressure (IOP) lowering effect of travoprost 0.004% in patients previously treated with beta-blocker monotherapy.

Methods: Two open label, prospective trials were conducted under the S.T.A.R.T. and S.T.A.R.T. II protocols. These were 4-week, open-label studies, conducted at 956 sites, which enrolled a total of 9,386 patients. Exclusion criteria were in accordance with the product labeling. Demographics and baseline IOPs were collected and study medication was dispensed at the initial visit. Patients were instructed to discontinue the beta-blocker eye drops, instill one drop of travoprost q.d. in each affected eye at approximately 8 PM ± 2 hours, and return for a 4-week follow-up visit. Efficacy was assessed by the mean IOP change from baseline to follow-up.

Results: Of the 9,386 patients in the two studies a total of 857 patients, 590 and 267 respectively, were treated with beta-blocker monotherapy. Average baseline IOP with beta-blocker monotherapy was 21.5 mmHg (SD=4.6) in S.T.A.R.T. and 22.0 mmHg (SD=4.6) in S.T.A.R.T. II. Baseline IOPs decreased to 17.4 (SD=3.9) in S.T.A.R.T. and 17.6 (SD=4.1) in S.T.A.R.T. II after 1 month of travoprost monotherapy. The differences of 4.1 mmHg, a reduction of 19% for S.T.A.R.T. and 4.4 mmHg a reduction of 20% for S.T.A.R.T. II were statistically significant ($p < .0001$).

Conclusions: Results show that travoprost 0.004% significantly reduced IOP on average by at least 4.1 mmHg, which is a reduction of 19%, in patients in which beta-blocker monotherapy was replaced. Results from S.T.A.R.T. II are in accord with the efficacy data reported for TRAVATAN® in the S.T.A.R.T. Study.

■ 2448

A study of the intraocular pressure lowering efficacy of travoprost monotherapy in patients previously treated with latanoprost and adjunctive beta-blocker therapy

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Purpose: Assess the intraocular pressure (IOP) lowering effect of travoprost 0.004% in patients previously treated with latanoprost and adjunctive beta-blocker therapy.

Methods: Two open label, prospective trials were conducted under the S.T.A.R.T. and S.T.A.R.T. II protocols. Two, 4-week, open-label studies, conducted at 956 sites, enrolled a total of 9,386 patients. Exclusion criteria were in accordance with product labeling. Demographics and baseline IOPs were collected and study medication was dispensed at the initial visit. Patients were instructed to discontinue latanoprost and beta-blocker eye drops, instill one drop of travoprost q.d. in each affected eye at 8 PM, and return for a follow-up visit. Efficacy was assessed by the mean IOP change from baseline to follow-up.

Results: Of the 9,386 patients in the two studies, a total of 74 patients, 55 and 19 respectively, were treated with latanoprost and adjunctive beta-blocker eye drops. Average baseline IOP with latanoprost and adjunctive beta-blocker eye drops was 21.3 mmHg (SD=4.3) in S.T.A.R.T. and 20.2 mmHg (SD=7.0) in S.T.A.R.T. II. Baseline IOPs decreased to 19.2 (SD=5.3) in S.T.A.R.T. and 18.9 (SD=4.3) in S.T.A.R.T. II after 1 month of travoprost monotherapy. The difference of 2.1 mmHg, a reduction of 10% for S.T.A.R.T. was statistically significant ($p < .0072$). The difference of 1.3 mmHg a reduction of 6% for S.T.A.R.T. II was not statistically significant ($p < .1882$).

Conclusions: Results show that travoprost 0.004% significantly reduced IOP by at least 1.3 mmHg, in patients in which latanoprost and an adjunctive beta-blocker were replaced. Results from S.T.A.R.T. II approach convergence with the efficacy data reported for TRAVATAN® in the S.T.A.R.T. Study.

■ 2451

The OCT-Ophthalmoscope, basic principles and histological correlation

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Purpose: In contrast to conventional OCT, which scans perpendicular to the retinal surface, the OCT-Ophthalmoscope provides a transversal OCT image (C-scan) parallel to the retinal surface. Simultaneously it makes a confocal SLO image showing pixel to pixel correspondence with the OCT image. To better understand and interpret these C-scans we used a porcine eye to correlate the OCT C-scan images to histological transversal cross-sections of the retina.

Methods: With a prototype OCT-Ophthalmoscope (OTI, Toronto, Canada) a stack of 30 to 60 C-scans over a depth of 2 mm was made of the retina of a fresh porcine eye, which was enucleated within 15 minutes after death. After completing the imaging sequence, the eye was fixated in 4% glutaraldehyde and 10% formalin. After embedding the eye in paraffin, transversal cuts were made at intervals of 5 micron.

Results: The histological cross-sections clarified the way the OCT images are build up, and helped to identify the different retinal layers, such as the nuclear and ganglion cell layers, the retinal pigment epithelium and the choriocapillaris, as seen in transversal cross-sections.

Conclusions: Correlating the histology to the transversal OCT image provides valuable information in the interpretation of transversal OCT images. It will also help us to better understand and interpret pathological retinal abnormalities in patients.

■ 2452

The OCT-Ophthalmoscope, clinical aspects in central serous retinopathy

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Purpose: The OCT-Ophthalmoscope produces a transversal OCT, C-scan, and a confocal SLO image simultaneously showing pixel to pixel correspondence. A stack of C-scans provides a unique 3D insight of the macular area. Our goal was to describe different aspects in central serous retinopathy as seen in C-scans and to validate the use of the OCT-Ophthalmoscope in the follow up of these patients.

Methods: A prototype OCT-Ophthalmoscope (OTI, Canada) is used to scan patients with central serous retinopathy in both C- and B-scan mode. A stack of 30 C-scans was taken of each patient at a scan rate of 2 frames/second. A single image covers a area of 20 degrees. Fluorescein angiography was used to confirm the diagnosis.

Results: Sixteen patients were scanned. Different aspects of a serous detachment could be detected, such as retinal epithelial detachments, broadening of a hyperreflective layer underneath the serous detachment corresponding to fibrinous exudates, and small precipitates seen as hyperreflective irregularities at the border of the serous detachment. Moreover the extent of the detachment could be more appreciated in the C-scan images. For follow up, the SLO images were used to align two consecutive OCT scans. A proper comparison between the consecutive OCT images could be made by measuring increase or decrease in extent of the detachments.

Conclusions: The OCT-Ophthalmoscope provides valuable additional diagnostic information in patients with central serous retinopathies. The extent of the serous detachment can be better appreciated and additional features are easier to see. By using the SLO image a reliable follow up is possible. With the 3D reconstructions provided by the OCT-Ophthalmoscope, this device is a promising diagnostic tool.

■ 2453

Retinal thickness mapping using the optical coherence tomograph with increased sampling

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Purpose: To improve the retinal thickness maps based on the Optical Coherence Tomography (OCT). The original thickness maps produced by the OCT present, as a major drawback, a lack of lateral resolution because they are made based on 6 radial lines that are expected to go through the fovea. The newly generated maps overcome these problems, allowing any number of either line or radial line scans to be mixed in the same map.

Methods: Data exported from the OCT includes fundus image and retinal thickness measurements along the scan line (shown on the fundus image). Eye movement compensation for both inter-scan and saccadic is achieved by image registration and scan line interpolation, respectively. The retinal thickness map is then built by cubic interpolation.

Results: The newly generated retinal thickness maps present an increased lateral resolution when compared with original maps. Moreover, this new system allows for the integration of any number of scan lines with any positioning, orientation and length in the eye fundus. The user is thus allowed to increase the lateral resolution of the retinal thickness map by adding any number of scans as necessary. This will permit the computation of cyst volumes e.g., and the monitoring of the evolution of the cystoid macular edema based on a new set of parameters. This system also allows for detailed retinal thickness mapping after cataract surgery, lens opacification or any other reasons that indicate the OCT for retinal thickness mapping.

Conclusions: This new retinal thickness mapping, based on the OCT, compares favourably with the OCT original mapping. It presents increased lateral resolution, consequently allowing for detailed mapping where the OCT has indications for use.

■ 2454

Subclinical macular oedema detected by Optical Coherence Tomography (OCT) in HLA B27 anterior uveitis. A pilot study

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Purpose: to investigate by Optical Coherence Tomography (OCT), a possible increase of the foveal thickness during an acute anterior uveitis, in HLA B27 positive patients.

Methods: prospective, internal controlled case series. HLA B27 patients referring for a unilateral acute anterior uveitis underwent a complete ophthalmological evaluation and OCT assessment. Foveal thickness was measured at the first visit (3 +/-2 days from the onset of ocular symptoms), and after 30 +/-5 days. Data of the affected eye were compared to those of the fellow healthy eye.

Results: 8 patients (mean age: 40 +/-16 yrs) were enrolled. At the first examination, the mean foveal thickness (+/- SEM) was 183 +/-12 microm in the affected eyes, and 165 +/-5 microm in the fellow eyes (paired Student t test p <0.05). After 1 month, a further increase in the mean foveal thickness was documented, despite the clinical resolution of the uveitis in 7 eyes, and the development of an evident cystoid macular edema in only one eye. Mean foveal thickness (+/- SEM) rose to 217 +/-40 microm in the uveitic eyes (161 +/-9 microm in the healthy eyes).

Conclusions: in all the examined cases of HLA B27 anterior uveitis, an increased foveal thickness was observed in the affected eye, if compared to the fellow healthy one. Such an increase persisted for at least 1 month, despite the clinical healing of the inflammation. These evidences suggest that some functional alterations, occurring during an anterior uveitis, may determine a subclinical macular edema lasting over the acute phase of the disease.

■ 2455

Optical coherence tomography investigation of incomplete visual recovery following surgery for proliferative vitreoretinopathy*BENSON SE, GRIGOROPoulos V, CHARTERIS DG, SCHLOTTMANN P*
*Vitreoretinal Research Department, Moorfields Eye Hospital, London***Purpose:** To assess the causes of incomplete visual recovery using Optical Coherence Tomography (OCT) in patients who have undergone successful surgery for proliferative vitreoretinopathy.**Methods:** Eligible patients were identified from all Vitreoretinal out patient clinics from the period April to August 2002. Inclusion criteria: Patients who had undergone successful surgery for proliferative vitreoretinopathy but had poor visual outcome without an identifiable cause on clinical examination. 18 patients were recruited and were scanned using standardized technique with the OCT2000 at 3 to 86 months postoperatively. Relevant data was collected from the patients casenotes.**Results:** Although ophthalmoscopically the retina appeared attached in all eyes, OCT revealed cystoid macular oedema with evidence of a confluent fluid cavity in the outer retina in 5 eyes (28%) and localised macular oedema in 5 eyes (28%).**Conclusions:** OCT is a useful diagnostic tool in assessing poor postoperative visual acuity, and can reveal additional pathology undetected on clinical examination. This may influence patient treatment and eventual visual outcome. In this series 4 out of the 5 patients who had a confluent retinal fluid cavity on OCT went on to have further surgery.

■ 2456

Inner limiting membrane and retinal pigment epithelium detection on optical coherence tomograph*BERNARDES R (1), MONTEIRO R (1), CUNHA-VAZ J (1, 2)*
*(1) AIBILI - Association for Biomedical Research and Innovation on Light and Image, Coimbra, (2) IBILI - Institute of Biomedical Research on Light and Image, Faculty of Medicine, University of Coimbra, Coimbra***Purpose:** To develop a robust method for the determination of the Inner Limiting Membrane (ILM) and Retinal Pigment Epithelium (RPE) of the human retina based on the Optical Coherence Tomograph (OCT) measurements. Currently available software presents incorrect determinations on the ILM and RPE, mostly in the presence of pathology, thus producing erroneous retinal thickness measurements.**Methods:** Raw data is exported from the OCT. OCT scanning is similar to a B-scan ultrasound systems and consists of 100 ultrasound like A-scans, that represent the reflection of different structures at different depths. The whole data may be regarded as an image of 500 by 100 pixels. As the determination of the ILM is shown to be sensitive to noise, different approaches were taken: a region growing algorithm and; an active contour algorithm. The RPE is, this way, searched on a reduced data set, as it is located under the ILM. This improves its detection as the strength of the optical reflectivity highly depends on the anterior structures. Nevertheless, a weighted set of criteria was chosen, considering not only the strength of the RPE interface but also the neighbourhood and the distance from the determined RPE location to the expected location, considering its position on the whole range of the B-scan.**Results:** This new algorithm for detecting the ILM and RPE interfaces from the OCT measurements appears to be a promising technique that allows not only reliable retinal measurements but also the possibility to extract new information currently hidden and/or misinterpreted.**Conclusions:** This new system allows better, reliable and extended data reporting based on the raw data collected from the OCT.

■ 2457

Tele-screening for diabetic retinopathy with an advanced Retinal Thickness

Analyzer (RTA)

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Purpose: To compare the effectiveness of tele-screening using a novel enhanced retinal thickness analyzer (RTA) with onsite routine ophthalmologic examination for diabetic retinopathy.

Methods: A consecutive series of 31 eyes from diabetic patients were included. All underwent stereoscopic dilated funduscopy and scanning with the RTA. The RTA reports consisted of a wide-angle, red-free fundus photograph and a macular-region retinal thickness map. Reports were graded by three independent graders in a masked manner. The diagnoses of proliferative retinopathy (PDR), macular edema, and treatment decisions made by the RTA graders and the clinical examiner were compared.

Results: On clinical examination 5 of 31 eyes were diagnosed with PDR. All 5 were referred for treatment by two graders and 4 eyes by one grader. All eyes with PDR and 12 of the 26 eyes with non-proliferative diabetic retinopathy showed severe macular edema. Seven of the 12 eyes with macular edema were clinically eligible for focal laser treatment, all of them were detected by all RTA graders. Macular thickening was detected in 8 eyes by RTA where no treatment was necessary judged by clinical examination. Sensitivity was mean 93% for detecting PDR and 100% for detecting macular edema with a specificity of 58 to 96% depending on the grader. In only one case the RTA did not allow valid assessment due to poor image quality.

Conclusions: Screening for diabetic retinopathy combining wide-angle fundus photography and macular thickness mapping offers the pre-requisites for establishing a successful tele-screening program. The high sensitivity for macular thickening detects more diabetes patients, which need further ophthalmologic examination.

■ 2461

Dinucleotides in the eye: presence, receptors and physiological actions

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Purpose: The purpose is to revise the extracellular role of the naturally occurring compounds diadenosine polyphosphates on ocular physiology. Three aspects will be specially studied: tear secretion, corneal wound healing and intraocular pressure (IOP).

Methods: All the experiments were performed with New Zealand white rabbits. For the tear secretion experiments, Schirmer tests and HPLC analysis were used. In the healing studies, wounds were done with paper disks soaked in n-heptanol. A slit lamp and the IMAGENet software was used to follow the healing process. IOP experiments were performed by means of a TONOPEN XL.

Results: Diadenosine polyphosphates, Ap_nA (where n varies from 2-7) are present in tears both in rabbits and humans. When they were tested in their ability to modify tear production in rabbits, Ap₄A (diadenosine tetraphosphate), Ap₅A (diadenosine pentaphosphate) and Ap₆A (diadenosine hexaphosphate), increased tear secretion. Ap₄A was the best increasing tear production 60 % above control. These dinucleotides activate a P₂Y₂ receptor present in the lachrymal gland. In the wound healing experiments, Ap₄A was the only dinucleotide able to accelerate the rate of corneal healing, changing from 72 μm/h (control) to 96 μm/h. This dinucleotide acts through a P₂Y receptor coupled to the MAPKinase cascade. IOP was modified in a different way depending on the dinucleotide tested. Ap₂A and Ap₃A, clearly produced an increase in IOP. Ap₄A produced a reduction of IOP of 30 %, presumably mediated by a P₂X receptor.

Conclusions: Diadenosine polyphosphates are naturally occurring substances present in tears and aqueous humour. the application of some of these compounds may help to revert pathological situations such as dry eye or ocular hypertension.

■ 2462

INS37217, a P₂Y₂ Receptor Agonist, for the Treatment of Retinal

Detachment: From Lab to Clinic

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Purpose: To provide an overview of preclinical and early clinical studies associated with the development of INS37217 for the treatment of retinal detachment.

Methods: Retinal detachments were induced in mouse, rat, and rabbit by injection of saline into the subretinal space, and INS37217 was evaluated for its ability to stimulate subretinal fluid reabsorption and retinal reattachment. Retinal reattachment and function were evaluated using fundus examination, optical coherence tomography (OCT) imaging, histological, TUNEL staining, and electroretinographic (ERG) approaches. A double-masked, randomized, placebo-controlled Phase 1/2 clinical study was conducted to evaluate the safety and preliminary efficacy of INS37217 in patients with rhegmatogenous retinal detachment.

Results: INS37217 was shown to activate the P₂Y₂ receptor in retinal pigment epithelium (RPE) cells and stimulate fluid absorption across RPE in vitro and in vivo. In mouse, rat, and rabbit models of induced non-rhegmatogenous retinal detachment, INS37217 was shown to be efficacious in stimulating reattachment when compared with vehicle. In mouse eyes, INS37217 enhanced recovery of ERG function and reduced photoreceptor apoptotic cell death following retinal reattachment. In the Phase 1/2 clinical study, INS37217 was well-tolerated at all three doses tested, and was shown to improve the extent of retinal reattachment when compared with placebo.

Conclusions: By stimulating subretinal fluid reabsorption across RPE cells, INS37217 is a promising compound for further clinical development in the treatment of retinal detachment.

Extracellular Nucleotides

■ 2463

ATP Release by Mechanical Stimulus

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Purpose: This study has examined ATP release in response to mechanical stimulation of corneal epithelium/endothelium, and cells of the trabecular meshwork (TM).

Methods: ATP release from primary cells grown on petri dishes was assayed by the Luciferase-Luciferin (LL) technique. Shear stress (SS) was applied as a mechanical stimuli by rotating an aluminum disk over cellular monolayers. Membrane deformation was induced by cell swelling. MLC phosphorylation in response to ATP was assessed by Western blotting.

Results: All cell types showed expression of P2Y1, P2Y2, and CD39 and ATP release in response to cell swelling. The hydrodynamics of the tear film in response to blink action led to a time-averaged SS of 14 dynes/cm² over one blink period. Exposure of epithelial cells to SS (0.34 dynes/cm²) resulted in ATP release 50 to 60 fold over the resting release. Exposure to ultrasound pulses simulating conditions of phacoemulsification led to massive ATP release from endothelium in everted bovine corneas. Acute exposure to exogenous ATP caused MLC dephosphorylation in endothelial cells. A model of outflow across TM as flow through a bed of parallel capillaries led to an estimate of SS = 0.6 dynes/cm². Exposure of TM cells to SS induced ATP release.

Conclusions: 1) Since the release of ATP was also induced by cell swelling, the mechanism of release in response to SS seems to involve stretch-activated conductive channels. 2) Autocrine activity by the released ATP through the activity P2Y subtypes is opposed by the degradation of ATP. 3) ATP-induced dephosphorylation of MLC in the endothelium could be due to adenosine formation mediated by ecto-ATPases.

■ 2464

Effects of Diquafosol, P2Y2 receptor agonist on ocular surface staining in dry eye patients

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Purpose: To compare the results of 2.0% diquafosol tetrasodium (INS365) Ophthalmic Solution vs. placebo (0.9% saline) on ocular surface staining in patients with dry eye in two clinical trials.

Methods: Two randomized, double-masked, placebo-controlled clinical trials comparing diquafosol tetrasodium to placebo (4 times daily for either 6 or 24 weeks). The extent of corneal (fluorescein) and conjunctival (lissamine green) staining was evaluated every 1-2 weeks post-randomization. Observed values were compared between treatment groups for each clinic visit adjusting for investigative site and baseline values.

Results: Corneal staining scores were significantly lower among subjects taking 2.0% diquafosol tetrasodium than placebo at most study visits during the 24-week trial. In the 6-week trial, a similar reduction in corneal staining was observed, however, the smaller sample size led to fewer statistically significant treatment group differences. Results for improvement in conjunctival staining were consistent with those observed for corneal staining, with significant differences observed in both trials.

Conclusions: The 2.0% diquafosol tetrasodium Ophthalmic Solution was effective at reducing corneal and conjunctival staining in patients with dry eye.

■ 2471

The tear film lipid layer forms a pleated sheet on eye closure

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Purpose: Conventional models of the human tear film assume that the lipid layer oil mixes with the lid margin lipid reservoir with each blink. We recently noticed a constancy of the interference pattern over a series of blinks, suggesting a high degree of structural integrity. We therefore studied the dynamics of the lipid film in spontaneously blinking adults.

Methods: Interference patterns were examined in the left eyes of 14 normal subjects (8 male, 6 female, aged 30-67), using the Keeler Tearscope Plus and the Kowa DR1 ocular interference microscope. With the Tearscope, video recordings of lipid film interference patterns were made using a Sony digital camcorder and downloaded onto a computer using Studio 7 Pinnacle software. Individual frames of stable images, captured at the end of the up-phase of successive blinks, were compared.

Results: There was a remarkable topographical constancy of the interference patterns following successive blinks. The mean number of blinks (SD) over which a pattern was repeated was 3.8 (1.7), with older subjects showing repeatability over a longer sequence, ie. 4.3 (2.3) blinks in the older group (49-67 yrs), compared to 3.4 (0.9) in the younger group (30-48 yrs).

Conclusions: Lipid from the meibomian layer of the tear film does not mix freely with the marginal lipid reservoir during the down-phase of the blink, but retains a structural integrity over the course of several blinks. This observation was made earlier in the rabbit, by Mishima and Maurice (1961). We postulate that the tear lipid film folds up at the lid margin like a pleated sheet, during lid closure. This probably reflects an interaction with lipid-binding molecules, such as lipocalins, at the oil-water interface.

■ 2472

Reduction of symptoms associated with ocular dryness by a new lubricating eye drop

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Purpose: Evaluate a new lubricating eye drop that contains a novel in situ gelling agent, HP-Guar, to relieve the symptomatic feelings of ocular dryness compared to a carboxymethylcellulose based product.

Methods: 87 subjects with a need to use ocular lubricants at least some of the time were enrolled in this randomized, double masked, parallel clinical study. The test product (SYSTANE[®], Alcon) contained Polyethylene Glycol 400, Propylene Glycol, HP-Guar and was preserved with Polyquad[™]. The control contained 0.5% carboxymethylcellulose and was preserved with Purite[™]. Both test and control were used a minimum of 4 times/day for six weeks. Symptom measures included: a) Drop Instillation Comfort, b) Frequency of Ocular Comfort for the previous 3 days (dryness, burning, stinging, blurry, gritty, scratchy, foreign body sensation) c) Experience with the treatment drops using a 5 point Likert format scale and d) 3 minute blur profile.

Results: The test product performed better than control with subjects reporting feelings of less morning dryness (p=0.015), less end of day dryness (p=0.023) and eyes that felt refreshed longer (p=0.023). Frequency of Ocular Comfort showed less foreign body sensation (p=0.033). At Day 42, visual blur profile was comparable between products within 30 seconds.

Conclusions: This study demonstrated that a new lubricating eye drop containing HP-Guar as a novel ingredient (SYSTANE[™]) provides longer-lasting relief from the symptomatic feelings of ocular dryness compared to a carboxymethylcellulose based product.

Tear film

■ 2473

CCL11: a marker in seasonal allergic conjunctivitis?

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Purpose: To follow patients presenting a seasonal allergic conjunctivitis and compare CCL11 (eotaxin-1) levels in their tears during the pollen period (inP) and the out of pollen period (outP).

Methods: First, 5 patients, allergic to grasses and rye, underwent a skin prick test (scale: 0 - 4+) and a specific serum IgE determination (class 0-6). Tears were collected during the outP and the inP, respectively. Patients were scored according to 5 allergic symptoms. Secondly, tears of 11 patients with a history of seasonal ocular allergy were collected during the inP and compared to tears of 6 control volunteers. CCL11 was analysed in tears by ELISA, using purified mouse anti-human eotaxin antibodies and recombinant eotaxin. The Wilcoxon/Kruskal-Wallis and t test were used for statistics.

Results: Seasonal allergic patients showed specific IgE with a positive class from 2 to 5. Their prick test for grasses was 3+ or 4+. Clinical score (sum of itching, hyperaemia, chemosis, eyelid swelling and tearing) reached 4 ± 0.8 during the inP and 0.4 ± 0.48 during the outP ($P=0.0043$). CCL11 mean concentration in tears was 2135 ± 784 [\pm SEM] pg/ml (range: 1147-3150) during the inP and 1743 ± 663 pg/ml (range: 1033-2622) during the outP. The difference is significant ($P=0.024$). On another hand, CCL11 concentration in tears of allergic patients collected during the inP was significantly different from CCL11 concentration in normal subjects ($P=0.0049$).

Conclusions: CCL11 concentration in tears increases during the inP versus the outP. CCL11 level could be used as a marker of seasonal ocular allergy to either confirm a diagnosis or monitor the response to a therapy.

■ 2474

Surgical approach to treat chronic watery eye in patients with permeable lacrimal drainage system

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Purpose: To evaluate the success rate of surgery to treat chronic epiphora and/or mucopurulent discharge.

Methods: Retrospective, noncomparative, consecutive, interventional case series of 17 patients from an ophthalmologist's office referred to a private clinic to either treat chronic epiphora (8/17), mucopurulent discharge (7/17), or both (2/17) by surgery. All patients had at least six months of unsuccessful medical treatments (antibiotic, tears substitution, anti-allergic, and/or anti-inflammatory) and a permeable lacrimal drainage system (irrigation test). In 13 patients with bilateral involvement only one side was analyzed. Piffaretti's transpunctal endoscopic diagnostic (17/17) and non-laser surgical lacrimal drainage procedure to remove partial obstructions of either the lacrimal canaliculus (3/17), the ductus nasolacrimalis (7/17), or both (3/17). All but one patient (16/17) underwent a posterior lacrimal ampullectomy. Some patients had in addition a conjunctivochalasis plasty (5/17), a lateral canthoplasty (1/17), or both (3/17). Main outcome measures were patient's self-assessment of symptoms' improvement and irrigation test outcome at least one year after surgery.

Results: The great majority (15/17) of patients had a marked improvement of their symptoms, many of them (11/17) even being symptoms free. One patient had no improvement and another one was lost to follow-up. When conducted (14/17), irrigation always revealed a permeable lacrimal system.

Conclusions: Surgery can be an option for patients who have a permeable lacrimal drainage system and suffer from chronic epiphora and/or mucopurulent discharge that do not respond to conventional conservative medical therapies.

■ 2475

The use of confocal microscopy for evaluation of cell densities in diabetic patients

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Purpose: To quantify and compare cell densities in six corneal layers of diabetic patients and healthy controls by in vivo confocal microscopy.

Methods: The left cornea of 15 diabetic patients (NIDDM, grade of retinopathy: 20 ETDRS) and 15 healthy controls was examined with confocal microscopy. The cell density in six corneal layers was determined manually after validation of the methods applied.

Results: The average surface cell density and standard deviation in the superficial, basal and endothelial layers was 725 ± 291 , 5950 ± 726 and 2694 ± 322 cells/mm² in controls, respectively, and 815 ± 310 , 5082 ± 427 and 2655 ± 385 cells/mm² in diabetics, respectively. The volumetric cell density and standard deviation in anterior, mid- and posterior stroma was 26307 ± 5994 , 19425 ± 3996 and 25641 ± 6327 cells/mm³ in controls, respectively, and 27528 ± 5217 , 21978 ± 4440 and 25752 ± 6438 cells/mm³ in diabetics, respectively. Both in diabetic patients and healthy controls the cell density in the mid-stromal layer was significantly lower than in the anterior or posterior stromal layers ($P < 0.025$). The difference between diabetics and controls was significant in the basal epithelial layer (-14.6%, $p < 0.001$) and in mid-stroma (+13.1%, $p < 0.029$).

Conclusions: The lower cell density in the mid-stroma of both groups can be attributed in part to differences in oxygen concentration in the stromal layers. The lower basal epithelial and the higher or equal superficial cell densities in diabetics compared to controls suggest a longer turnover time of the superficial cells or a shorter turnover time of the basal epithelial cells in diabetes. The higher mid-stromal density in diabetics may be attributed to a higher glucose concentration in the cornea of these patients.

■ 2476

Stromal hydration dynamics during long-term extended wear of soft contact lenses

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Purpose: To evaluate the thickness profile of the corneal stromal layer associated with long-term wear of low transmissibility soft contact lenses, in order to discuss the physiological and clinical implications in corneal hydration control during contact lens fitting.

Methods: Total and stromal thickness were evaluated in thirteen subjects who had worn one of two types of low oxygen transmissibility (Dk) hydrogel lenses on an extended wear basis for an average of 8 years (range 3 to 16 years). A group of fifteen age and gender matched control subjects who had never worn contact lenses acted as controls. Total and stromal thickness was evaluated at the centre, four mid-peripheral and four peripheral locations using a modified optical micro-pachometer.

Results: Superior cornea of lens wearers was significantly thicker than the inferior. Apparent stromal edema in the group of lens wearers was 2.1%, 1.2%, 0.7% thicker than in the control group ($F = 1.7$; $p = 0.19$). Stromal thickness changes demonstrated association with the number of nights in extended wear ($F = 22.6$; $p < 0.001$), and lens material ($F = 3.8$; $p = 0.05$). Significant effect of length of time in extended wear schedule ($F = 11.3$ $p < 0.001$) could reflect the pachymetric manifestations of different stages in stromal hydration control during extended wear of low-Dk soft contact lenses.

Conclusions: Vertical asymmetry of total and stromal corneal thickness is present in chronically hypoxic corneas supporting previously reported short-term effects. Corneal apparent edema displays a peak after the first decade in extended wear which is interpreted as a pachymetric evidence of corneal hydration fail which has been previously reported as corneal exhaustion syndrome.

■ 3101

Uveal melanoma regression rate can help forecast metastases

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Purpose: To analyze the post-brachytherapy ultrasonographic dynamics of tumor height and internal reflectivity in patients with uveal melanoma, and correlate them with the five-year incidence of metastatic disease and the five-year survival rate.

Methods: We examined 147 patients (147 eyes) with posterior uveal melanoma having a mean age of 61 years, who were treated with Ru-106 brachytherapy. Patients were followed clinically and ultrasonically using both A and B mode standardized echography every 6.7 ± 0.3 (mean \pm SE) months. On average, each patient was examined 5.8 times. The echographic parameters included tumor base size, height, internal reflectivity, regularity, vascularity and extra-scleral extension. The clinical follow-up included ocular examination and metastatic screening.

Results: At the time of brachytherapy the mean height of the tumors was 5.2 mm (range 2.2 - 11.8 mm). After brachytherapy, 142 tumors (96.6%) responded with a decrease in height. This decrease was at an initial mean rate of approximately $6.1 \pm 0.8\%$ per month in the metastatic group vs. $4.3 \pm 0.4\%$ in the non-metastatic group. Tumors higher than 6 mm, tumors with an internal reflectivity of less than 50%, and tumors with an initial rate of height regression larger than 0.7 mm/month or 10% per month had a higher five-year melanoma-related mortality. Kaplan-Meier survival analysis and the multivariate Cox proportional hazards model showed a significant role for tumor height and tumor regression rate in patient survival.

Conclusions: The post-brachytherapy tumor regression rate can be useful in estimating the prognosis for metastatic spread of uveal melanoma, deciding on the follow-up regimen, and, in the future, possible preventive measures.

■ 3102

CSE1L/CAS in human retina

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Purpose: CSE1L/CAS is a recently described protein associated with proliferation and apoptosis. It has first been found in breast cancer but has now been demonstrated in several other cancers and tissues as well. In this study, we have investigated human retina for the presence and distribution of CSE1L/CAS.

Methods: Paraffin sectins of 4 human eyes (16, 26, 52, and 79 years, respectively) were labeled with an antibody against CSE1L/CAS. The eyes had been removed for either choroidal melanoma (98-291, 99-065) or for end-stage glaucoma (97-040, 03-143). Immunoreactivity was visualized using AEC.

Results: In eyes harbouring a choroidal melanoma, prominent immunoreactivity was seen in the RPE, in photoreceptor inner segments, synaptic terminals of the outer plexiform layer, individual cells in both outer and inner nuclear layers, in ganglion cells, nerve fibers and in Mueller cells. Tumour cells also revealed moderate labeling particularly in the periphery although one tumour stained rather inhomogeneously. In addition, very intense labeling was also observed in an epiretinal membrane present in one of the melanoma eyes. - The 2 specimens without tumour exhibited hardly any immunoreactivity.

Conclusions: CSE1L/CAS is present in human retina and localizes most prominently to the RPE, to ganglion cells and to Mueller cells. Its precise function, however, remains as yet unclear. Possibly tumours such as choroidal melanoma can upregulate its expression, and one might speculate on its implication for retinal proliferative disease.

■ 3103

EMMPRIN, a Matrix Metalloproteinase (MMP) Inducer, is Expressed in Uveal Melanoma

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Purpose: MMPs are involved in extracellular matrix degradation and angiogenesis, critical for tumor growth and invasion. Stromal cells (e.g. fibroblasts or endothelial cells) rather than tumor cells per se usually produce MMPs, a process stimulated by tumor cell expression of EMMPRIN (Extracellular Matrix Metalloproteinase Inducer). EMMPRIN expression in human primary uveal melanomas and uveal melanoma cell lines was studied.

Methods: EMMPRIN-immunoreactivity (-IR) was examined in sections of mixed morphology uveal melanomas (n=13). EMMPRIN mRNA expression was assessed in uveal melanoma cell lines (OCM-1, -3 & -8) and primary choroidal fibroblasts using RT-PCR. Enzymatic activity in media from melanoma cell/choroidal fibroblast co-cultures was examined using zymography.

Results: Uveal melanomas displayed variable EMMPRIN-IR. Regions of tumors showed moderate to intense cell surface immunolabelling (7/13). Weak EMMPRIN-IR was visible in some cases (3/13). Generally stromal cells did not display obvious EMMPRIN-IR, but did express intense MMP-2-IR (previous study). RPE cells showed intense EMMPRIN-IR in all cases, and Muller cells displayed localised areas of EMMPRIN-IR. Intense EMMPRIN-IR was also seen on RPE and glial cells that extended across the inner tumor surface, replacing normal retina, and on tumor cells occasionally seen invading the retina. EMMPRIN mRNA was detected in uveal melanoma cell lines. Preliminary zymography from melanoma/fibroblast co-cultures did not show obvious proMMP-2 activation, although MMP-1 activity may be modulated in OCM-3 or -8 (but not OCM-1)/fibroblast co-cultures.

Conclusions: The presence of EMMPRIN, an MMP inducer, on melanoma cells may be involved in stimulating the expression of certain MMPs in uveal melanomas.

■ 3104

Proteomics: a new era in ophthlmo-oncology?

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Purpose: To evaluate the use of proteomics (protein profiling) in aqueous humour of uveal melanoma patients compared with controls.

Methods: Aqueous humour of 24 uveal melanoma patients and 24 control patients was thawed, applied to a Strong Anion Exchange (SAX2) surface Protein Chip array (CIPHERGEN Biosystems, Fremont, CA) and analyzed. The arrays were analyzed on a PBS-II mass reader (CIPHERGEN Biosystems) using Biomarker patterns software.

Results: On the basis of two proteins, aqueous humour of melanoma eyes and control eyes could be distinguished in 89% of cases.

Conclusions: Proteomic evaluation could be a useful tool in finding diagnostic markers in uveal melanoma.

■ 3105

Human papillomavirus and epithelial tumours of the conjunctiva

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Purpose: To establish the role of human papillomavirus (HPV) in the development of pterygia, carcinoma in situ- and carcinomas of the conjunctiva.

Methods: 50 pterygia, 14 carcinoma in situ conjunctiva and 19 conjunctival carcinomas were investigated with polymerase chain reaction (PCR) technique. All HPV positive specimens underwent DNA sequence analysis in order to establish the HPV type. Furthermore, all specimens were investigated with mRNA in situ hybridisation (ISH) for HPV mRNA. 20 normal conjunctival biopsies served as control.

Results: Eight percent of the pterygia, thirty-three percent of the carcinoma in situ specimens and eight percent of the conjunctival carcinomas harboured HPV. mRNA ISH demonstrated viral transcription in all three types of conjunctival lesions. All specimens containing normal conjunctiva were HPV negative.

Conclusions: Only a fraction of pterygia, conjunctival carcinoma in situ and conjunctival carcinomas are associated with human papillomavirus in contrast to cervix uteri carcinomas.

■ 3106

Is Helicobacter the cause of conjunctival MALT lymphomas ?

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Purpose: To evaluate the association between infection with helicobacter and mucosa associated lymphoid tissue (MALT) lymphoma of the conjunctiva.

Methods: 21 cases of conjunctival MALT lymphoma and 10 cases of conjunctival lymphoid hyperplasia were investigated for the presence of helicobacter pylori and other helicobacter strains with polymerase chain reaction (PCR) technique. Helicobacter positive specimens were investigated with DNA sequence analysis in order to establish the helicobacter strain. Furthermore, helicobacter pylori DNA in situ hybridisation was performed. 20 biopsies from normal conjunctival tissue served as control.

Results: Helicobacter was identified in two of 21 conjunctival MALT lymphoma and in one of ten conjunctival lymphoid hyperplasia. DNA sequence analysis identified all infections to be caused by helicobacter pylori. DNA in situ hybridisation was positive in a single specimen. The biopsies from normal conjunctival tissue were all helicobacter negative.

Conclusions: The association between helicobacter pylori and lymphoid tumours of the conjunctiva appears to be only minor. This in contrast to the known connection between Helicobacter pylori and gastrointestinal associated lymphoid tissue (GALT) lymphoma.

■ 3111

History and technique

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History: Few would recall the operation proposed by Krasnov in the late fifties, sinustomy, which is in fact the first seed of non-penetrating surgery. Believing that the maximum resistance to outflow resides at the level of sclera in glaucoma patients, Krasnov proposed excising the bulk of sclera overlying the Schlemm's canal. The obvious difficulty of surgical technique, and the lack of modern microscopes at that time hindered the popularization of his operation. Many years later Thom J Zimmerman, benefiting from a better available understanding of outflow mechanisms, proposed what he termed ab-externo trabeculectomy. The procedure differs from sinusotomy in the fact that Zimmermann advocated the removal of the inner wall of Schlemm's canal and the Juxtacanalicular trabeculum (JCT), as well as the creation of a superficial scleral flap guarding the sinusotomy. Kozlov suggested extending the dissection anteriorly to excise a portion of corneal stroma together with the deep sclera, exposing Descemet's membrane aiming at creating percolation through the membrane as well. Stegmann on the other hand augmented the operation by injecting viscoelastic material in the two cut ends of Schlemm's canal, thus dilating it, and termed the procedure viscocanalostomy.

■ 3112

Potential mechanisms of function

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There are several points of interest when studying the potential mechanisms of function of non-penetrating surgeries (NPGS). Namely the removal of the inner wall of Schlemm's canal (SC) and the adjacent trabecular tissue, the aqueous humor flow through the Trabeculo-Descemet's membrane (TDM), the aqueous resorption after its passage through the TDM. Flow through the TDM: In NPGS the inner wall of the canal is peeled. Analysis of the peeled membrane provided conclusive evidence that includes aside from the inner wall of SC the Juxta-canalicular trabeculum (JCT), and to a lesser extent the corneoscleral trabeculum. In that respect the procedure attempts to remove the external portion of the trabecular meshwork responsible for the main aqueous outflow resistance in a glaucomatous eye. The TDM offers resistance to aqueous humor outflow that allows for a slow decrease in IOP during surgery and will account for the reliable and reproducible IOP on the first postoperative day. Thus the main advantage of the TDM is to reduce the immediate post-operative complications such as hypotony, flat anterior chamber, choroidal detachments and induced cataract. Aqueous resorption: After aqueous humor passage through the TDM, multiple mechanisms of aqueous resorption have been proposed; a subconjunctival bleb, an intrascleral bleb (intrascleral lake), suprachoroidal filtration, and an episcleral vein outflow via Schlemm's canal.

Non-penetrating glaucoma surgery

■ 3113

Intrascleral implants

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To avoid a secondary collapse of the created intrascleral space after non penetrating filtering surgery, surgeons have attempted to insert devices in the scleral space . These devices differ in material being used, size, shape, design, consistency, hydration ability and whether or not being absorbable.

■ 3114

Phaco-deep sclerectomy

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Although trabeculectomy is currently considered the standard surgical procedure combined with cataract surgery, it is associated with potentially vision threatening complications including hyphaema, excessive filtration leading to shallow or flat anterior chamber, choroidal detachment, hypotony maculopathy, suprachoroidal hemorrhage bleb related problems and increased risk of endophthalmitis. Non-penetrating glaucoma is a solid alternative to trabeculectomy with advantages of decreased early postoperative complications. Results have been satisfactory in comparative studies, where IOP control was statistically comparable and complications were significantly lower with combined Non-penetrating-Phacoemulsification surgery.

■ 3115

Clinical Results

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NPGS has been proven to be a safer technique than other available surgical modalities including trabeculectomy and drainage devices. The question of efficacy, nevertheless, is far from resolved. Controversial, often contradictory results are available in the literature. One should keep in mind though as he browses between results that it is all about technique. Issues related to which technique is superior to which in the wide spectrum of NPGS is of paramount importance. The fact of an existing long learning curve could not be over stated. It is neither meaningful nor scientifically sound to compare ones last twenty cases of trabeculectomy to ones first twenty deep sclerectomies.

■ 3116

Controversies

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Perhaps nothing has been more controversial in the last few years, regarding glaucoma surgery, as the question of exactly where does non-penetrating surgery (NPGS) stands in our armamentarium of glaucoma therapies. The procedure attempts to remove the external portion of the trabecular meshwork as well as the inner wall of Schlemm's canal, responsible for the main aqueous outflow resistance in a glaucomatous eye. Not penetrating the eye, the main advantage of NPGS is the reduction of the immediate post-operative complications, but safer technique that offers lesser success rates will certainly not be embraced with open arms by many glaucoma surgeons. In a published report¹ of long term results of deep sclerectomy with collagen implant complete success rates (less or equal to 15 mm Hg with additional medical treatment) have been achieved in 45% of cases after 5 year follow-up. Yet not all reports in the literature provide similar success rates^{2,3}. This disparity in results can be attributed to a number of factors. First, the availability of different techniques; under the broad genre of NPGS, different surgeons perform fundamentally different procedures. Secondly, it is of paramount importance to understand that given the long learning curve associated with deep sclerectomy, it is neither fair nor scientifically sound to compare a surgeon's last 20 cases of trabeculectomy with his first 20 cases of deep sclerectomy. Proper surgical technique is the corner stone of achieving success with this procedure, and achieving the proper depth of dissection could not be overstated. Then finally comes the issue of goniopuncture. NPGS has been criticized for requiring a goniopuncture in almost half of the cases at long-term follow-up. The fact remains that even with subsequent perforation of the TDM in almost 50% of cases at long-term follow up, NPGS still offers a safer option. With respect to efficiency one study that uses a cut-off of 16 mm Hg for complete success, reported that NPGS offers 5% success rate as compared to 29% success of trabeculectomy in a 4-year follow-up. That is if one considers goniopunctured cases as failures. If goniopunctured cases are considered successful the numbers dramatically jump to 27% for NPGS versus 29% for trabeculectomy, therefore not statically significant. What was still significant is the fact that NPGS is associated with less complications, among which lower perturbation of lens nuclear transparency and a lower incidence of "need-for" cataract extraction (18% vs. 69%, $p < 0.001$).

Keratoprosthesis 1

■ 3141

Advantages of Strampelli's Osteo-Odonto-Keratoprosthesis

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Purpose: Considerations on the author's experience with keratoprotheses since 1955 (starting with a model designed by Dorzee).

Methods: Description and discussion of Strampelli's Osteo-Odonto-Keratoprosthesis (designed in 1963), emphasizing the advantages of this type of heterotopic autograft support. Illustration of important technical details.

Results: In rigorously selected cases the osteo-odonto-keratoprosthesis is satisfactorily tolerated and retained for prolonged periods of time, if all goes well. Acceptable visual results can be expected in favorable cases. Investigation must continue in this field to further reduce the number of early and late extrusions.

Conclusions: Although the technique is very complex and a number of special preoperative conditions are required, Strampelli's osteo-odonto-keratoprosthesis represents a very encouraging biological alternative in the field of corneal prosthetics.

■ 3142

Personal Innovations and Experience of Falcinelli OOKP in the last 30 years

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The author describes his experience of OOKP surgery including innovations in technique, results, complications and their management from the last 30 years.

■ 3143

Visual Rehabilitation by OOKP in amblyopic patients with end-stage ocular surface disease

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Purpose: Osteo-odonto-keratoprosthesis (OOKP) is the procedure of choice for sight restoration in patients with bilateral, end-stage severe inflammatory eye disease. The operation is generally offered as a last resort and visual results are limited by potential visual acuity depending on the status of the posterior segment of the eye. An assessment of the presence of pre-existing amblyopia is limited to knowledge of the age of onset of the visual deprivation and anticipation of amblyopia may influence a decision not to operate or present a grim prognosis. To analyze the results of OOKP performed in individuals with visual impairment from childhood and believed to be amblyopic and correlation of visual gain with pre-operative assessment of visual potential.

Methods: Retrospective analysis of patients who underwent OOKP in Brighton from 1996 to 2003. Case selection: Patients who had impaired vision in the affected eye from early (< 5 years) and late (> 5 years) childhood. Patients who went blind in later life, but who had a history of onset of primary disease in childhood with some visual deprivation were also included. Those who had 'normal' vision in childhood but developed visual impairment and subsequent blindness in later life and had been blind in the operated eye for more than 10 years were arbitrarily selected as a control group for comparison.

Results: Of a total of 28 adults who underwent OOKP surgery in Brighton, 11 met the selection criteria. There were 6 males and 5 females. Six patients had had visual impairment or onset of sight threatening ocular disease from early childhood and five from late childhood. Six patients met the criteria for serving as controls for comparison. 91% cases and 100% controls gained vision after the operation. 67% cases with visual deprivation from early childhood and 100% of those affected in late childhood attained a visual acuity of > 6/60. One year 'survival' rates of the implanted OOKP were 91% in cases and 100% in controls. More detailed statistical analysis comparing results between the groups with analysis of various contributory factors will be presented.

Conclusions: OOKP serves as an effective procedure for sight restoration in patients with pre-existing amblyopia. Onset of visual deprivation in early childhood need not be a major contraindication for the operation.

■ 3144

Imaging of Osteo-odonto-keratoprosthesis (OOKP) by Electron Beam Tomography (EBT) and High Resolution Computed Tomography (HRCT)

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Purpose: To describe our experience of using EBT and HRCT in imaging of OOKP to identify early bone loss which may threaten the viability of the globe.

Methods: EBT has been performed on 7 patients with OOKP and the results are compared to HRCT. The OOKP lamina dimensions were measured on EBT and HRCT and compared to the manual measurements at the time of surgery.

Results: There was a high degree of resolution of the OOKP lamina noted with EBT. In particular, it identified 2 patients with a marked degree of thinning of the inferior part of the lamina. This was not identified on HRCT. These areas of thinning were not present at the time of surgery. Compared to HRCT, the advantages of EBT include speed of scanning, associated 3D computer software allowing volumetric reconstruction and low dose of radiation exposure.

Conclusions: It is important to monitor regularly the dimensions and stability of the OOKP lamina as it will help detect cases that are at risk of extrusion of the optical cylinder and consequent endophthalmitis. Prophylactic measures can then be taken to prevent such serious complications from occurring. In our small series, we have found EBT to have excellent resolution and speed and we would support regular scanning of the OOKP lamina in all patients. Problems in defining the actual dimensions of the prosthesis relate to a) the wide range of densities of the alveolar and denticular bone involved with a high degree of inter prosthesis density variability and b) the effects of Partial Voluming with objects close to the spatial resolution limitations of the imaging modality.

Oxygenation of the fundus

■ 3151

Clinical Relevance of Ocular Oxygenation

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Purpose: Oxygen is a basic requirement of life and impaired oxygenation and blood flow plays a role in many blinding diseases and their treatment. Treatment regimens already in use influence the oxygenation of the eye and treat ischemic eye diseases such as diabetic and other vascular retinopathies and glaucoma. Physiological studies have shown that retinal laser treatment and vitrectomy improve retinal oxygenation and recent studies have shown that carbonic anhydrase inhibitors do the same.

Methods: We will review studies on the oxygenation of the retina following retinal laser treatment and vitrectomy. Transvitreal polarographic oxygen electrodes were placed over the optic disc and retina in pigs and the oxygen tension measured as Dorzolamide and Acetazolamide was injected intravenously.

Results: Panretinal photocoagulation significantly increases the oxygen tension of the retina. Vitrectomy improves the oxygen tension of the retina significantly in retinal areas where a branch vein occlusion has been induced. Dorzolamide and Acetazolamide also significantly increase the oxygen tension of the retina and optic nerve in pigs. The mechanism of this effect is through CO₂ accumulation in the tissue and vasodilatation leading to increased blood flow.

Conclusions: Laser treatment and vitrectomy are accepted treatment modalities for diabetic and other ischemic retinopathies and physiological studies have conclusively shown that this improves the oxygenation of the retina. Recent studies with carbonic anhydrase inhibitors suggest that the oxygenation of the retina and optic nerve may also be improved pharmacologically. We propose that three therapeutic pathways (laser, vitrectomy and drugs) exist to improve the oxygenation of the ischemic retina and optic nerve in addition to oxygen breathing.

■ 3152

Studies of optic nerve head oxygenation

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Purpose: To evaluate the variations of the optic nerve head PO₂ during normoxia, hyperoxia (100% O₂), carbogen breathing (95% O₂, 5% CO₂), and after intravenous administration of acetazolamide.

Methods: PO₂ measurements were obtained within the optic nerve head and at intervascular areas of the optic disc in the intact eyes of anaesthetized miniature pigs using oxygen-sensitive microelectrodes introduced through the vitreous cavity by an electronic micromanipulator. PO₂ was measured continuously during 10 minutes under systemic normoxia, hyperoxia and carbogen breathing, and under pharmacological variations of the perfusion pressure, as well as after intravenous injection of acetazolamide (bolus of 500 mg).

Results: Within the optic nerve head, hypoxia, hyperoxia, and the variations of the perfusion pressure did not alter significantly the PO₂. Carbogen breathing induced a significant increase in both systemic PaCO₂ and PaCO₂. Furthermore, it increased significantly the optic disc PO₂ (DPO₂=16.0±6.2 mmHg; p<0.0001; n=10). Acetazolamide did not affect significantly the PaCO₂ and the optic disc PO₂, unless carbogen breathing was simultaneously administered (DPO₂=18.9±6.2 mmHg; p=0.002; n=5). In the latter case, the optic disc PO₂ was still elevated twenty minutes after the interruption of carbogen breathing (DPO₂=4.3±3.2 mmHg; p=0.042; n=5) in parallel with a high systemic PaCO₂.

Conclusions: Carbogen breathing increases the optic disc PO₂ significantly in miniature pigs more than systemic hyperoxia. Acetazolamide alone is not sufficient to increase the optic disc PO₂. The association of acetazolamide injection with carbogen breathing could increase the oxygenation of the optic disc significantly through a postulated vasodilatory effect of elevated systemic PaCO₂.

■ 3153

Partial Pressure of Oxygen in Retinal Veins and Optic Disk Blood Vessels of Monkeys with Experimentally Induced Glaucoma.

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Purpose: Blood flow is reduced in the optic disk of monkeys with experimentally induced glaucoma (Percicot, ARVO Abstract, 1996). Our goal was to study in the same model the variations of the blood oxygen pressure (pO₂) in the vasculature of the optic nerve head (ONH).

Methods: Two different groups of male monkeys had an argon laser photocoagulation (ALP) of the trabeculum in one eye resulting in an increase of the intraocular pressure (IOP). In both control and experimental glaucoma (EG) eyes, two-dimensional maps of the blood pO₂ were recorded with the phosphorescence quenching method. The first group of 5 monkeys (Group I) was measured 6 years after ALP whereas the second group of 8 monkeys (Group II) was measured 2 months after ALP.

Results: For both groups, the IOP was significantly higher in EG eyes (15 ± 3 versus 47 ± 9 , and 17 ± 4 versus 45 ± 16 (mean \pm SD), respectively). Group I and II showed in the control eyes a blood pO₂ of 41 ± 5 mmHg in the retinal veins and a blood pO₂ of about 55 ± 7 mmHg in the ONH capillaries. In the EG eyes of Group I, blood pO₂ decreased by an amount of 21 and 24 % in retinal veins and ONH capillaries, respectively. In the EG eyes of Group II, blood pO₂ increased by an amount of 14 and 22 % in retinal veins and ONH capillaries, respectively.

Conclusions: Six years after ALP, reduced blood flow and blood pO₂ suggest an altered oxygen delivery to a largely atrophic ONH tissue. Two months after the ALP, increase in blood pO₂ suggests a slower metabolism due to less activity. This work is supported in part by the Swiss National Science Foundation (Grant # 31-65093)

■ 3154

Reflectance change at the optic nerve head in response to flicker

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Purpose: To study the effect of light flicker on dual-wavelength reflectance ratio images of the human eye fundus.

Methods: The retina of 6 normal volunteers was illuminated with the halogen lamp of a fundus camera filtered with a dual-band pass filter (569/600 nm). Pairs of retinal images centred at the optic nerve head were acquired simultaneously using an intensified CCD video camera mounted on an image splitter. 3 consecutive phases of 60-90 s duration each were performed. The measuring light was maintained steady during phases 1 and 3 and flickered at 8 Hz using a chopper wheel during phase 2. This cycle was repeated at least 3 times. After applying image corrections and automatic image registration, images acquired under identical physiological conditions were added and the ratio (R) of images measured at the two different wavelengths computed (569-nm image divided by 600-nm image). Changes in percent of R between the 3 phases were calculated. Then, mean variations of several areas were determined for each person (in vessels, in tissues inside and outside the optic disc).

Results: The data obtained for one subject were excluded because of technical reasons. No significant changes were found a) between the two baselines (phase 1 and phase 3) for all areas, b) for the tissue outside the optic disc in response to flicker. Light flicker caused a decrease in R of $(-2.9 \pm 1.7 \%)$ for the retinal vessels and of $(-5.0 \pm 1.5 \%)$ for the tissue of the optic disc (mean change \pm C.I.). Variations of vessel diameters in response to light stimulation were also observed.

Conclusions: Light flicker has a significant effect on R of the optic disc tissue and of the vessels. This activity-evoked response could be due to a change in blood volume or tissue scattering.

The yellow IOL

■ 3161

The transmittance of the human lens

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Purpose: To provide an overview of current knowledge of the transmittance of the human lens to optical radiation.

Results: An important function of the ocular lens is to block electromagnetic energy entering into the eye, that may otherwise cause damage to the retina. The physical entity describing the transparency to optical radiation is transmittance. The transmittance of a medium is defined as the ratio between the power exiting a medium and the power entering a medium. The human crystalline lens attenuates wavelengths shorter than 300 nm very strongly and then progressively increases the transmittance towards longer wavelengths. In the young lens, there is a peak transmittance of a few % in the ultraviolet (UVR) region at 320 nm. Towards longer wavelengths, the transmittance decreases to a minimum at around 365 nm. Reaching longer wavelengths, the transmittance progressively increases to close to 1 at 450 nm. This transmittance spectrum is believed to mirror the lens content of the tryptophan metabolite 3-OH-kynurenine-glucoside (3-HKG). In the aging lens, the window of transparency around 320 nm disappears and the peak absorption at around 365 nm disappears. This is believed to reflect a progressive loss of 3-HKG. Simultaneously, progressive absorption develops in the blue part of the spectrum, decreasing the transmittance up to 550 nm.

Conclusions: It is concluded that the normal human lens effectively blocks UVR and at increasing age also increasing amounts of blue light.

■ 3162

The blue light hazard and AMD: a case for a yellow IOL

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Purpose: To review risk factors in AMD and, in particular, the published data demonstrating the hazardous nature of retinal exposure to ultraviolet and blue light and to relate this to endogenous and novel exogenous filters pre- and post-cataract surgery.

Methods: An evaluation of the ophthalmic and biophysical databases undertaken together with a review of codes of practice for safe exposure to lasers and other sources of light. These data were then analysed in relation to their impact upon the cell biology of ageing in AMD.

Results: The database demonstrated that retinal exposure to blue light was a thousand times more hazardous than comparable exposures in the red and infrared. Attenuation of retinal irradiance in the blue was demonstrated as a consequence of yellow intraocular filtration and, in the aphakic eye, adequate attenuation was achieved by the use of AcrySof Natural, a yellow IOL.

Conclusions: The study demonstrated a case for prevention of excessive blue light exposure of the retina and the need for blue attenuating filters in microscopes during cataract surgeries and in IOLs. The AcrySof Natural lens provided adequate protection against blue light in the post-cataract eye.

■ 3163

How to protect against blue light damage to the eye

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Purpose: To review the current knowledge regarding solar retinitis resulting from staring at the sun. This was once referred to as "eclipse blindness" and associated "retinal burn." However, solar retinitis (or solar maculopathy) results from a photochemical injury mechanism following exposure of the retina to shorter wavelengths in the visible spectrum, i.e., violet and blue light.

Methods: The action spectrum for blue-light photoretinopathy peaks at 440 nm and this data was employed with environmental sunlight exposure data to determine the adequacy of filtration. Conclusive animal experiments in 1976 showed that an intense exposure to short wavelength light causes acute photic maculopathy, and this is not a thermal injury mechanism.

Results: Blue light retinal injury (photoretinitis) can result from viewing either an extremely bright light for a short time or a less bright light for longer exposure periods. It is relatively safe to view the sun near sunset when it appears as a bright orange disk--largely devoid of blue light--but is hazardous to view at noontime when the bright white disk is rich in blue light.

Conclusions: A filter with a theoretical short-wavelength cutoff at approximately 500 nm will transmit 90% of the photopic visible spectrum, but block 98% of the hazardous blue light from a white light source. However, the protective filtering against blue light needs to be balanced against visual requirements and circadian response needs.

■ 3164

The theoretical concepts of the yellow IOL

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Prior to the advent of UV-blocking IOLs, cataract surgery would result in not only the removal of the cataractous lens but also the removal of the protection that the crystalline lens normally affords the retina. During the early 1980's, the use of UV light-attenuating IOLs became widespread. The IOL manufacturers recognized that the normal adult human lens was an effective UV absorber and thus, to effectively replace the lost function, an IOL would have to likewise absorb UV light. IOLs manufactured today contain an UV chromophore that effectively protects the retina from exposure to light up to 400 nm. The natural human lens not only provides protection from UV light exposure, but also decreases the retinal exposure to blue light in the 400-500 nm range. Some authors have speculated that the spectral transmission characteristics of the ideal IOL would approximate that of the adult crystalline lens. Thus the logic to replace the natural human crystalline lens with an IOL that approximates its UV absorption properties could be extended to the replacement of the crystalline lens by an IOL capable of attenuating blue light filtration as well. Alcon Laboratories, Inc. has developed the AcrySof Natural IOL to approximate the UV and blue-light attenuating properties of the human crystalline lens. This IOL contains a covalently bound chromophore that absorbs light in the 400-500 nm range, adding this light protection to that already provided in the UV range. The AcrySof Natural IOL is made of the same material as the AcrySof multipiece and single piece lenses but adds the blue-light-attenuating properties of the human crystalline lens.

The yellow IOL

■ 3165

The physics and the chemistry of the yellow IOL

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Approximate replication of the transmission characteristics of the human crystalline lens required the development of a unique, state-of-the-art, polymerizable yellow chromophore. Following a rigorous experimental process, a proprietary compound was created that could be covalently bonded into the polymer backbone of the AcrySof IOL material. The covalently bound chromophore provides the desired transmission spectrum, and following the normal extraction process used for the AcrySof IOL material, no detectable (< 1 ppm) unbound chromophore remains within the material. The transmission spectrum of this new IOL has been compared to that of various IOLs available to the market (AcrySof MA60BM IOL, Sensar AR40E, CeeOn Edge 911A, Phacoflex II S140NB), as well as to the 4-year old and 53-year old human crystalline lens. In addition, various physical and optical tests have been performed to demonstrate equivalence to the traditional AcrySof material.

■ 3166

Anatomical and functional results with the new yellow IOL SN60AT : first results

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Purpose: To studied anatomical result and functional results with the yellow IOL after uneventfull cataract surgery.

Methods: We prospectively included patient for cataract surgery and yellow IOL implantation. All patients had no other pathology except standard cataract. Pre and postoperative evaluation included anatomical evaluation, visual acuity under normal condition, lox contrast visual acuity, contrast sensitivity, glare test, color vision evaluation. we added quality vision questionnaire. Evaluation was programmed at 2 months and 6 months postoperatively.

Results: First result showed very good postoperative visual acuity, normal contrast sensitivity test and color vision evaluation.

Conclusions: Yellow IOL implantation is safe for patient. Functional evaluation gave good results. Anatomical evaluation need longer follow-up evaluation.

■ 3171

Evaluation of leukocyte dynamics diabetic retinopathy

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Purpose: To investigate leukocyte-endothelial interactions in the streptozotocin diabetic mouse model.

Methods: Leukocytes were isolated from spleens of syngeneic mice and labelled with the fluorescent tag calcein-AM. Cells were transferred to syngeneic mice and imaged in the retina using a scanning laser ophthalmoscope (SLO). Images were recorded on video tape (S-VHS) for later analysis of leukocyte adhesion to the retinal vessels. After imaging animals were infused with 2% Evans Blue, killed and eyes removed and fixed in 2% paraformaldehyde. Retinas were flat mounted and examined for cells and vessel leakage under a confocal microscope.

Results: Normal cells flowed freely in the circulation of normal mice and did not adhere to retinal vessels in the SLO. Cells from animals with diabetes infused in to diabetic mice adhered to the retinal vessels, as did normal cells infused in to diabetic animals. In addition cells from animals with diabetes adhered to retinal vessels of normal mice. These results were confirmed in flat mount preparations of excised retinas. There was an increase in the number of cells present in the retinas of diabetic mice that had received cells from diabetic or normal cells mice, and also in the normal retinas of mice that had received cells from diabetic mice.

Conclusions: These results confirm previous reports indicating that leukocyte adhesion is, in part at least, responsible for the capillary occlusions that occur in diabetic retinopathy. Furthermore, the data show that the alterations in leukocyte-endothelial cell adhesiveness reside not only in changes to the retinal vascular endothelium but are properties of the diabetic leukocyte. This work is supported by Astra-Zenica.

■ 3172

Ascorbic acid influence the cell cycle of ARPE cells

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Purpose: Proliferation of RPE cells, sown out in the vitreous cavity, is seen responsible for the development of PVR. Application of cytostatic drugs with high side effects is the general medical treatment. In cell culture assays we could show that ascorbic acid not only inhibit proliferation but also allow cells to repopulate 120 h after exposure to ascorbic acid in high concentrations. To get more insights about the influence of ascorbic acid in regard to cellcycle control we stimulated ARPE cells with different concentrations of ascorbic acid and measure the proliferation, apoptosis and at least the necrosis rate by FACS

Methods: ARPE cells were stimulated with 0,125mM, 0,25mM, 0,5mM, 1mM, 2mM and 4mM ascorbic acid for 24h. After trypsination cells were marked with fitc-annexin and propidium jodid (using Bender Med Systems Kit) and measured coloured cells in fitc and pe channel in flow cytometer (Becton Dickinson).

Results: FACS analysis show that cellcycle phases of ARPE cells are directly correlated with rising concentrations of ascorbic acid. Low concentrations like 0,125mM and 0,25mM induce proliferation; higher concentrations upon 0,5mM inhibit proliferation and highest concentration of 2mM and 4mM induce both apoptosis and necrosis.

Conclusions: These results let us purpose that ascorbic acid, regularly known as antioxidant, react in unphysiological high concentrations like other reactive oxygen species (ROS).

■ 3173

Enhanced retinal neovascularization after intravitreal injection of dimeric ephrinB2

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Purpose: Eph receptors and their membrane tethered ephrin ligands comprise the largest family of receptor tyrosine kinases and are involved in the projection of growing axons. Studies on mice have revealed an important function of ephrinB2 during vascular development in embryonic life, but the impact on retinal neovascularization (RNV) has not been studied yet. To investigate the role of ephrinB2 ligand on RNV we injected dimeric ephrinB2 in the vitreous of mice using the murine model of retinopathy of prematurity (ROP).

Methods: RNV in the ROP-model was induced by exposing postnatal day 7 (P7) C57BL/6J mice to 75% oxygen for 5 days. On P12 they were returned to room air and treated with an intravitreal injection of mouse recombinant ephrinB2 (0,3microM) in the right eye and buffer solution in the left eye. RNV was visualised by perfusion with fluoresceine-dextran on P17. Retinal whole mounts were prepared and their blood vessel patterns were quantified blindly using a scoring system adapted from Higgins et al.

Results: Intravitreal injection of ephrinB2 treatment increased the retinal angioproliferative changes compared to the control eyes (n=27). Paired Wilcoxon test analysis of the RNV scores showed a statistical significant difference (p<0.05).

Conclusions: These findings provide evidence that the Ephrin/Eph-system is involved in pathologic retinal angiogenesis. A single injection of dimeric ephrinB2 increases vascular proliferation significantly. Blocking its action might open new possibilities in the treatment of angioproliferative retinopathy.

■ 3174

Macroglial changes in an experimental model of hypercholesterolemia; variations after normalization of cholesterol blood values induced by a normal diet

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Purpose: To evaluate (a) retinal changes in the Müller cells and the different types of astrocytes in an experimental model of hypercholesterolemia and (b) modifications induced by a period of normal cholesterol blood values induced by a normal diet.

Methods: Rabbits were administered a cholesterol enriched diet for ten months and thereafter a normal diet for 6 months. The retinas were processed using light microscopy and transmission electron microscopy. Retinal wholemounts were processed by immunohistochemistry (anti-GFAP).

Results: 1) Hypercholesterolemic rabbits: The end feets of the Müller cells exhibited intense GFAP immunoreactivity. In some areas, glial scars were observed. Type I astrocytes were hyper-reactive and formed sheaths over the blood vessels. In some areas, the number of type I astrocytes decreased. Type II astrocytes, which connected the blood vessels, were hyper-reactive and increased in number and size. The number of elongated astrocytes in the nerve fibre layer (NFL) was reduced and those remaining were hyper-reactive. 2) Rabbits with normal cholesterolemic values after normal diet: The strong hyper-reactivity of the Müller cells persisted. These cells filled the spaces left by dead retinal neurons. Type one astrocytes were replaced by type II astrocytes, which were hypertrophic and extended a network of processes over the blood vessel. Features of the elongated astrocytes of the NFL were unchanged.

Conclusions: After normalization of the cholesterol blood values induced by a normal diet, the reactivity of the retinal macroglia persisted as a consequence of the vascular changes induced by chronic ischemia.

■ 3175

Search for retinal antiangiogenic factors in a mouse model of oxygen induced retinopathy

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Purpose: In the mouse model of oxygen induced retinopathy it has been observed that a puncture of the eye per se reduces the retinal neovascularisation. It is the aim of this study to determine the factors involved based on differential display assays.

Methods: C57Bl6j mice were exposed to 75% oxygen from postnatal day 7 (P7) to P12 and then returned to room air. At P12 one eye was punctured with a glass pipette posterior to the limbus. The control eye was not manipulated. At P19 retinal flat mounts were evaluated for angioproliferation. Alternatively, total RNA was isolated from the retinae of control and injured eye at P17. After reverse transcription cDNA was amplified in a differential display PCR using P33-labeled nucleotides. The products were separated by gel electrophoresis. After autoradiography candidate bands were excised, purified, reamplified, and sequenced.

Results: Eyes punctured at P12 showed a significant reduction of retinopathy when compared to the contralateral uninjured control eye. So far, differential display analysis of retinal RNA from both eyes indicated up- and downregulation of more than twenty genes including Otx2, an activator of RPE differentiation.

Conclusions: The observation that trauma to an eye in the mouse model of oxygen induced retinopathy leads to inhibition of retinal angiogenesis, is a possible approach to identify new genes involved in regulating angiogenic homeostasis in the eye.

■ 3176

Distribution of various angiogenic factors in central and peripheral retinal pigment epithelium

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Purpose: Chorioidal neovascularisation (CNV) is a sprouting of vessels from the chorioidea through Bruch's membrane. Although responsible for most cases of vision loss in age related macula degeneration (AMD) its detailed mechanisms are unknown. In general, angiogenesis describes the sprouting of new vessels from preexisting ones. It is tightly regulated by factors that either promote or impede angiogenesis. Dysfunctional Retinal Pigment Epithelium (RPE) as a production site of these factors is believed to play a central role in the genesis of CNV. Aim of the study was to investigate if differences in the expression of various angiogenic factors in central and peripheral RPE are responsible for CNV occurring at the posterior pole.

Methods: Whole globes were obtained, post mortem time was less than 20 hours and donor age ranged between 27 and 50 years. RPE was extracted in a 36mm² area behind macula and close to ora serrata using a specially designed device. VEGF and PEDF concentrations were investigated using Northern-Blot technique. For examination of post-transcriptional modified angiogenic factors, Western-Blot analysis was performed.

Results: There was no difference in the expression of any examined angiogenic factors. VEGF, PEDF, Endostatin and Angiostatin were found in same concentrations in central and peripheral RPE.

Conclusions: A shift in the expression of angiogenic factors of central RPE is rather provoked by local processes. Since there is no difference in the expression patterns, the idea of RPE cell loss due to oxidative or ischemic damage is further supported.

■ 3177

Influence of hypoxia in the early and late changes of age related macular degeneration

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Purpose: Early age related macular degeneration (ARMD) is characterised by atrophy of the choroid and the thickening of Bruch's membrane by the accumulation of extracellular matrix (ECM). These findings could be related to hypoxia of the outer retina and retinal pigment epithelium (RPE). One factor known to increase the formation of ECM is connective tissue growth factor (CTGF). Additional CTGF has been proposed to be involved in the angiogenesis. The enzyme plasminogen activator inhibitor-1 (PAI-1) inhibits the degradation of ECM. In the present study we investigated the effect of hypoxia on the expression of CTGF and PAI-1 in cultivated human RPE cells.

Methods: The expression of CTGF and PAI-1 in native RPE cells of 6 donors was investigated by RT-PCR, northern-blot and western-blot analysis. Cultivated RPE cells from 5 human donors of the third passage were: 1. 4 - 12 hours under hypoxic conditions (1% O₂) incubated 2. 1 - 16 hours re-oxygenated. The induction of CTGF and PAI-1 were quantified by using immunohistochemical staining, western-blot and northern-blot analysis.

Results: Native RPE cells constitutively express CTGF und PAI-1. Hypoxia increases the expression of CTGF in cultivated RPE cells about the factor 2-3x. Following re-oxygenation there was an increase about 3-4x fold. Hypoxia increased the expression of the enzyme PAI-1 about the factor 3-4x, while the phase of re-oxygenation lead to a 5-6x fold increase.

Conclusions: Local hypoxia in ARMD seems to induce the expression of CTGF und PAI-1 in the RPE. This cascade may play an important role in the observed early and late changes of ARMD like thickening of Bruch's membrane and formation of choroidal neovascularisations.

■ 3201

Molecular signalling pathways

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Purpose: Deregulated signaling pathways are important during tumorigenesis.

Therefore, we investigated the signaling pathways controlling the growth of human normal choroidal melanocytes and cells from the choroidal melanoma and their link to the cell cycle machinery.

Methods: We used human choroidal melanocytes and human choroidal melanoma cells lines for in vitro studies and primary human choroidal melanoma tumors were used for biochemical and immunohistochemical studies.

Results: Analysis of the activation and involvement of intracellular signaling pathways in the control of cell proliferation were performed by using western blotting, and pharmacological and oligonucleotide antisense strategies respectively. We showed that c-kit receptor is expressed in most of the choroidal melanoma tumors and may be implicated in the proliferation of choroidal melanoma. We also demonstrated that the MEK/ERK signaling pathway plays a key role in melanoma cell proliferation through the modulation of the expression of transcription factors like c-myc and proteins of the cell cycle like the Cdks inhibitory protein p27.

Conclusions: The understanding of signaling pathways controlling the growth of human normal choroidal melanocytes and cells from the choroidal melanoma is necessary for the development of future potential target for therapeutic strategies.

■ 3202

Role of protein kinases

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Purpose: Protein tyrosyl phosphorylation is an essential component in intracellular signalling, including functions like mediation of cell proliferation, survival, apoptosis, differentiation, migration and attachment. Our interest has recently been focused to the role of insulin-like growth factor-1 receptor (IGF-1R), c-met and c-kit.

Methods: Samples of uveal melanoma were used in conjunction with established cell lines. Protein kinases were detected by immunohistochemistry and Western blot analysis. Tumor cell growth was further assessed by cell counting, thymidine incorporation and cell viability assays. The polymerase chain reaction followed by direct sequencing was used to detect c-kit gene abnormalities. Survival data was used for Cox proportional hazard models.

Results: High IGF-1R expression was independently correlated with poor outcome. Moreover, in vitro inhibition of IGF-1R activity was associated with a drastic decrease in uveal melanoma cell viability. Interestingly, expression of IGF-1R and c-met were intimately inter-correlated. In contrast to skin melanoma, c-kit was upregulated in 75% of fresh frozen and 60% of paraffin-embedded uveal melanoma. However, sequencing of exon 11 of the c-kit gene failed to reveal mutations in any of 7 studied tumors, suggesting that post-translational mechanisms are involved. Currently, 2 patients with c-kit upregulated metastatic uveal melanoma are being treated with imitinab, a c-kit blocking agent. Disease has remained stable for 1 and 2 months respectively.

Conclusions: The pathways used by some protein kinases constitute potential targets for future therapeutic efforts, and specifically the IGF-1R and c-kit may offer significant therapeutic advantages. Animal models need to be pursued to clarify the molecular mechanisms involved.

■ 3203

Uveal Melanoma and Growth Inhibition Pathways

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Recent genetic and molecular studies of uveal melanoma have enhanced understanding of the molecular biology of this unusual type of cancer. An insensitivity to anti-growth signals is one of the critical attributes of a cancer cell: this loss of control allows the cells to grow without respect to surrounding tissue or distant organs to which it may metastasise. This loss may occur at any stage in the cytokine or cell-cell contact mediated growth inhibition pathways, from cytokine via receptor signaling to tumour suppressor gene (TSG) loss or mutation. The molecules most likely to be affected are those critical to the function of more than one growth inhibition pathway within uveal melanocytes. Current understanding is imperfect, but there is evidence that several such pathways are abrogated in uveal melanoma, and that these differ substantially from those altered in most cutaneous melanomas. While abnormalities of the p16INK4a TSG have been reported in uveal melanoma, silencing by methylation is only present in a minority of tumours, and mutation is very rare indeed. Our group has found that the TGF beta pathway is abnormal in the majority of uveal melanomas, and there is loss of p27Kip1 expression, a downstream effector of this pathway. Again, no mutations have been described in this pathway. Abnormal phosphorylation of RB is common in uveal melanomas, but mutation appears to be rare. There are a lot of negative studies: the p53 gene is not over-expressed and is probably wild-type in uveal melanoma, but there are conflicting data about mdm2 which influences its function. No relationship with melanocortin receptor allele type or MITF expression has been described in uveal melanoma. PTEN is not mutated in uveal melanoma cell lines. Cell-cell interaction leads to the phenomenon of contact inhibition, which is lost in cancer cells. There have been few studies of the pathways involved in this interaction in uveal melanoma, but beta-catenin expression is abnormal in some cutaneous melanomas. The lack of mutations in uveal melanoma means that future work should include exploration of epigenetic mechanisms of gene silencing, including methylation.

■ 3204

Interaction between the melanoma cell and its environment

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Purpose: To determine the molecular pathways that play a role in the development of metastases of uveal melanoma. Although many parameters are related to prognosis, the direct biological relationship between these parameters (e.g. tumor size, monosomy 3) with the development of metastases is often as yet unclear. New types of analyses may help us to define the specific molecules involved in the tumor cell escape from the eye or tumor cell growth in the liver.

Methods: Expression profiles of primary uveal melanoma were compared by microarray analysis with normal melanocytes and cell lines obtained from metastases (PA van der Velden *Int J Cancer* 2003, 106, 472-9). Interesting molecules were studied further.

Results: The analysis of the data showed that one molecule, TIMP-3, an inhibitor of metalloproteinases, was downregulated during tumor progression, but was not a prognostic marker. This made us realise, that one can separately look at prognostic markers (tumor size etc.), and at markers of tumor progression. Progression markers show a different expression in melanocytes, melanoma cells and metastases, and may be targets for tumor growth inhibition. A further interesting set of progression markers are the HLA Class I and II molecules. As shown by C. Ericsson (*IOVS* 2001, 42, 2153-6), HLA Class II expression can also be a prognostic marker, but since these molecules are not expressed on melanocytes, they are also progression markers. Theoretically, they may be important in the anti-tumor immunological defense.

Conclusions: The differences between prognostic markers and progression markers, with special attention to the possibility of HLA Class II molecules functioning as either immune stimulators or tolerance inducers, will be discussed.

■ 3205

ERK1/2 activation during choroidal melanoma cell proliferation and tumorigenesis: constitutive activation and growth factor autocrine loop of activation

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Purpose: To analyze the role of constitutive activation and growth factor-mediated activation of signaling pathways involved in the control of choroidal melanoma cell proliferation and tumorigenesis.

Methods: The activation of the ERK/MEK signaling pathway was investigated in primary cultures of normal human choroidal melanocytes and various human cell lines of choroidal melanoma were cultured by using western blot analysis. Pharmacological and oligonucleotide antisens (ODN AS) strategies were performed to analyze the role of the MEK/ERK activation in cell proliferation and in in vitro tumorigenesis. The role of various growth factors in the activation of the MEK/ERK signaling pathway was investigated by immunoblocking, pharmacological and ODN AS strategies.

Results: The MEK/ERK signaling pathway is highly activated in the absence of choroidal melanoma cell proliferation due to activated somatic mutation in the kinase upstream of MEK/ERK. But, this module is also under the control of a parallel signaling pathway which is regulated by the activation of endogenous growth factors secreted by the choroidal melanoma cells. Both constitutive activation and growth factor autocrine loop of activation control cell proliferation and tumorigenesis. This control is mediated through activation and inhibition of proteins involved in the regulation of the cell cycle.

Conclusions: These data may have important implications for the development of more selective methods for the inhibition of choroidal melanoma.

■ 3206

Ribosomal protein genes are differentially expressed between uveal melanoma with monosomy 3 and disomy 3

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Purpose: Almost every second patient with uveal melanoma will develop metastatic disease, which is strongly associated with loss of one chromosome 3 in the primary tumour (monosomy 3). Global gene expression analysis may contribute to identify the biological mechanisms that underlie the differential metastasizing potential of uveal melanoma with and without monosomy 3.

Methods: Gene expression analysis on RNA from 20 primary tumours was performed by using microarrays containing approx. 12500 genes (Affymetrix). The resulting expression measurements of 7902 significantly expressed genes were subjected to hierarchical cluster analysis and Wilcoxon rank sum test.

Results: Cluster analysis identified two distinct classes of tumours. Intriguingly, all tumours in one of these classes showed disomy 3 while in the other all but one tumour had monosomy 3. We also tested if the expression profiles of several groups of functionally related genes contribute more or less to the dissimilarity of the two tumour classes. Only a subset of 98 genes involved in protein biosynthesis showed a distinct expression profile. When breaking down the components of this subset we found that the observed deviation is almost exclusively based on genes coding for cytoplasmatic ribosomal proteins whereas the remaining genes of this subset, mostly aminoacyl-tRNA-synthetases, show no differential expression.

Conclusions: These results strongly indicate that there are two entities of uveal melanoma. Among all groups of functionally related genes tested, genes coding for ribosomal proteins showed the most significant contribution to tumour dissimilarity of the two tumour classes.

Keratoprosthesis 2

■ 3241

Coralline Hydroxyapatite Keratoprosthesis: First Human cases

LEON C

Purpose: The ideal prosthesis will let the tissue grow into the supporting material and have a similar curvature as the recipient cornea.

Methods: We developed a new support for keratoprosthesis made of porous hydroxyapatite. They were implanted unilaterally in the eyes of 12 New Zealand rabbits for 12 months (1995) before the first human cases.

Results: We observed good vascularization. There were no signs of infection or extrusion, epithelial downgrowth, or any adverse tissue reaction. Human cases are presented individually.

Conclusions: This biocompatible keratoprosthesis with a biointegrable, nonbiodegradable, and colonizable haptic can help many people to see again.

■ 3242

AlphaCor™ : Outcome data update

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Purpose: To provide an overview of the AlphaCor artificial cornea and summarise outcome data.

Methods: AlphaCor is a synthetic hydrogel cornea implanted within the corneal plane by means of a 2-stage procedure. Human clinical evaluation commenced in 1998. Following regulatory approvals, device assessment continues using a web-based data collection system. Pre-operative, operative and post-operative data is collected 3-monthly.

Results: Current outcome data will be presented. To end May 2003, 59 devices had been implanted with follow-up of up to 55 months, mean 15 months, with a 1-year probability of survival of 80%. Mean pre-operative visual acuity was Hand Movements and the mean gain in visual acuity was 2 lines. Complications encountered were mainly related to corneal melting or related to optic deposition. The single most significant risk factor for melt-related complications after AlphaCor implantation is a history of herpes simplex viral infection. Topical medroxyprogesterone 1% appears to be protective against melt onset. Risk factors for loss of optic clarity have also been identified and include certain topical medications and smoking.

Conclusions: Early outcomes with AlphaCor (previously known as the Chirila KPro) suggest that the device is a promising alternative to donor tissue in high risk cases. Case selection is important for good outcomes, and herpetic patients are excluded. Further, avoidance of risk factors that cause deposition within hydrogels is important for sustained good outcomes. Clinical evaluation continues.

■ 3243

Aachen type III keratoprosthesis ready for implantation

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Purpose: The first type of the Aachen Keratoprosthesis is used for vitreoretinal surgery since 5 years now. We developed the new type III prosthesis for prolonged up to permanent implantation. This goal seems to be near now after development of special materials, forms and application techniques.

Methods: With surface modified silicone, and Polyvinylidendifluoride we form a composite keratoprosthesis being constructed from a highly woven skirt and a soft silicone optic. both parts are glued together by a special melting process that allows mechanical and biological homogenous construction. The skirt is highly tolerated and feasible for cellular and ligamental attachment.

Results: The new designed type III Aachen Keratoprosthesis is now ready for implantation. Its parts have been tested alone and assembled on biocompatibility and mechanical properties. The implantation procedure has been tested on animal eyes and gives a reliable and save procedure.

Conclusions: The use of the type I keratoprosthesis in vitreoretinal surgery and its first Type II application for a prolonged time gives us reason to try the Type III prosthesis on humans in near future. First implantation in a desperate case of severe endophthalmitis after loosening of a PMMA prosthesis showed the principal applicability of the system.

■ 3244

Supradescemetic synthetic cornea: preliminary results of a non-penetrating keratoprosthesis in vascularized rabbit corneas

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Purpose: To assess feasibility and biocompatibility of a supraDescemetic Synthetic Cornea (sDSC) implanted in rabbits following a corneal injury.

Methods: Severe corneal injury was created in the right eye of 15 rabbits by application of 1-heptanol and complete surgical removal of the limbus. After development of corneal vascularisation and scarring (mean: 45 ± 5 days) implantation of an sDSC (7mm diameter, 450µm thick optical zone, 100µm thick outer flange) was performed. The KPro's were implanted with their central optic part positioned on a completely exposed Descemet's membrane (DM), the outer flange was located in deep stroma (85%). 3 different materials were tested in this series: hydrophobic PMMA (n=5), hydrophilic HEMA-MMA (n=5) and HEMA-NVP (n=5), with a water content of 34% and 75%, respectively. Following KPro implantation, the corneal surface (including the outer surface of the KPro optics) was covered with a conjunctiva-Tenon flap. A central flap opening was made after the eyes became completely quiet (mean: after 63 ± 7 days).

Results: Implantation of the sDSC could be performed successfully in all 15 consecutive cases, without any perforation of DM. Repair of the conjunctival flap had to be performed in 5 rabbits. At time of the flap opening, 3 of the corneas with a PMMA implant and 3 with a HEMA-NVP75 implant had developed neovascularisation to some degree in the interface between DM and KPro optic. Corneas that received an sDSC made of HEMA-MMA34 displayed a complete clear DM without any vessels or scarring. DM was found firmly attached to the posterior surface of the optic.

Conclusions: Implantation of a non-perforating synthetic cornea on top of an exposed DM is feasible; the transparency of DM can be maintained at last over a several months observation period and was limited by the ingrowth of vessels in the Descemet-KPro interface, with HEMA-MMA34 showing the most promising results. A lamellar supraDescemetic Synthetic Cornea would theoretically reduce the risks of a penetrating KPro, as opening of the anterior chamber is not required.

Keratoprosthesis 2

■ 3245

The Dacron Tissue KPro: design, technique and results

PINTUCCIS

In 1979 Pintucci developed a new KP (International Patent Pending) with a Dacron fabric supporting element. Since 1954, Dacron fabrics have been successfully used in permanent cardiovascular prostheses which were colonized by host tissues. In our Kpro the Dacron supporting element is fixed to a medical grade PMMA optic. The free spaces within the fabric allow the biological colonization of the tissue. On the basis of our in vivo and in vitro experiments, we started to implant our new KP in patients with corneal blindness who were not amendable by a corneal transplant. After 0extensive in vitro and in vivo testing of different Dacron tissues, the Sauvage filamentous Dacron fabric was chosen for its good colonizability and mechanical performance. (softness and pliability), thus preventing aseptic corneal necrosis by mechanical stress; it is chemically inert, and not subject to reabsorption; it does not activate the complement; it can be autoclaved; it is easily cut into the desired shape and it can be sutured. With the colonized Dacron tissue supporting element, the reparative epithelial proliferation along the optical cylinder is stopped, owing to contact-inhabitation and to lack of empty spaces which have been filled by the newly formed connective tissue. We do not graft the supporting flange in the corneal stroma thickness but we suture it on the corneal surface. In this way the colonized Dacron tissue plays a trophic and amechanical role. It is important to emphasize that extremely thin and vascularized corneas may be successfully treated in this manner. The colonized Dacron tissue KP implant can be considered and behaves as an autotransplant placed between the cornea and a oral mucous graft. Over the past few years, we employed hard palate mucous to cover the Dacron in extremely dry eyes. The hard palate mucous has fewer elastic fibers and much more keratinization, is thicker and stronger than the labial mucous, and performs better in dry eyes. Over 1000 Pintucci's Kpro were implanted in different continents in over two decades.

■ 3251

A rabbit model to investigate the role of dopamine in choroidal blood flow regulation

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Purpose: The goal of this study was to investigate the role of dopamine as a potential modulator of choroidal autoregulation in anesthetized New Zealand white rabbits.

Methods: Rabbits were instrumented with hydraulic occluders around the inferior caval vein and the descending aorta for manipulation of blood pressure. Intraocular pressure, mean arterial pressure, orbital venous pressure, choroidal laser Doppler flowmetry (LDF), carotid blood flow were measured and vascular resistance was calculated. As a step of verification of LDF in the choroid, we performed LDF and microsphere injection assessment of choroidal blood flow simultaneously in a first group of animals under control conditions and after injection of a vasoconstrictor (L-NAME 15mg/kg). In a second group all variables were measured at baseline and during changes of blood pressure (1) under control conditions (2) during dopamine infusion (15 µg/min/kg) and (3) after D1 receptor block (SCH23390, 0.5mg/kg).

Results: LDF measurements and microsphere injection assessment of choroidal blood flow are comparable qualitatively but not quantitatively. LDF detected smaller changes in blood flow after L-NAME application than the microsphere injections, however, both results show a substantial reduction of choroidal perfusion. Dopamine infusion resulted in a vasodilation, that was reversible after the infusion pump was turned off. The effect could be blocked by the D1 receptor antagonist SCH23390. Also the pressure flow relationship of the choroid shifted upward with dopamine. The upward shift was blocked by SCH23390.

Conclusions: Our data show that dopamine modulates choroidal autoregulation in anesthetized rabbits via a D1 receptor mediated mechanism.

■ 3252

Measuring subfoveal choroidal blood flow by laser doppler flowmetry: theoretical and experimental considerations

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Purpose: To review the basic principle, range of applications and limitations of near-infrared laser Doppler flowmetry (LDF) for the measurement of subfoveal choroidal blood flow in humans.

Methods: Subfoveal choroidal blood flow was measured in normal volunteers by focussing a near-infrared (810 nm) laser beam at the fovea (power < 90 micro-W at the cornea) and detecting the light scattered by the red blood cells (rbc) in the choriocapillaris to obtain the spectrum of Doppler shifts. From this spectrum and using a signal synchronized with the heart pulse, the pulsatile and non-pulsatile components of the mean velocity, volume and flux (ChBF) of the rbc in the illuminated volume were determined and expressed in relative units. The technique allows continuous measurements of these LDF parameters, so that their responses to various physiological stimuli can be precisely monitored.

Results: In response to a moderate decrease in mean ocular perfusion pressure (OPP), ChBF demonstrated autoregulatory capability. Increasing the OPP by either static (isometrics) or dynamic exercise (biking) reveals the presence of an active mechanism that maintains ChBF close to its basal value. During dark adaptation of the retina, ChBF was found to decrease by almost 20%.

Conclusions: The demonstrated feasibility of near-infrared (810 nm) LDF to quantify the pulsatile and non-pulsatile components of subfoveal choroidal blood flow responses to physiological provocation has opened new avenues in the investigation of the physiology of the choroidal vascular system.

Choroidal circulation in health and disease

■ 3253

Choroidal blood flow in age-related macular degeneration

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Purpose: Several studies have suggested that choroidal blood flow is decreased in patients with age-related macular degeneration (AMD). We have now investigated the choroidal circulation in AMD patients with CNV in the fellow eye.

Methods: Nine AMD patients with drusen in one eye (study eye) and CNV in the fellow eye were included in this study. Mean age was 73 ± 6 years (1SD) and visual acuity was 20/40 or better in the study eye. Circulatory measurements obtained in these patients were compared with those of 10 normal controls with a mean age of 67 ± 9 years and a normal eye examination. Following pupillary dilatation, laser Doppler flowmetry (Oculix) was used to assess relative choroidal blood velocity (ChB Vel), volume (ChB Vol) and flow (ChB Flow) in the center of the fovea of the study eye of each subject. Mean arterial blood pressure (BPm), heart rate and intraocular pressure (IOP) were monitored following blood flow measurements. All circulatory measurements are shown in arbitrary units (AU).

Results: Mean ChB Vel, ChB Vol, and ChB Flow were 0.40 ± 0.11 AU, 0.22 ± 0.06 AU, 7.3 ± 2.0 AU in AMD patients and 0.47 ± 0.09 AU, 0.25 ± 0.1 AU and 10.5 ± 4.3 AU in normal controls. In comparison to controls, a significant decrease of 30% in ChB Flow (student's t-test, $p=0.05$) was seen in AMD eyes. The decreases in ChB Vel, and ChB Vol were not statistically significant ($p=0.14$, and $p=0.36$, respectively). BPm was significantly higher by 23.7% ($p=0.03$) in AMD subjects. A significant correlation was observed between BPm and ChB Flow in AMD patients ($r=0.859$, $p=0.003$) and a nonsignificant correlation was detected in normal subjects ($r=0.098$, $p=0.79$). When all subjects were analyzed together, a significant inverse correlation between age and ChB Flow was also observed ($r=-0.631$, $p=0.003$).

Conclusions: Our results suggest that choroidal blood flow is reduced in eyes of AMD patients that have CNV in the fellow eye. This raises the possibility that decreased foveolar choroidal blood flow in AMD eyes may have a role in the development of CNV in the same way as decreased flow is associated with neovascularization in other vascular tissues of the body.

■ 3254

Choroidal blood flow regulation during isometric exercise in humans

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Purpose: There is recent evidence that the choroid regulates its blood flow during isometric-exercise induced changes in ocular perfusion pressure. The mediators of this choroidal vasoconstrictor response are, however, unknown. In the present experiments we investigated a number of potential systems involved in these responses.

Methods: All studies were performed in healthy male subjects. These subjects performed squatting for 6 minutes and choroidal blood flow was measured continuously with laser Doppler flowmetry. Blood pressure and intraocular pressure were measured to calculate ocular perfusion pressure. These experiments were done in the absence or presence of an endothelinA-receptor antagonist, an angiotensin converting enzyme inhibitor, a nitric oxide synthase inhibitor, an alpha receptor agonist, a muscarinic receptor antagonist and a beta receptor antagonist.

Results: Characteristic pressure-flow curves of the choroid were obtained. Under physiological conditions blood flow in the choroid started to increase at ocular perfusion pressures approximately 60% above baseline. These pressure curves were altered by the endothelinA-receptor antagonist and the nitric oxide synthase inhibitor, but not by the other administered drugs.

Conclusions: Our data confirm that the choroid regulates its blood flow during exercise induced changes in perfusion pressure. In addition, our data indicate that the endothelin and the L-arginine/nitric oxide system are involved in this response.

■ 3255

Experiments on choroidal hemodynamics; blood flow regulation and coupling to neuro-retinal function

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Purpose: Visual stimulation of the human retina and acute changes in the ocular perfusion pressure (OPP) have been reported to elicit changes in the retinal blood flow which point to vascular regulation and neuro-vascular coupling. The effects of altered OPP and photic stimulation on the choroidal blood flow are less well elaborated. The studies reported here were carried out to demonstrate choroidal vascular regulation and photic driving of the choroidal blood flow (ChBF).

Methods: Healthy adult volunteers between 20 and 42 years of age participated in these studies. In one experiment (n=7), the OPP was changed through 20 minutes of aerobic exercise. Resultant changes in the sub-foveal ChBF were measured by laser Doppler flowmetry (LDF). Concomitant changes in the OPP were calculated from the IOP and real-time measurements of the systemic BP with a Colin 7000 system. In a second study (n=12), the ChBF was measured as the retina was stimulated by diffuse red flashes at 1, 30 and 60Hz. The neuroretinal responses were quantified by electroretinograms (ERGs).

Results: During aerobic exercise, the OPP increased by a maximum of 40%, but the ChBF remained within 7% of resting values. During photic stimulation of the retina, the ChBF increased but the ERGs decreased at higher flicker frequencies.

Conclusions: 1) The ChBF is kept constant by some mechanism during large physiological increments in the OPP. 2) The ChBF is augmented by increased flicker frequency, but the neuroretinal response (ERG) is not linearly linked to the change in blood flow. Support: Natural Sciences and Engineering Research Council (NSERC) of Canada.

■ 3256

Choroidal blood flow in age-related macular degeneration. Effect of photodynamic therapy

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Purpose: To study the changes of the regulation of the subfoveal choroidal blood flow (ChBF) in eyes with age-related macular degeneration (AMD) and within a neovascular tissue before and after photodynamic therapy (PDT), in response to an acute increase in systemic blood pressure.

Methods: Laser Doppler flowmetry (LDF) was used to determine changes of subfoveal choroidal blood velocity, volume and flow during isometric exercise. Measurements were performed, at baseline and end of exercise, in one eye of 18 young healthy volunteers (mean age 314 years CI, group I), of 25 older healthy volunteers (mean age 694 years CI, group II), and of 24 subjects with subfoveal classic neovascularisation (mean age 675 years CI, group III). In 10 eyes of the group III, LDF parameters were measured before and after PDT.

Results: The acute increase in systemic blood pressure induced a similar increase in the mean perfusion pressure (PPm) for all groups. We did not record any significant changes of the LDF parameters of the groups I and II, but in group III the ChBF within the neovascular tissue increased significantly (DChBF=12.13.7 CI). In patients of group III treated by PDT (n=10), isometric exercise induced a significant increase in PPm (18.75.7 mmHg CI) before PDT, resulting in an increase of ChBF within the neovascular tissue (17.413.5 CI). However, after PDT, the PPm increase did not change significantly the LDF measurements.

Conclusions: Similarly to normal younger controls, the acute elevation of systemic blood pressure does not affect the ChBF in elderly subjects with non-exudative stages of AMD. In contrast, the ChBF within the subfoveal neovascular tissue increases significantly during isometric exercise. PDT partially restores the affected regulation of ChBF.

Choroidal circulation in health and disease

■ 3257

Choroidal blood flow reaction to handgrip stress in vaso-spastic subjects and its relevance to glaucoma

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Purpose: To assess the impact of vascular dysregulation on choroidal blood flow response to the hand-grip test and the relevance of this response to glaucoma.

Methods: Eighty healthy volunteers underwent a hand-grip test while choroidal blood flow was measured by means of laser Doppler flowmetry. Blood pressure, heart rate, and intraocular pressure (IOP) were monitored. Choroidal blood flow changes were compared between subjects with a positive history of cold hands and control subjects by means of analysis of variance. The relationship of the vascular response to the level of IOP at which progressive damage occurred was analyzed in 21 patients with primary open-angle glaucoma who had progressive damage despite normal or normalized IOP.

Results: Blood pressure and heart rate increased and IOP decreased in response to a hand-grip test. Healthy subjects with a positive history of cold hands (n: 36) demonstrated a decrease in choroidal blood flow during the hand-grip test, whereas control subjects (n: 44) demonstrated an inverse response pattern (P: 0.039). Glaucoma patients with a decrease of at least 10% in choroidal blood flow during the hand-grip test had lower IOP (14.67 ± 3.83 and 13.50 ± 2.59 mm Hg in the right and the left eyes, respectively) compared (P: 0.032) with those without such a decrease (16.54 ± 3.85 and 16.92 ± 2.95 mm Hg in the right and the left eyes, respectively).

Conclusions: A hand-grip test elicits a different blood flow response in subjects with vasospasm compared with control subjects. Damage by glaucoma in patients with a decrease in choroidal blood flow during a hand-grip test may progress at a relatively lower IOP.

■ 3261

Refractive surgery and aberrations

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Purpose: 1) To evaluate the changes induced by standard LASIK for myopia and hyperopia in both the aberrations of the corneal front surface and total aberrations. 2) To understand the causes for the increased spherical aberration after corneal ablation. 3) To assess the relationship between optical and visual degradation after LASIK.

Methods: Total aberrations were measured with Laser Ray Tracing (sequentially scanning the pupil and collecting the corresponding aerial images). Corneal aberrations were obtained from videokeratographic corneal elevation maps. Pupil size was 6.5 mm. Wave aberrations were described as Zernike polynomial expansions. Root Mean Square wavefront error (RMS) was used as optical quality metric. The wave aberration for the internal optics was computed as the difference of total and corneal wave aberrations. Other measurements included axial length, anterior chamber depth, visual acuity and contrast sensitivity. Computer simulations of the standard ablation algorithms were performed on the pre-operative corneas using clinical data of optical zone and correction and the aberration predictions compared to the actual data.

Results: 1) Total 3rd and higher order aberrations increased by a factor of 1.96 on average for myopic and 2.20 for hyperopic LASIK. 2) Spherical aberration increases toward positive values after hyperopic LASIK and toward negative values after myopic LASIK. Corneal spherical aberration increases more than the total spherical aberration in both cases. For the same absolute correction, corneal spherical aberration increases 3.86 times more after hyperopic than after myopic LASIK. 3) Computational predictions show that these changes are not inherent to the theoretical standard algorithm. 4) Changes in contrast sensitivity after LASIK are well accounted by changes in the optical modulation transfer function.

Conclusions: 1) Aberrometry (corneal and ocular) is a good tool to understand the optical changes induced by LASIK refractive surgery. 2) Optimization of ablation algorithms require a better understanding of laser-tissue interaction and biomechanical effects. None. Spanish Ministry of Science and Technology Grant BFM2002-02638.

■ 3262

Measurement of scattered light and its effects on visual performance

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Purpose: High levels of intraocular light scatter (LS) can affect the quality of the retinal image and can also give rise to discomfort glare. It is therefore of interest to measure the LS function of the eye and to relate its parameters to some measure of visual performance. Clinical methods assume a fixed pattern for the angular distribution of LS. This incorrect assumption has the great advantage of making it possible to estimate the amount of LS using a single ring, scatter source. The purpose of this study was twofold: First, we wanted to establish the errors involved when the measurement of LS employs a single ring. Second, we wanted to develop an appropriate measure of visual performance that reveals the effects of increased LS and aberrations in the eye.

Methods: The LS test used in this study employs either a single annulus or 5 discrete annuli of varying eccentricity. The full scatter test measures both the amount and the angular distribution of LS in the eye.

Results: 25 normal subjects were investigated (mean, sd: 31 years). Each subject carried out both the single annulus and the full scatter test, as well as assessment of wavefront aberrations. Visual performance was measured using a novel contrast acuity test developed to assess changes in visual performance in pilots post refractive surgery (Aviat. Space. Environ. Med. 74: 551-559, 2002). The results reveal large variation in the angular distribution of scattered light and the corresponding contrast acuity.

Conclusions: The single ring estimates of LS can be vastly different to the actual values when the angular distribution of LS deviates significantly from that assumed for the single annulus.

Visual assessment post refractive surgery

■ 3263

Night flying and visual performance post refractive surgery

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Purpose: Determine the impact of refractive surgery (PRK and LASIK) on the ability of helicopter pilots to fly at night and whether visual performance measures and/or subjective reports correlate with night flight performance.

Methods: 20 pilots (mean pre-op refraction -1.60 diopters) completed vision and flight performance testing prior to refractive surgery (11 PRK, 9 LASIK) and at least 1-month post-op; 12 completed 6-month post-op testing. Vision testing includes measurement of wavefront aberrations, high and low contrast (5%) acuity, contrast sensitivity, and night vision goggle (NVG) performance (VA & CSF). Flight testing includes airfield maneuvers, confined area operations and instrument flight under night unaided and NVG conditions in a flight simulator and in the Black Hawk aircraft.

Results: Initial analysis indicates measurable decreases in visual performance, especially ocular aberrations and contrast sensitivity, however flight performance in terms of flight maneuver score and variability either remains the same or improves. Subjects report and instructors rate improved performance and analysis of objective flight data shows no significant difference from baseline performance under night and NVG conditions. Only the one-week post-op subjects have decreases in flight performance that correlate to visual performance, most likely due to the effects of transient healing.

Conclusions: This small sample of pilots maintained flight performance levels after refractive surgery despite measurable reductions in visual performance. Pilot experience and increased field of view may counter the effects of aberrations and contrast changes. The implications of clinical and functional testing will be discussed.

■ 3264

Clinical correlation between higher order aberrations and visual performance after LASIK

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Purpose: To correlate higher order aberrations (HOA) with clinical measures of visual performance after LASIK.

Methods: Thirty-two subjects underwent standard LASIK to correct myopia and astigmatism. Best corrected visual acuity (BCVA), 5% low contrast visual acuity (LCVA), and night driving simulation (NDS) test performance were measured preoperatively and at six months postoperatively. The preoperative to postoperative differences in these measurements were compared to the corresponding differences in HOA measured with a Hartmann Shack wavefront device.

Results: Postoperative BCVA, 5% LCVA, and NDS test scores were significantly different from preoperative measurements ($p < 0.0008$). There were significant changes in Zernike terms $Z(3,-3)$, $Z(3,-1)$, $Z(4,0)$, and HOA RMS (3rd to 6th order). Changes in HOA RMS and $Z(4,0)$ were significantly correlated with changes in 5% LCVA ($R^2 = 0.213$ and 0.175 , $p = 0.0001$ and 0.0006 respectively). There were only weak correlations between changes in HOA and changes in BCVA and NDS test scores.

Conclusions: While wavefront technology appears to offer a more complete measure of the human optical system, the Zernike expansion may not be adequate to describe the relationship between HOA and changes in visual performance that are apparently due to changes in the optical system. Better metrics are needed to accurately assess the changes induced by refractive surgery and their effect on visual performance.

■ 3265

Conventional vs wavefront-guided treatments & effects on visual performance

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Purpose: Claims have been made that customised, corneal ablations enable the average refractive surgery patient to achieve 20/10 vision by reducing their higher-order, preoperative aberrations. Conventional ablations have been shown to increase aberrations. No significant difference in high contrast acuity (CA) for the average patient has, however, emerged from previous studies designed to compare the two techniques. The purpose of this investigation was to use a more sensitive, contrast acuity test to detect any differences in visual performance between the two techniques (Aviat. Space. Environ. Med. 74: 551-559, 2002).

Methods: A prospective randomised, double-masked pilot study was designed in which 10 patients (preoperative MSE $-4.6 \pm 0.2D$) underwent conventional LASIK in one eye (VISX Star S4) and wavefront-guided LASIK in the other (Wavescan v3.0). Monocular CA thresholds were measured under both photopic and mesopic conditions, before and 1 and 3 months after surgery.

Results: At 1 month, both techniques led to a reduction in visual performance, i.e., a significant increase in CA thresholds under both photopic and mesopic conditions. On average, there was no significant difference between eyes undergoing conventional or wavefront-guided ablation. The preliminary results obtained so far suggest that some patients exhibit lower CA thresholds following wavefront-guided ablation, probably as a result of smaller, surgically-induced aberrations.

Conclusions: This pilot study suggests that wavefront-guided ablations can be of some advantage, but may not be necessary for the average refractive surgery patient (i.e., low/medium myopes).

■ 3266

Epithelial and Biomechanical Changes in LASIK: Are they significant, and can we characterize them for improving the accuracy of custom ablation?

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Purpose: Purpose: To characterize the epithelial and biomechanical components affecting the accuracy of LASIK.

Methods: Methods: Routine LASIK was analyzed by 3D very high-frequency digital ultrasound (VHFU) and Orbscan pre- and (min) 3-month post-op. Epithelial and residual stromal thickness (RST) profile maps were produced. Anterior and posterior corneal best-fit sphere (BFS) were obtained by Orbscan. Bowman's surface curvature was calculated from anterior BFS and epithelial thickness profile. Back surface curvature change was used to dissociate the total anterior surface curvature change into epithelial and biomechanical components. Gradient optics and lens formulae were used to calculate epithelial and total corneal power. Corneal power change (CPC) from pre- to post-op was calculated with permutations removing biomechanical, epithelial changes or both.

Results: Results: Minimum RST observed was 262- μ m. Below a RST of 290- μ m, post-op back-surface curvature change correlated with RST ($R^2=0.5$). Mechanical changes accounted for a 15% decrease in intended decrease in central corneal power ($p<0.001$). Epithelial changes produced 5% decrease in intended corneal central flattening ($p<0.001$). Significant non-linear correlations were found between myopia level treated and epithelial ($p<0.001$) or biomechanical ($p=0.011$) power shift measured.

Conclusions: Conclusions: True customized ablation may require epithelial and biomechanical predictive modeling for achieving aberration free vision.

■ 3271

Infracyanine green (IFCG) assisted peeling of the internal limiting membrane (ILM) in macular surgery is safe for the inner retinal layers: a study of the ILM by electron microscopy.

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Purpose: The use of indocyanine green (ICG) facilitates the peeling of the internal limiting membrane of the retina (ILM) in cases of surgery for macular hole, pucker and macular oedema. Because of conflicting results about the possible toxic effects of ICG on the internal retinal layers, electron microscopy of the peeled membranes was performed.

Methods: ILM peeling is facilitated in our department by the use of infracyanine green (IFCG), dissolved in glucose 5%, which differs from the common used indocyanine green (ICG) by the fabrication method and by the solubility and osmolarity. 46 pieces of ILM, removed after IFCG assisted peeling, for diabetic or non-diabetic macular oedema, preretinal membranes, or macular holes were investigated by electron microscopy. The ILM fragments were fixed in glutaraldehyde, imbedded in epon and viewed with a Zeiss electron microscope.

Results: The green stained pieces showed to be thick layered basement membrane material corresponding to the internal limiting membrane of the retina and were found to be present in all cases. The ILM showed a smooth vitreal surface and a rough undulating internal retinal surface. In all cases this retinal surface did not show any presence of retinal tissue or membrane structures of Müller's cells.

Conclusions: Electron microscopy of removed internal limiting membranes (ILM) of the retina after infracyanine green (IFCG) assisted peeling, confirms the safety of the use of IFCG for the internal retinal layers in macular surgery.

■ 3272

Visual improvement in eyes with diabetic macula edema following vitrectomy

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Purpose: To test the hypothesis that ppv can reduce diabetic macular edema (DME) and improve visual acuity in eyes affected by DME

Methods: 30 consecutive eyes from 21 patients suffering from DME underwent ppV.

The posterior hyaloid had to be detached in all eyes. If present epiretinal membranes were peeled. One eye was excluded from further analysis after developing a retinal detachment leaving 29 eyes to be analysed. The eyes were monitored for a median interval of 14 months (1-60) months.

Results: The macula flattened in 22/29 (76%). Angiographically DME diminished or disappeared after 3 or more months in 16/17 eyes. Visual acuity improved by 2 to 6 lines in 17/29 (59%) eyes. Visual acuity improved by 1 line in 8/29 (28%) eyes and remained constant in 4/29 (14%) eyes. Visual acuity did not decrease in any eye following ppv.

Conclusions: Almost always ppV results in a slowly developing reduction and often complete disappearance of DME as evidenced by ophthalmoscopy and fluorescens-angiography, accompanied by a lasting at times large increase in visual acuity. For some patients this leads to a dramatic improvement in the quality of life.

■ 3273

Management of complicated retinal detachment using a new heavy silicone oil (Oxane Hd) as temporary internal tamponade

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Purpose: To assess the efficacy and safety of a new heavy silicone oil (HSO), with a density of 1.03 and a viscosity of 3000 centistokes, as temporary internal tamponade in selected cases of retinal detachments.

Methods: In this prospective study, thirty patients were operated on [inferior or posterior breaks: 17, proliferative vitreoretinopathy ? C1: 18, anterior proliferative vitreoretinopathy : 10] with a minimum of 12 months of follow-up. Heavy silicone oil was injected directly at the end of the operation in 3 cases, by air-HSO exchange in 1 case, and by perfluorocarbon liquid-HSO exchange in 26 cases. Heavy silicone oil was removed in all cases, after 6 weeks to 10 months.

Results: Anatomic success was achieved in 23/30 cases, in 8 cases after one or more reoperations due to redetachment. Elevated intraocular pressure persisted in two cases after HSO removal, controlled by medical therapy. Eight of sixteen phakic eyes developed posterior subcapsular cataract, which required extracapsular extraction in six cases. Emulsification of HSO was observed in two cases.

Conclusions: In view of the results of this prospective study, this new heavy silicone oil appears safe and effective in the management of complicated retinal detachments. We think it can improve the success rate of surgery in selected cases of retinal detachment, especially in inferior or posterior breaks, or when large inferior retinectomy is needed.

■ 3274

Complications after perfluorohexyloctane (F6H8) internal tamponade

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Purpose: To analyze complications after transient use of F6H8 as internal tamponade in patients with complicated retinal detachment.

Methods: F6H8 was applied in 23 eyes (23 patients, 10 female, 12 male, median age: 66 years (31-88y) with tractional/rhegmatogenous detachment in the lower quadrants (study period 2.5 years). Most eyes had been subject to major surgical interventions (median 2, range: 1-6). F6H8 was used for a median period of 24 days (range: 21-38). F6H8 tamponade was discontinued immediately 1x and after 7 days in another case. Median follow-up after F6H8 removal: 30 months (range: 6-42).

Results: Complications during F6H8 internal tamponade: dispersion 21/22 (95%), posterior segment pigmented "fluffy" proliferation 21/22 (95%), severe cellular debris on IOL/lens capsule/crystalline lens/iris/cornea 12/22 (55%), endothelial touch: 10/22 (45%), transient IOP increase: 9/22 (41%), severe PVR 2/22 (9%), phakogenic uveitis 1/22 (1/5 phakic eyes). Primary retinal reattachment was achieved in 19/22 eyes (86%). Complications after F6H8 removal: hypotony 20/22 (91%) (5/22 transient, 15/22 (68%) permanent). IOP decreased from 13.3 +/- 4.7 mm Hg preoperatively to 6.9 +/- 6.5 mm Hg finally. Hypotony was related to cyclitic or pericapsular membrane formation. Further complications: keratopathy 10/22 (45%) (4 transient, 6 permanent), cataract 5/5, retinal redetachment within 6 months 10/22 (45%), progressive capsular fibroses was a general finding in all pseudophakic eyes.

Conclusions: With regard to the high incidence of severe and functionally relevant complications further use of F6H8 has to be discussed critically.

■ 3275

Duration of macula off retinal detachments can affect visual outcome

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Purpose: To determine the association between the duration of macula off detachment and the visual outcome following corrective surgery.

Methods: Retrospective review of the medical records of patients who underwent surgery for macula off detachment over a 5 year period (April 1994- March 1999).

Results: There were 104 patients in the study. Patients with macula off retinal detachments wait a mean of 2.6 weeks (± 0.3 SE mean) before presentation and 1.8 weeks (± 0.2 SE mean) before surgery. Only 4.8% of patients presented the same day as the onset of their symptoms. The mean duration of detachment was 4.2 weeks (± 0.3 SE mean). 78% of patients achieved a postoperative improvement in visual acuity. 36.5% achieved functional visual success of 6/12 at 3 months. There was no significant difference in visual outcomes for patient undergoing internal vs external procedures ($p=0.188$). The preoperative visual acuity was the most significant predictor of post op visual acuity ($p<0.0005$). Less than 40% of macula off detachments > 6 weeks duration achieved a visual acuity of 6/12 or better compared with 68.2% of those of < 1 week duration.

Conclusions: Patients < 60 years old are more likely to achieve visual improvement despite the duration of the detachment. The best mean post operative vision was seen in patients with detachment < 1 week duration. Macula off detachments > 6 weeks duration have a significantly poorer visual prognosis. Awareness of this visual prognosis can assist in planning the timing of surgery to ensure an acceptable result.

■ 3276

Intravitreal injection of triamcinolone for retinal vein occlusion

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Purpose: To evaluate the clinical outcome of the intravitreal injection of triamcinolone acetate for treatment of macular edema due to retinal vein occlusion.

Methods: Ten patients with clinically significant macular edema due to retinal vein occlusion were enrolled in this prospective study. Six patients had branch retinal vein occlusion and 4 patients had central retinal vein occlusion. The patients were given a single intravitreal injection of triamcinolone acetate (4mg/0.1ml). Visual acuity, intraocular pressure (IOP) measurements and fundus fluorescein angiography (FFA) findings were recorded before the drug injection and throughout follow-up period.

Results: Five men and 5 women with a mean follow-up period of 5.6 \pm 2.5 months, showed a significant clinical resolution of macular edema and decrease of fluorescein leakage on FFA at an average time of 3 weeks. Visual acuity increased from 0.2 \pm 0.15 to a maximum of 0.5 \pm 0.2 during the follow-up period. IOP exceeded 21mm Hg in 4 patients. Two patients with an IOP over 30mm Hg received topical antiglaucomatous treatment and IOP control was achieved during the follow-up period. No other complications were noted. Two patients received reinjection of triamcinolone due to the recurrence of macular edema.

Conclusions: Despite the need of reinjection in order to achieve complete resolution of cystoid macular edema in patients with retinal vein occlusion, intravitreal injections of triamcinolone may be beneficial to improve visual acuity.

■ 3277

Pars plana vitrectomy with and without Radial Optic Neurotomy (RON) in the treatment of central retinal vein occlusion

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Purpose: Radial optic neurotomy has recently been described as a surgical management for central retinal vein occlusion. The purpose of this study is to compare the anatomical and functional result of surgical cases of vitrectomy with radial optic neurotomy and only vitrectomy in patients with central retinal vein occlusion.

Methods: A consecutive series of 20 patients who have undergone vitrectomy and radial optic neurotomy (10 patients) or only vitrectomy (10 patients) for central retinal vein occlusion were evaluated. All patients had at least 6 months of follow up, and mean follow up was 10 months. Pre and post operative visions, fluorescein angiograms and OCT foveal thickness were reviewed and analyzed.

Results: In radial optic neurotomy group 7 patients (%70) , in only vitrectomy group 1 patient (%10) had improved in visual acuity. In radial optic neurotomy group no patient, in only vitrectomy group 3 patients (%30) had deterioration in visual acuity. Nine patients (%90) in radial optic neurotomy group, 4 (%40) patients in only vitrectomy group had clinical improvement as determined by fundus examination, photography, and fluorescein angiography. In patients who underwent radial optic neurotomy, average foveal thickness was decreased from 610.2 μm to 236.9 μm within 3 months. In only vitrectomy group preoperative mean foveal thickness and postoperative mean foveal thickness at 3 month were 592.5 μm and 416.5 μm respectively.

Conclusions: Anatomical and functional outcomes obtained after radial optic neurotomy was superior than only vitrectomy. Early resolution of macular edema seems to be an important factor for the visual prognosis.

■ 3301

The T lymphocyte chemoattractant mig is highly expressed in vernal keratoconjunctivitis

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Purpose: To examine the expression of the 3 interferon-g (IFN-g)-inducible CXCR3-binding chemokines CXCL10 / IP-10 (IFN-g-inducible protein of 10 kDa), CXCL9 / Mig (monokine induced by IFN-g), and CXCL11 / I-TAC (IFN-inducible T-cell chemoattractant) in the conjunctiva of patients with vernal keratoconjunctivitis.

Methods: Conjunctival biopsy specimens from sixteen patients with active VKC, and nine control subjects were studied by immunohistochemical techniques. The phenotype of inflammatory cells expressing chemokines was examined by double immunohistochemistry.

Results: In the normal conjunctiva, very weak Mig immunoreactivity was observed on basal epithelial cells and on vascular endothelial cells in the upper substantia propria. There was no immunoreactivity for the other chemokines. In all VKC specimens, strong immunoreactivity for Mig was expressed by epithelial cells, vascular endothelial cells, and inflammatory mononuclear cells. Inflammatory mononuclear cells expressing IP-10, and I-TAC were noted in 10, and 9 specimens, respectively. The numbers of Mig+ inflammatory cells were significantly higher than the numbers of IP-10+, and I-TAC+ inflammatory cells ($p < 0.001$). Inflammatory cells expressing Mig were CD4+ T helper/inducer cells ($71.6 \pm 3.2\%$), CD8+ T cytotoxic/suppressor cells ($19.5 \pm 1.5\%$), and CD68+ monocytes/macrophages ($5.3 \pm 5\%$). All inflammatory cells expressing IP-10, and I-TAC were CD68+ monocytes/macrophages.

Conclusions: The CXC chemokine Mig is selectively and highly expressed in VKC suggesting a pathogenic role of the chemokine receptor CXCR3 and the ligand Mig in the recruitment of activated T lymphocytes.

■ 3302

Behcet's disease or cerebral lymphoma?

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Purpose: To present a case of Behcet's disease (BD), initially misdiagnosed as cerebral lymphoma to illustrate one of the pitfalls in this group of patients.

Methods: Case report

Results: A 37 yo woman was referred with a suspected diagnosis of cerebral lymphoma presenting with right hemiplegia, dysarthria and mental confusion. TC scan and MRI showed a mass in the left hypothalamus area. A brain biopsy showed a picture that was classified as a lymphoproliferative disorder. One year after the patient was referred to us for diplopia accompanied by a recurrence of the neurologic symptoms, fever and malaise. Inflammatory markers were high. A detailed history revealed that the patient, before the onset of the neurologic signs and symptoms, had presented recurrent oral and genital aftosis, arthralgias with episodes of erythema nodosum and fever of short duration. Recent history included an HSV infection. We made a clinical diagnosis of BD. Treatment with CsA was started with resolution of the systemic and ocular picture. 8 months later the patient spontaneously interrupted the treatment and showed a recurrence of diplopia, hemiparesis and aftosis. At the present time the pt is under treatment with CsA and oral prednisone and she is free of symptoms.

Conclusions: BD is a chronic recurrent inflammatory disease on an autoimmune basis with multisystem involvement. No specific diagnostic test is available, but the diagnosis is based on clinical grounds and linked to a detailed history, as shown by our case. Many microbial agents could trigger the abnormal inflammatory processes involved in the onset of BD. In spite of its poor distribution in CNS, CsA is effective to reduce the steroid dosage. The serious flare up of the disease after treatment interruption confirms its therapeutic effectiveness.

■ 3303

Systemic CD4+ T cell phenotype and activation status in intermediate uveitis

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Purpose: To investigate peripheral blood CD4+ T cell phenotype of patients with intermediate uveitis using chemokine receptor expression and cytokine production and to evaluate systemic CD4+ T cell activation.

Methods: Peripheral blood lymphocytes of 18 patients with idiopathic intermediate uveitis and 6 patients with presumed sarcoid intermediate uveitis were evaluated for CD4+ T cell expression of CD69, CCR4, CCR5, CXCR3 and the intracellular cytokines IFN γ , TNF α and interleukin (IL)-10 by flow cytometry, and for IL-2, IL-4, IL-5, IL-10, IFN γ and TNF α production following unstimulated and activated culture using the Cytokine Bead Array and compared with 23 healthy control subjects.

Results: CD3+CD4+CD69+ expression and CD3+CD4+TNF α + expression were greater in patients with idiopathic intermediate uveitis and presumed sarcoid intermediate uveitis than control subjects ($p=0.002$ and $p<0.05$, respectively). CD3+CD4+CD69 expression did not correlate with disease activity. There was no difference in chemokine receptor expression between groups. The ratios of IL-2/IL-5 and IFN γ /IL-10 in supernatants of activated peripheral blood lymphocyte cultures were significantly higher in patients with presumed sarcoid intermediate uveitis than control subjects.

Conclusions: This study implicates TNF α in the expression of intermediate uveitis, highlighting the potential role of anti-TNF therapies for this disease. Idiopathic intermediate uveitis was accompanied by systemic CD4+ T cell activation but in this cohort CD69 was not a useful surrogate marker of uveitis activity. Studies of Th1/Th2 cytokine ratios suggested predominant Th1 activity in sarcoid intermediate uveitis even when the systemic disease was clinically quiescent.

■ 3304

Vitreous involvement in Fuchs' uveitis at the origin of diagnostic delay with potential deleterious consequences

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Purpose: Vitreous involvement in Fuchs' uveitis is a frequent sign of which clinicians are not sufficiently aware and which therefore often leads to a diagnostic delay. The aim of the study was to analyse the proportion of cases with vitreous involvement, the proportion of other signs, the proportion of undiagnosed Fuchs cases, the mean delay of diagnosis and the initial erroneous diagnosis.

Methods: Patients seen at the Uveitis Clinic at la Source Eye Centre, Lausanne, Switzerland (62/742 cases; 8.3%) and in the Ocular Immunology Unit, SM Nuova Hospital, Reggio Emilia, Italy (17/221 cases; 7.6%) with the diagnosis of Fuchs' uveitis were analysed. The proportion of cases with vitritis and the other parameters cited above were recorded.

Results: The most frequent sign found was vitritis in 80/82(97.5%) eyes, followed by stellate keratic precipitates in 73 (89%), posterior subcapsular opacities or cataract in 47 (55%), heterochromia in 33(42.6%), Koeppel nodules in 12(14%) eyes. In 61/79 cases (77%) diagnosis was delayed for a mean of 3.67+/-4.34 years. The original erroneous diagnosis was intermediate uveitis in 37/61 cases (60%) and posterior or panuveitis in 13 (22%) of cases, for which the presence vitritis was probably responsible. 27 patients were treated with systemic steroids and 7 patients with additional immunosuppressive drugs. In one case this therapy was nearly lethal because of a subsequent missed Toxoplasma Gondii primoinfection.

Conclusions: Fuchs' uveitis is a largely undiagnosed uveitis probably because the predominant vitreous involvement is ignored by many ophthalmologists. This situation needs to be publicised in order to avoid possibly severe deleterious effects following erroneous systemic corticosteroid and/or immunosuppressive therapy.

■ 3305

Importance of early diagnosis and adequate treatment of Vogt-Koyanagi-Harada disease on preventing evolution toward recurrences and complications

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Purpose: We compared Vogt-Koyanagi-Harada disease (VKH) patients for whom the diagnosis was reached within 3 weeks of the onset of symptoms and the adequate treatment started within this interval to a group of patients for whom the diagnosis was reached more than 3 weeks after the onset of symptoms and/or who did not receive adequate treatment at this time.

Methods: Retrospective study at La Source Eye Center in Lausanne from 1.1995 to 4.2003. Evaluation of the delay from symptoms to diagnosis and instauration of adequate treatment in all VKH patients and comparison of the clinical evolution, treatment duration, complication rate of the early versus late group.

Results: 16 patients with VKH were seen at La Source Eye Center in Lausanne. 5 patients (31%) were diagnosed and treated within 3 weeks (early group), in the other 11 cases (69%) the diagnosis was reached after 3 weeks and/or the adequate treatment was not introduced within this time (late group). The mean delay from symptoms to diagnosis was of 16 ± 2.2 days in the early group and 3.2 ± 4.2 years in the late one. In all early group patients, inflammation was controlled after treatment and 3 patients with a follow-up without recurrence of at least 7 years were considered as healed. No severe complications occurred in the early group but 5/11 patients in the late group developed complications (cataract, glaucoma, neovascular membrane).

Conclusions: By showing that the proportion of inactive cases is significantly more frequent, the duration of disease is shorter and the complication rate is lower in the early group, this study confirms that it is essential to rapidly reach the diagnosis of VKH and treat the patients vigorously without delay.

■ 3306

Pauci-immune Retinal Vasculitis

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Purpose: We describe a case series of pauci-immune retinal vasculitis. The literature was reviewed in relation to classification, pathophysiology and associated systemic features. The significance and diagnostic value of positive anti-neutrophil cytoplasmic antibodies is discussed as well as the importance of early recognition and treatment in this spectrum of disease entities.

Methods: Three cases of P-ANCA positive pauci-immune retinal vasculitis are presented. The first case is that of Microscopic Polyangiitis with systemic features including fatigue, arthralgia, weight loss, bilateral retinal vasculitis, mononeuritis multiplex, finger-pulp haemorrhages and loin pain with haematuria. A renal biopsy demonstrated an interstitial lymphocytic infiltrate. Two further cases are described with lesser systemic features.

Results: Positive P-ANCA assays should always raise the suspicion of an underlying systemic vasculitis. We discuss the assay results of our cases in relation to the International Consensus Statement on testing and reporting of ANCA. ANCA testing where specificities are not positive for PR3 or MPO demonstrate variable and often low antibody levels, with usually no clear correlation for disease activity. ANCA titres and positivity decline with immunosuppressive treatment and disease quiescence.

Conclusions: We want to draw attention to the small vessel vasculitides. We discuss the importance of the ANCA assay, and describe the associated features of these conditions, so that they can be recognised earlier and morbidity prevented.

■ 3307

Levels of pro-inflammatory cytokines in lacrimal fluid in patients with diabetic retinopathy

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Purpose: To study of levels of pro-inflammatory cytokines (interleukins (IL) 1 alpha and beta, 8, tumor necrosis factor alpha (TNF alpha) in lacrimal fluid and blood in patients with proliferative diabetic retinopathy.

Methods: The study was performed in 12 patients with proliferative diabetic retinopathy. Levels of cytokines in lacrimal fluid and serum were determined by ELISA.

Results: It was established that concentration of IL 1 alpha in lacrimal fluid was less than 50 pg/ml and corresponded with its level in blood. Larimal concentration of IL 1 beta increased up to 130-150 pg/ml, though its level in blood was normal. IL 8 content in lacrimal fluid was the same. Lacrimal concentration of TNF alpha exceeded in 2-3 times a level in blood and at some patients achieved up to 600 - 900 pg/ml. Significant changes after treatment in the contents of cytokines in plasma of the patients with proliferative diabetic retinopathy were not revealed. However the appreciable increase of concentration of IL - 1 alpha, IL - 1 beta in lacrimal liquid was determined, and it was especial of IL-8 and TNF alpha. So some patient's level of IL-8 in lacrimal fluid achieved up to 600-800 pg/ml, and TNF alpha - 1800 pg/ml after treatment, that in 20 times surpassed its level before treatment, though in blood the significant difference did not observe.

Conclusions: We regard that elevation of pro-inflammatory cytokines is positive fact. It supports activation of eye's local immune system in patients with diabetic retinopathy. Cytokine secretion may play an important role in retinal neovascularization.

■ 3311

Epidemiology and demographs of endophthalmitis in Europe

BARRY P

■ 3312

Early diagnosis of endophthalmitis with PCR and new microbiological real time testing

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Purpose: To determine whether the PCR decreases the time devoted to the diagnosis and identification of the pathogens in eyes developing endophthalmitis versus classical microbiological test.

Methods: Thirty-four samples, 24 aqueous (HS) and 6 vitreous samples (VS), were collected from 23 patients showing clinical signs of endophthalmitis. Ocular samples were taken for microbiological study, including classical cultures, gram and giemsa stains and PCR. ITS-5.8S rDNA and 16S rDNA were amplified by PCR to detect fungal and bacterial DNA. Amplified DNA were sequenced and aligned against sequences in GenBank at National Institutes of Health to identify the pathogen.

Results: The results were PCR positive for fungal or bacterial primers in 91% of the AS and in 100 % of the VS from the patients with infectious endophthalmitis.

Microbiological methods could detect the pathogen in 50% of AS and 83% of VS. The pathogen agent of the 19 patients was identified by microbiological or molecular methods. The PCR result was obtained in 5 hours after the sample was taken and the molecular identification 2 to 3 days afterwards. Cultures needed an average of 1 to 13 days to grow. Gram and giemsa stains were the quickest method to detect the pathogen but the sensitivity was only 23% in AS and 66.6% in VS.

Conclusions: Microbiological and molecular methods showed similar efficiency in the case of acute endophthalmitis, however molecular methods had a higher rate of positive identification of the pathogen in delayed endophthalmitis.

■ 3313

Emerging therapies in the medical treatment of endophthalmitis

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Purpose: Endophthalmitis is a rare but catastrophic complication of intraocular surgery and penetrating injuries. Emerging resistance of organisms to standard antibiotic therapy has forced clinicians to continually evaluate the best intraocular antibiotics for the treatment of endophthalmitis. This study was designed to investigate the efficacy of intravitreal levofloxacin in an experimental model of endophthalmitis.

Methods: 75 New Zealand rabbits received injections of either *Pseudomonas aeruginosa* (10 CFU/50 ml), *Staphylococcus aureus* (40 CFU/50 ml) or *Staphylococcus epidermidis* (30.103 CFU/50ml) into the vitreous cavity of the right eye. Three subgroups were formed; those treated with 100 ml levofloxacin 0.5% (10 rabbits), those treated with 100 ml of vancomycin 1% and ceftazidime 2% (10 rabbits) and the untreated control group (5 rabbits). The Peyman classification was used to assess the severity of endophthalmitis. Eye examinations were performed until day 8 from inoculation. The microbiological status of the vitreous samples was tested at day 2, 5 and 8.

Results: 60% (4/10) of the rabbits treated with levofloxacin in the *Pseudomonas* group did not develop severe endophthalmitis, versus 80% of the rabbits treated with the combined therapy. In the *S. aureus* group both treatments (combined and monotherapy) showed the same result, 90% did not develop severe endophthalmitis. In the last group (*S. epidermidis*) the result was 80-100% monotherapy - combined treatment.

Conclusions: This low dose of levofloxacin (0.5%) was considered as effective in treating Gram-positive (*Staphylococci*) infections as a combination of vancomycin and ceftazidime. However the combined therapy was more effective in combating a Gram-negative (*Pseudomonas*) infection.

■ 3314

New approaches to the surgical treatment of endophthalmitis

SANCHEZ DE FIGUEROA M

Update in the diagnosis and treatment of endophthalmitis

■ 3315

Summary and conclusions

ALIO y SANZ JL

■ 3341

Disposable Barriers for Ophthalmic Contact Devices

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Purpose: The problems of cross-infection associated with contact ophthalmic devices are addressed by the proposed design fabrication and clinical evaluation of a unique, inexpensive, sterile barrier system. The problems are of particular importance given the recommendations from the UK Department of Health with respect to the potential of CJD transmission via ophthalmic devices.

Methods: It is generally accepted that commonly used ophthalmic contact devices pose a problem for the potential risk of cross-infection. Established clinical standards of sterilisation, most commonly wiping with an alcohol swab, are inadequate and ineffective. There are several examples of contact devices that are susceptible to these problems, including: the Goldman tonometer head, Gonioscope lenses and A-Scan probes. None of the currently available solutions (for example disposable tonometer heads and silicone sheaths) are popular, efficient or cost effective. Additionally they are only concerned with tonometry. Our approach comprises a disposable, optically transparent, laminate barrier consisting of two thin layers: one a barrier film that contacts the eye and the other a hydrogel adhesive that enables the barrier film to be readily and reversibly attached to the tonometer head. Polyurethanes, which are available in an extensive range of structures appear to offer best all round solution to the barrier problem; laminates for preliminary clinical design evaluation are now being produced from a range of polyurethane barrier films coated with hydrogel adhesives. To enable a useable device to be fabricated barrier and adhesive need to be: (a) assembled with readily removable front and back surface coverings (b) reproducibly cut into device shape (b) packaged and sterilised. Prototype fabrication and clinical evaluation are now in progress with polyurethane films that meet the specification that has evolved from initial design studies, i.e.: (a) thickness (<25 μ uncoated, 250 μ coated; weight (<0.01g, i.e. 0.1g coated) (b) mechanical properties (Emod < 50 Mpa; Eb > 250%) (c) optical transmission characteristics (T_{wet} > 40%) (d) "wettability" and surface energy (GAMMA p > 4mn/m).

■ 3342

Evolution, Characterization and Modification of Novel Contact Lens

Materials: PVA and Fluorosilicone and Hydrogels

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A radical shift in polymer synthesis and processing has enabled the development of novel hydrogel chemistries with a range of interesting and unique properties. Both materials are characterized by very low protein deposition levels. An increased understanding of tear film and ocular physiology coupled with a wide variety of intended wearer regimens has been a catalyst for further improvement in these novel contact lens materials. Such modifications to these ophthalmologic biomaterials have not only provided the basis for improved clinical performance but also increased their potential for use in biomedical fields other than vision correction.

■ 3343

Enhancing corneal epithelial cell response to surfaces

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Purpose: To investigate methodologies for enhancing the corneal epithelial cell response to various modified surfaces.

Methods: Base hydrogel materials were surface modified by direct chemical grafting of tethered extracellular matrix proteins and peptides. Modified materials were tested in primary rabbit cell and organ culture for cell growth rate. The degree and strength of cell attachment to the modified surfaces was then determined using immunochemistry and jet impingement.

Results: Tethered attachment of proteins/peptides significantly enhanced epithelial cell growth on surfaces as compared to solution coating (> 80% confluence within 10 days versus < 25% confluence over 15 days). Specifically, tethered laminin, a fibronectin/laminin combination, and FAP (peptide) modified surfaces gave the most significant increase in epithelial cell response. Additionally, passively adsorbed proteins from the media onto the surface did not interfere with the function of the tether-proteins in enabling epithelial cell attachment to the tether-modified hydrogel surfaces. And jet impingement showed that a mean shear force of 479 dynes/cm² was not sufficient to break the adhesive bonds between the corneal epithelial cells and laminin or FAP surface-modified hydrogels.

Conclusions: It is possible to significantly enhance the corneal epithelial cell response to hydrogel surfaces by tethering specific extracellular matrix proteins or peptides onto the surfaces.

■ 3344

Polymer membranes for RPE transplantation

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Purpose: The design and modification of polymer membrane materials required to provide a monolayer of RPE cells suitable for transplantation.

Methods: Polymer materials: Two commercially available polyurethanes were used; Pellethane and Tecoflex. These were cast into thin films or electrostatically spun to form porous films. Surface modification: The materials were air plasma treated using a commercially available capacitor plate plasma system (Emitech K1050 X Plasma Asher). Immediately after plasma treatment the samples were placed in sterile distilled water and stored for at least 24 hours. Surface analysis: The effect of the plasma treatment was analysed using dynamic contact angle measurements (Camtel Ltd. CDCA 100 contact angle analyser). Cell culture: Treated and untreated samples were incubated with an established but non-immortalised human RPE cell line (ARPE-19) for 1, 2, 3 and 7 days. After each time period the cells were stained with phalloidin and propidium iodide and evaluated using fluorescent microscopy.

Results: Dynamic contact angle analysis demonstrated that the plasma treatment increased the hydrophilicity of the materials. For Pellethane the advancing angle decreased from 93° to 61.5° and the receding angle decreased from 80° to 34.5°. The untreated Pellethane and Tecoflex films did not support RPE adhesion and proliferation whereas on the treated surfaces cell cultures reached confluency by day 3. Staining revealed that these cells had a characteristic mosaic monolayer appearance.

Conclusions: Polyurethane substrates have suitable mechanical properties for this application and can be surface modified to enhance RPE monolayer formation. (Acknowledgements: Dunhill Medical Trust)

■ 3345

DNA Genotyping demonstrates that cultured limbal corneal allografts are short-lived, but still promote long-term corneal healing

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Purpose: Limbal stem cell deficiencies destabilize the corneal surface and cause severe visual impairment. For patients with bilateral disease, allogeneic donors offer the only source of replacement tissue. We have begun investigating whether sheets of cultured allogeneic limbal epithelium can repair limbal defects and whether they survive on the corneal surface.

Methods: Epithelial cells were released from cadaveric limbal tissue by enzyme digestion. Sheets of multilayered cells were grown and released from tissue culture plastic with the enzyme dispase, and applied to patients' debrided corneas. Amniotic membrane, functioning as a biological bandage, was then applied. Samples of all the tissues used on the patients' eyes were collected and subjected to PCR Short Tandem Repeat DNA genotyping to monitor the fate of the cultured cells.

Results: 10 eyes have been grafted in 3 time-cohorts ranging from 4 months to 42 months. Central corneal buttons, from 3 patients in the earlier time cohorts, were subsequently removed during penetrating keratoplasty to restore vision. Immunohistochemistry revealed well-developed corneal epithelia in all 3 cases. PCR STR genotyping of these tissues, and surface impressions from all other patients, showed that after 3 months to corneal surface no longer contained cells from the cultured limbal sheets but cells with only the patients' own genotypes. Cultured cell genotypes could be demonstrated at 8-10 weeks.

Conclusions: Cultured limbal epithelial allografts were able to contribute to the repair of corneal surfaces which previously demonstrated prolonged and complete limbal deficiencies. The DNA genotype results suggest that the application of the cultured cells contributed to the restoration of the patients' own limbal function.

■ 3346

Novel biomimetic materials for ophthalmic applicationsLLOYD A, ANDREWS CS, BARRAUD JE, HANLON GW
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Purpose: To prepare and evaluation of a range of novel biomimetic phosphorylcholine-based polymers for ophthalmic applications

Methods: A range of phosphorylcholine-based hydrogels were prepared by free-radical polymerisation. The composition of the materials was modified through the addition of various hydrophobic monomers. The properties of the materials were examined with respect to mechanical strength, refractive index and surface wettability. The biocompatibility of these materials was assessed with respect to cytotoxicity, lens epithelial cell adhesion and proliferation, inflammatory cell adhesion and activation and bacterial adhesion.

Results: The materials were shown to have a range of physicochemical and mechanical properties. The adhesion of mammalian and bacterial cells was shown to be dependent on the composition of the polymers with improved biological properties with increasing phosphorylcholine content.

Conclusions: A range of novel biomimetic polymers with controlled physicochemical, mechanical and biological properties have been developed which may have application in the fabrication of intraocular lenses, contact lenses and other ocular implants.

■ 3351

Diagnostic accuracy of the Retinal Thickness Analyzer: differentiation between normal eyes and eyes with glaucoma or macular pathologies

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Purpose: To determine diagnostic values of the Retinal Thickness Analyzer (RTA) for eyes with manifest glaucoma or macular pathologies.

Methods: We examined 133 eyes of 71 patients from three clinically well-defined groups: 46 eyes with typical glaucomatous visual field or optic disc changes, 34 with various forms of established macular diseases and 53 normal eyes. All data were collected by the same person (P. M.). RTA experts from the manufacturer evaluated the analysis results from all the examinations in a completely blinded fashion. We then compared the expert's interpretation with the actual diagnosis to determine sensitivity, specificity, positive (PPV) and negative predictive values (NPV).

Results: From the 133 examinations 24 (18%) were indeterminate, with the majority in the glaucoma group (30%) and in the macula group (21%), compared to 6% in the normal group. When censoring for indeterminate results, the following test properties were calculated: For the detection of glaucoma: sensitivity 81%, specificity 57%, PPV 44% and NPV 88%; for the detection of macular diseases: sensitivity 59%, specificity 96%, PPV 84% and NPV 88%.

Conclusions: Conclusion: The Retinal Thickness Analyzer might be a helpful tool for diagnostic decisions, particularly when used in a clinical context with high pre-test probabilities. However, since many scan results, particularly in patients with glaucoma, were judged indeterminate, the RTA still needs further improvement to be useful in a non-research setting.

■ 3352

In-vivo visualisation of lipofuscin in the parapapillary area in normals, ocular hypertensives, and primary open angle glaucomas

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Purpose: Lipofuscin accumulation in the atrophic parapapillary zones of glaucoma eyes was described in 1996 by Kubota. We investigated the lipofuscin-induced increased fundus autofluorescence in-vivo.

Methods: Controlled cross sectional prospective analysis of 79 consecutive eyes (15 normals, 21 eyes with OHT and 38 with primary open angle glaucoma). The confocal scanning laser ophthalmoscope (HRA, Heidelberg Retina Angiograph) was used after excitation with argon green laser (488 nm) to detect parapapillary autofluorescence in a spectrum above 500 nm. Size, extension of the parapapillary autofluorescent area and its mean distance to the optic nerve head were measured using the HRA-standard-software.

Results: Very small autofluorescent areas were found in normal vital discs in the parapapillary atrophic area ($0.04 \pm 0.05 \text{ mm}^2$) in contrast to OHT ($0.11 \pm 0.14 \text{ mm}^2$) or POAG ($0.42 \pm 0.96 \text{ mm}^2$, $p < 0.03$). Lipofuscin was found more distant from the optic disc in POAG ($0.36 \pm 0.38 \text{ mm}$) than in OHT ($0.14 \pm 0.16 \text{ mm}^2$) or normals ($0.12 \pm 0.10 \text{ mm}$, $p < 0.01$).

Conclusions: The distance of lipofuscin from the optic nerve head is quite similar in normals and OHT. But the size, width and length are higher in OHT than in normals. Manifest glaucomas have more distant and larger lipofuscin areas in contrast to OHT or normals. The parapapillary fundus autofluorescence may help the ophthalmologist to detect "active" autofluorescent areas in the parapapillary atrophic zone in OHT or POAG.

■ 3353

In-vivo measurement of lipofuscin as a degenerative product in the parapapillary atrophic zone of optic discs with and without glaucomatous atrophy

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*Department of Ophthalmology, Erlangen***Purpose:** To assess the level of autofluorescence (lipofuscin) of atrophic parapapillary zones in different stages of glaucomatous optic disc atrophy.**Methods:** Controlled cross sectional prospective analysis of 79 consecutive eyes (41 eyes with vital optic discs, 38 with primary open angle glaucoma). Eyes with retinal diseases or retinal pigment epithelial pathologies were excluded. The confocal scanning laser ophthalmoscope (HRA, Heidelberg Retina Angiograph) was used after excitation with argon green laser (488 nm) to detect parapapillary autofluorescence in a spectrum above 500 nm. Size, extension of the parapapillary autofluorescent area and its mean distance to the optic disc were measured using the HRA-standard-software. Additional optic nerve head photographs taken with the 15° Zeiss telecentric fundus camera were examined from two experienced ophthalmologists to determine the stage of glaucomatous optic disc atrophy (stages 0 to 4).**Results:** Small autofluorescent areas were found in vital discs (optic nerve glaucoma stage 0) in the parapapillary atrophic area ($0.08 \pm 0.12 \text{ mm}^2$) in contrast to glaucomatous discs in stage 1 ($0.24 \pm 0.26 \text{ mm}^2$) and stages 2, 3 and 4 ($0.59 \pm 1.29 \text{ mm}^2$, logistic regression analysis $r=0.71$; $p=0.029$). The circular length of lipofuscin correlated borderline with the stage of the glaucomatous disc atrophy (higher glaucoma stages: $r=0.82$; $p=0.09$).**Conclusions:** The lipofuscin accumulation is correlated with the stage of progression of glaucoma and the stage of optic disc atrophy. The detection of active parapapillary autofluorescent areas especially in POAG may offer the ophthalmologist a promising option for diagnosis of early glaucoma.

■ 3354

Techniques of choice in glaucoma surgery

BARRAQUER J

*Instituto Barraquer, Barcelona***Purpose:** Evaluation and update of different procedures for surgical management of glaucoma**Methods:** Goniotomy, YAG laser iridotomies, trabeculectomy, deep non-perforating sclerectomy and diasclear cyclophotocoagulation with Nd:YAG laser are considered and important technical details are briefly illustrated.**Results:** Clinical results from the author's personal casuistics are briefly referred and illustrated.**Conclusions:** Goniotomy is still the primary intervention of choice in congenital glaucoma. YAG laser iridotomy is curative in early angle closure glaucoma and may be used prophylactically in the fellow eye. Trabeculectomy and its modern modification, deep non-perforating sclerectomy are of choice in primary open-angle glaucoma when medical treatment fails. Diascleral photocoagulation with Nd:YAG laser is the last recourse in refractory glaucoma.

■ 3355

Randomised controlled trial comparing a new PMMA implant to the collagen implant in deep sclerectomy

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Purpose: To compare the intraocular pressure lowering effect and the safety of a new cross-shaped, non-absorbable Polymethyl-Methacrylate (PMMA) implant with the collagen implant during deep sclerectomy procedure.

Methods: 60 patients with medically uncontrolled primary and secondary open angle glaucoma were randomly assigned to receive either a PMMA implant (30 eyes) or the collagen implant (30 eyes). The patients were examined before and after the operation at 1 day, 1, 2, 3 weeks and 1, 2, 3, 6, 9 and 12 months. At each visit, the following examinations were performed: slit lamp, tonometry, visual acuity and visual field

Results: The mean follow-up period was 11.5 (SD 2.8) months. The mean preoperative IOP was 21.9 (SD 8.9) for the PMMA and 20.9 (SD 7.6) for the collagen implant. The mean post-operative IOP 7.6 (SD 4.7) mm Hg (PMMA) vs. 5.8 (SD 5.8) mm Hg (C) at day 1, 13.9 (SD 3.0) mm Hg (PMMA) vs. 15.2 (SD 2.3) mm Hg (C) at month 9, and 14.1 (SD 3.5) mm Hg (PMMA) vs. 14.9 (SD 5.1) mm Hg (C) at month 12. Complete success rate at 12 months, defined as an IOP lower than 21 without medication, was 54% (PMMA) vs. 59% (Collagen). Qualified success rate, defined as IOP below 21 with or without medication was at 96% (PMMA) vs. 100% (C). There were no significant differences between the two groups in postoperative and transient complications.

Conclusions: Deep sclerectomy with the new cross-shaped PMMA implant provides comparable results in efficiency and safety to the collagen implant.

■ 3356

Intravitreal Triamcinolone Acetonide as Treatment of Neovascular Glaucoma

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Purpose: To evaluate safety and efficacy of intravitreal triamcinolone for treatment of neovascular glaucoma.

Methods: The clinical interventional comparative case series study included 18 patients (18 eyes) with neovascular glaucoma due to proliferative diabetic retinopathy (n=11) or ischemic central retinal vein occlusion (n=7). All patients received an intravitreal injection of 25 mg of triamcinolone acetonide. The total study population was divided into a study group consisting of 5 eyes in which the intravitreal injection was not combined with a retinal destructive procedure; and into a control group consisting of 13 eyes in which the intravitreal triamcinolone injection was combined with a peripheral circumferential transscleral retinal cryocoagulation. Mean follow-up was 5.4 ± 4.2 months. Main outcome measures were visual acuity, intraocular pressure, and degree of iris neovascularization.

Results: In the total study population, intraocular pressure was significantly (p=0.003) reduced from 35.9 ± 12.7 mm Hg preoperatively to 20.6 ± 11.0 mm Hg postoperatively. Degree of rubeosis iridis decreased significantly (p=0.02) from 2.7 ± 1.2 relative units to 1.3 ± 1.2 relative units. All patients demonstrated a regression of iris neovascularization. Study group and control group did not vary significantly in the decrease in iris neovascularization (p=0.85), increase in visual acuity (p=0.70), best postoperative visual acuity (p=0.78), reduction in intraocular pressure (p=0.85), and intraocular pressure at the end of follow-up (p=0.44).

Conclusions: Intravitreal triamcinolone acetonide in combination with, or without, a transscleral retinal cryocoagulation may be an additional treatment option for neovascular glaucoma.

■ 3357

Principles of a new glaucoma implant for ocular drug delivery*SCHRAGE NF (1, 2), FRENTZ M (1), GRIES T (3)**(1) University Clinic of Ophthalmology, Aachen, (2) Aachen Center of Technologytransfer in Ophthalmology, Aachen, (3) RWTH- Institute for Textile technic, Aachen*

Purpose: Intraocular drug application needs developements of slow releasing systems and ways to insert the drug into the eye. Many proposals of extraocular drugs and galenics have been made but still intraocularly mostly the vitrasert is the only used application.

Methods: With a small extraocular depot and an implanted drainage tube we are able to pump against the intraocular pressure by means of an osmolar pump. The combination of those two principles leads us to intraocular drug delivery by means of a special new device.

Results: With a simulation of drug release by means of fuoresceine we are able to fill height of considerable height by means of osmotic pressure and transports through a wick. The transport itself is slow and of first order. Thereby we can produce dependent on the tubings and the wicks systems with drug release from 4 days up to several months. Estimations of those were given by means of a simulator system in a cuvette system mounted in a photometer.

Conclusions: A combination of textile structures used as a wick and the glaucoma implant developed by our group will lead to a intraocular drug delivery system in near future.

■ 3361

The importance of epidemiology in the effort of reducing world blindness due to cataract

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Purpose: To review epidemiologic contributions to our understanding of the causes of cataract.

Methods: In epidemiologic studies of non-communicable diseases such as cataract, six factors are considered in the argument to prove causality of a risk factor for a particular health outcome: 1) consistency of results, 2) strength of the association, 3) dose response relationship, 4) temporality, 5) biological plausibility, and 6) specificity.

Results: Case control and cohort studies conducted in many countries have confirmed the following risk factors for cataract prevalence and incidence: ocular UV-B exposure, low antioxidant intake, certain medications, cigarette smoking, diabetes and gout, and family history (genetics). All six major epidemiologic criteria for causality have been satisfied and this association has been confirmed in laboratory experiments. Simple ecologic study designs have yielded a wealth of information to quantify the association of ambient levels of UV-B and cataract.

Conclusions: Epidemiology will continue to advance our understanding of the causes of cataract with the ultimate aim of reducing blindness due to cataract worldwide. It has been clearly shown that risk factors for cataract vary by cataract type, thus it is important to distinguish cataract type in the design and analysis of epidemiologic studies. Investigators need to work together to identify tools that can be used across populations to allow comparison of results between studies.

■ 3362

Risk factors for lens opacification in Icelanders 50 years and older. Reykjavik Eye Study

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Purpose: To examine risk factors for cortical and nuclear lens opacification in citizens of Reykjavik.

Methods: 1045 persons, 583 females and 462 males age 50 years and older were randomly sampled from the national population census for eye examination including slit-lamp microscopy and Scheimpflug photography. They also answered a 26-item questionnaire. The photographs were used for the diagnosis of lens opacification. The data was analysed using a logistic regression model.

Results: An increased risk was found with ageing for nuclear (OR=1.23; 95% CI 1.19-1.26; $p<.001$) and grade II & III cortical lens opacification (OR=1.19; 95% CI 1.16-1.22; $p<.001$). Cigarette-smoking more than 20 pack-years increased the risk for nuclear lens opacification (OR=2.52; 95% CI 1.52-4.13; $p<.001$) as did pipe- or cigar-smoking (OR=2.48; 95% CI 1.20-5.12; $p<.05$). Spending more than 4 hours/day outside on weekdays in their 20's - 30's and 40's and 50's had increased risk of grade II & III cortical cataract, OR=2.80 (95% CI 1.01-7.80; $p<.05$) and 2.91 (1.13-9.62; $p<.05$) respectively. Systemic corticosteroid use was a risk factor for cortical cataract (OR=3.70; 95% CI 1.43-9.56; $p<.05$). Moderate alcohol use decreased the risk of cortical cataract grade I (OR=0.56; 95% CI 0.35-0.91) and possibly also for grade II & III.

Conclusions: Ageing is the main risk factor for both cortical and nuclear lens opacification. Important modifiable risk factors are smoking for nuclear- and systemic corticosteroid use and outdoor exposure for cortical lens opacification.

■ 3363

The prevalence, incidence and progression of cataract using the WHO cataract classification system

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Purpose: To provide prevalence, 5-year's incidence and progression rates of age-related lens opacities in the Icelandic population > 50 years of age using the WHO cataract classification system (WHO system) and to show the reproducibility and the corresponding visual acuity in this system.

Methods: Of 1045 subjects who participated in the Reykjavik Eye Study (RES) in 1996, 846 or 88.2% of the survivors were reexamined 5 years later in 2001. Changes in the crystalline lens were examined and photographed under maximal pupillary dilation. Classification of nuclear (N), cortical (C) and posterior subcapsular cataract (S) was determined from photographed images using the WHO system. Visual acuity (VA) was tested on a Snellen chart at 6 meters.

Results: Prevalence in 2001, five year's incidence and progression were 16.1%, 8.1% and 41.7% for N, 29.7%, 17.6% and 26.2% for C and 4.7%, 4.5% and 33.3% for S, respectively. Reproducibility of judgment by the same examiner was over 90% and agreement between two examiners was about 80%. The mean VA of eyes with pure N, pure C and pure S were 20/26.7, 20/26.3 and 20/37, respectively. The mean VA with and without central cortical opacity were 20/32.8 and 20/22.

Conclusions: Applying the WHO system, although prevalence and incidence were highest in C, the five year's progression rate was highest in N for the Icelandic population. The relationship between the different types and severity of cataract, according with the WHO system, and VA in the Icelandic population was established. We confirmed the usefulness of the WHO system as a new diagnostic standard for determining lens opacity with reliable reproducibility.

■ 3364

The fact about environmental UVR as a cause of cataract

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Purpose: To evaluate the available evidence for ultraviolet radiation (UVR) as an etiologic factor in age-related cataract.

Methods: The geographical variation in the incidence of different types of cataract was examined relative to changes in ambient UVR exposure and ambient temperature as an environmental co-factor. Other ocular changes such as presbyopia and diseases such as pterygium and droplet keratopathies were checked as indicators of intense environmental UVR exposure.

Results: The actual distribution of sunlight exposure and the determination temperature variations of different tissues within the anterior segment of the eye are shown to be dependent upon only ambient temperature and the geometrical factors that influence selective UVR exposure. The result highest UVR exposure occurs during light overcast where the horizon is visible and ground surface reflection is high. Exposure in a high mountain valley (lower ambient temperature) with green foliage results in a much lower ocular dose.

Conclusions: The collective evidence suggests that UVR plays an etiological role in cortical cataract, but temperature may play a more important role in the etiology of nuclear cataract.

■ 3371

Prognostic value of Short-Wavelength Automated Perimetry (SWAP) in age-related maculopathy

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Purpose: To investigate the correlation of functional changes assessed by short-wavelength automated perimetry to visual outcome in age-related maculopathy (ARM).

Methods: 119 patients with age-related maculopathy (mean age 71 ± 6 years) were included. Follow-up examination was performed 28 ± 15 months after baseline (fundus photograph, blue-on-yellow-perimetry to assess short-wavelength sensitive (SWS) cone mediated sensitivity; Humphrey field analyzer program 10-2).

Results: In 85 eyes (71%) fundus changes remained stable and no visual loss was observed (ARM). 34 eyes (29%) had a significant visual loss due to choroidal neovascularization or atrophy (AMD). At baseline these groups did not differ in presence of soft drusen or focal hyperpigmentation and in visual acuity (stable ARM: logMAR 0.12 ± 0.13 , progressing: 0.13 ± 0.11 , $p = 0.57$). A significant difference was found for the mean sensitivity assessed with blue-on-yellow perimetry at baseline between the both groups (stable ARM: 21.0 ± 5.0 dB; progressing: 17.1 ± 6.2 dB; $p = 0.0005$). A loss of sensitivity below e.g. 17 dB had a much higher prognostic value compared to the prevalence of soft drusen or focal hyperpigmentation.

Conclusions: Baseline data of mean SWS sensitivity were correlated with the visual outcome of the 2 years follow-up. Short-wavelength sensitive (SWS) cone mediated sensitivity seems to be an important prognostic factor of AMD.

■ 3372

An analysis of autofluorescence imaging of CNV due to age-related macular degeneration

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Purpose: To describe the features of autofluorescence (AF) imaging of choroidal neovascularisation (CNV) at different stages of disease, in patients with AMD and to compare those of recent onset with fluorescein angiography (FFA).

Methods: Fundus AF images of 65 patients with CNV were recorded using a scanning laser ophthalmoscope (HRA or Zeiss). 20 patients had AF imaging of recent onset CNV (Group 1), 8 patients had AF imaging 1-6 months after onset (Group 2) and 37 had AF imaging of late stage CNV (Group 3). Digital FFA images from group 1, were compared to the AF images using Imagenet software. Data were analysed using paired t-tests and presented using a Bland-Altman plot.

Results: In group 1, the areas of hyperfluorescence on FFA corresponded spatially with areas of normal AF in 16 cases. In 18/20 cases, the extent of the total lesion on AF was larger than that on FFA (mean difference in size = $+1.63\text{mm}^2$; 95%CI = 0.58, 2.68; $p = 0.004$). 85% of cases demonstrated focal increases of AF. In groups 2 & 3, areas of decreased AF were seen.

Conclusions: Eyes in group 1 demonstrate areas of preserved AF within the lesion, indicating some preservation of RPE function initially, which has implications for visual prognosis. The greater extent of the lesion on AF implies more widespread damage to the RPE and may explain recurrences after laser treatment. In groups 2 & 3, decreased AF implies loss of RPE and photoreceptors. Focal increases of AF, suggests RPE functioning abnormally at these sites. AF imaging may increase our understanding of CNV, secondary to AMD.

■ 3373

Upregulation of NT4 in human retina from eyes with proliferative vitreoretinopathy

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Purpose: To examine the expression and localisation of BDNF and NT4 in normal human retina and to identify changes of expression in eyes with proliferative vitreoretinopathy (PVR)

Methods: Normal human retina from donor eyes and retinectomy specimens from patients with PVR were fixed in 4% PFA. Specimens were embedded in agarose gel and 100 µm retinal sections were cut using a vibratome. Immunostaining of sections was performed using mouse monoclonal antibodies to rod opsin, and rabbit polyclonal antibodies to BDNF and NT4. Donkey anti-mouse antibodies conjugated with FITC and donkey anti-rabbit antibodies conjugated with Cy3 were used as secondary antibodies. T0-pro3-iodide was used for nuclear staining. Sections were examined using laser scanning confocal microscopy (Bio-Rad).

Results: Minimal intensity staining for BDNF and NT4 was observed at the photoreceptor outer segments and the nerve fibre layer in the normal human retina. The pattern of staining did not alter for BDNF in the PVR specimens studied despite marked photoreceptor loss. Staining for NT4 was greatly increased in PVR sections, with the NT4 localisation being prominent throughout the entire retinal thickness.

Conclusions: BDNF and NT4 are detectable by immunostaining at the photoreceptor and nerve fibre layers in the normal human retina. In PVR retinectomy specimens there is an upregulation of NT4 staining with a widespread distribution in the retina corresponding to the Müller cell localisation. It is possible that NT4 expression by Müller cells is upregulated in PVR given previous reports of its production by these cells. This phenomenon may be indicative of a positive regulatory role that these supporting cells play in retinal regeneration.

■ 3374

Mouse fundus angiography with a scanning laser ophthalmoscope: visualization of angioproliferative retinopathy using indocyanin-green imaging

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Purpose: In a mouse model of oxygen induced retinopathy (OIR) the pathological vascular changes can only be quantified by sacrificing the animal at different stages of the disease. When using fluorescence angiography (FAG), in vivo-imaging of this angioproliferative disease is complicated by immediate leakage of the dye into the vitreous and anterior chamber. A possible alternative to FAG might be the use of indocyanin-green (ICG) in order to evaluate proliferation, leakage and the effects of local antiproliferative drugs.

Methods: C57BL/6J mice with and without OIR were anaesthetized with isoflurane. For fundus imaging and angiography with ICG a scanning laser ophthalmoscope (SLO, Heidelberg Engineering) with an adapted optical system and condensing lens was used. The dye was injected intraperitoneally.

Results: Angiography with ICG produces reproducible images of the retinal vessels as early as day 12. Retinal proliferations, tortuosity of the vessels and to a lower extent retinal capillaries could be observed.

Conclusions: Using the SLO the normal and pathological development of the murine vasculature can be examined in vivo. Images are of good quality and allow estimation of retinal angioproliferation over time without having to sacrifice the animal. A standard will be developed to quantify the retinopathy, e.g. to determine the effectiveness of drugs modulating angiogenesis.

■ 3375

Increased Endothelin-1 plasma level in younger patients with retinal vascular occlusive diseases

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Purpose: Retinal vascular occlusive diseases can occur even in younger patients. The pathogenesis of such perfusion disorders is poorly understood, which makes therapeutic decisions and prophylaxis difficult.

Methods: In a retrospective study we analyzed the clinical findings of six patients under 55 years of age (three men with retinal vein occlusion and three women with branch retinal arterial obstruction), examined for signs of a vascular dysregulation and measured the Endothelin-1 (ET-1) plasma level.

Results: In all our patients the ET-1 plasma level was markedly above the normal level in that age. In all cases an increased tendency for a vascular dysregulation could be found in the nailfold capillaroscopy. Furthermore migraine and coldness of the extremities was often mentioned whereas neither anatomical changes of the vessels in the carotis and ophthalmica region nor disturbances in the hemostasis and fibrinolysis could be found.

Conclusions: The increased ET-1 plasma level and the dysregulation of the vascular tone together with the history of migraine and tendency for cold extremities suggests a functional vascular dysregulation as a potential mechanism for such perfusion disorders. This should be taken into consideration in the therapy of such patients.

■ 3376

Fluphenazine maculopathy

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Purpose: To demonstrate the first reported incidence that fluphenazine has directly caused a maculopathy secondary to its accumulation at the retinal pigment epithelium.

Methods: A case report based on a 45 year-old lady presented with bilateral maculopathy with visual acuity of 6/18 on the right and 6/24 on the left 10 years ago. She had been on fluphenazine for the past 10 years for schizophrenia.

Results: Investigation including fluorescein angiogram, colour vision assessment and electrophysiological testing confirmed the nature of the retinal damages. Her maculopathy deteriorated further despite discontinuation of the medication.

Conclusions: Previous publication has reported maculopathy with fluphenazine in association with the welding arc injury, but she has not been exposed to welding flash or other extreme photochemical sources, we believe this is the first reported incidence that fluphenazine has directly caused a maculopathy secondary to its accumulation at the retinal pigment epithelium.

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