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CORNEAL TATTOO: ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL OBSERVATIONS OF A CASE

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Purpose: Tattooing is employed to provide unsightly corneas with a central artificial pupil or to reduce glare from a paracentral leucoma. The technique is to apply platinum (or gold) chloride to the designated area after an epithelial debridement and subsequently to apply a reducing agent. This procedure is nowadays uncommon in the western world, but is still popular in some countries. According to the literature of the last half of the century metallic salt granules are exclusively seen within the corneal stroma. However, as early as 1929 Bietti reported deposition of these granules also in the regenerated epithelium[1]. The aim of this study was to clarify this controversy using modern techniques.
Methods: The corneal button of 59 year-old male who received a corneal tattoo in Iran 20 years ago was examined by conventional light and electron microscopy. Immunohistochemistry was performed using CD 68 macrophage marker.
Results: Light microscopy revealed clumps of black granules in the keratocytes of the anterior and mid stroma and to a less extend between stromal lamellae. Deposition of these granules was also suspected in basal epithelial cells. Transmission electron microscopy confirmed a predominating intracytoplasmatic localisation of the salt granules in keratocytes, but also showed the deposition of identical electron dense particles in the cytoplasm of the epithelial basal cells.
Unlike inflammatory cells adherent to the posterior surface of the cornea, neither keratocytes nor epithelial cells containing metallic salt granules were positive for CD68.
Conclusions: There is ultrastructural evidence of metallic salt granules deposition in basal epithelial cells. Apparently, there is retrograde movement of these granules to the corneal surface with subsequent phagocytosis by the epithelium.

1.Bietti G. Histologische Untersuchungen und technische Bemerkungen über Hornhauttätowierung mit Platinchlorid, Silbernitrat und Goldchlorid + Silbernitrat. Klin Monatsbl Augenheilkd 1929, 82:741-51

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AN IMMUNOHISTOCHEMICAL STUDY OF THE CELLULAR INFILTRATE IN MOUSE CORNEAS WITH RECURRENT HERPETIC DISEASE

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Purpose To study HSV-1 reactivation and recurrent disease in mice.
Methods We have made quantitative observations *in situ* on the immunological events in the cornea during the development of recurrent corneal disease induced by UV irradiation of latently infected mice. Eyes with such disease were examined by immunohistochemistry using monoclonal antibodies to identify mouse immune cells and a polyclonal antibody to detect virus antigens.
Results On day 4, virus antigens were seen in the corneal epithelium of all mice, and in some animals antigens were also present in the iris and/or the conjunctival epithelium. The number of foci of infection ranged from 1-5. At this time, granulocytes were the predominant infiltrating cell; they were present throughout the corneas with large numbers associated with epithelial lesions. By day 7, in some corneas, ulcers had healed and associated stromal disease was limited to slight focal oedema and/or cellular infiltration (mild disease). In others, ulcers remained and stromal disease was severe with opacification and vessel ingress. On day 7, in corneas with mild disease there was a significant infiltrate of T cells and granulocytes were rare. In contrast, T cells were sparse in corneas with severe disease and large number of granulocytes were still present. These difference persisted until at least day 10.
Conclusions There was considerable variation in the amount of antigen in eyes with recurrent disease which may influence the magnitude of the immune response. The early presence of granulocytes, suggests that these cells play a role in initial clearance of virus. In this model, the severity of immunopathology is associated with the continued presence of large numbers of granulocytes rather than T cells.

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THE BLOOD-AQUEOUS BARRIER AFTER CORNEAL EXCIMER LASER SURGERY.

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Purpose: Intraocular complications following excimer laser surgery of the cornea (PRK and PTK) that are related to the disruption of the blood-aqueous barrier (BAB) have not been reported so far. The aim of the present study was to evaluate disturbances of the BAB after excimer surgery.
Patients and Methods: Laser flare measurements in the aqueous were performed before, one day, three days and seven days after surgery on 40 Patients aged between 28 and 50 years (mean:36.2±4.1) who underwent excimer laser surgery of the cornea. All patients were treated with topical antibiotics and diclofenac eye drops until the corneal epithelium had closed up completely. After epithelial closure therapy was continued with topical steroids. The Kowa Laser flare-cell meter FC-1000 was used for all flare measurements.
Results: One day after surgery flare was increased (11.7±5.6 photon counts/msec) compared to pretreatement measurements (7.1±3.2 photon counts/msec), but the difference was not significant (p>0.05). Seven days after laser treatment the aqueous flare was fond to be within normal range (9.2±4.3 photon counts/msec). It seems to exist a positive correlation between the applied laser energy and the postoperatively measured aqueous flare.
Conclusions: Photorefractive and phototherapeutic excimer laser surgery of the cornea does not affect the blood-aqueous barrier significantly. A moderate increase in blood-aqueous barrier permeability seems to correlate with the applied laser energy.

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IN VITRO AND *IN VIVO* FLUORESCENCE SPECTRAL STUDIES ON THE LENS CRYSTALLINES: CLINICAL STUDIES

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Purpose: The aim of this study was the characterisation of several endogenous lens fluorophores involved in aging processes and Diabetes, before cataract formation.

Methods: The fluorescence spectra of the lens were recorded with a Zeiss spectrometer with a 75 W high pressure Xenon lamp as light source. Monochromatic wavelengths are selected using interference filters. After the beam is focused on the lenses of the patients using a slit lamp microscope, the spectra are acquired by a optical multichannel analyser (OMA). The different excitation wavelengths are 365, 404, 436 and 485 nm.

Results: Preliminary results have been performed with normal and diabetic lenses *in vivo*. For each eye, 4 spectra are taken using the available excitation wavelengths. The spectra are corrected by the excitation and emission correction factors previously determined.

Conclusions: The experimental arrangement has successfully been used to study fluorescence spectra of lens crystallines *in vivo* and *in vitro*. Preliminary results in both cases show differences between spectra for normal and diabetic patients, and increase in fluorescence with the onset of cataract. While the fluorophores have not been identified, the results show that the technique will be valuable for following structural changes in the crystallines and may be useful to study prophylactic drugs which may be used in future as potential drugs to reverse the cataractogenesis process.