European Journal of Ophthalmology / Vol. 11 n. 2, 2001 / pp. 105-115

Review

Interactions between ocular surface fluid and cornea related to contact lenses

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PURPOSE. *To improve the quantification of damage to the ocular surface, metabolite levels, electrolyte concentrations, and enzyme activities were assayed in corneal epithelium, stroma and tears.*

METHODS. *In rabbits, rinsing or contact lenses were used to induce microtrauma. For more severe trauma, experimental injuries were induced with 1 N NaOH. Human accidents included epithelial lesions and mild chemical burns. Enzymatic test systems and electron dispersive X-ray analyses (EDXA) were employed. Corneal hydration was assessed by wet and dry weights. Interleukins were analysed with ELISA.*

RESULTS. *In contrast to normal eyes, in ocular surface trauma the interaction between tear fluid and cornea played an important part. After wearing contact lenses or rinsing, glucose and lactate levels in the cornea and in tears increased, and ATP and glycogen in the cornea decreased. After epithelial lesions, N-acetylglucose aminidase (NAcGA, E.C.3.2.1.50) was released into the tears. Epithelial defects alone and – much more – rinsing the denuded stromal surface produced an increase of lactate and glucose in tears and a dramatic fall in Na, Cl, and S levels in the stroma. Rinsing with phosphate induced corneal calcification. IL-1 and IL-6 were increased in human corneal buttons from patients with trauma and inflammation.*

CONCLUSIONS. *Biochemical analyses may be useful to quantify trauma to the ocular surface. (Eur J Ophthalmol 2001; 11: 105-15)*

KEY WORDS. *Electrolytes, Glucose, Lactate, Hydration, Alkali burn, Surface rinsing*

Accepted: February 7, 2001

INTRODUCTION

Since the optical quality of the eye depends to a large extent on the integrity of the cornea and the overlying tear film, the interactions of the ocular surface fluid with corneal epithelium and stroma have been widely investigated with various methods (1, 2). To understand the pathology of the cornea, the special structure of the epithelium and stroma has to be considered: The corneal epithelium has distinct layers of basal, intermediate and superficial cells. *Zonulae occludentes* form barriers preventing diffusion of even small molecules and penetration of microorganisms. The epithelial cells are attached to the basement membrane by hemidesmosomes (3). In addition, special anchoring complexes protrude from the epithelium into the stroma (4). From its anatomical structure, the epithelium consists mainly of cellular material, with only very little extracellular space.

The stroma forms the major part of the cornea. In contrast to the epithelium, about 90% of the stromal volume is extracellular space, filled with the delicate structure of collagens and glycosaminoglycans. Normally, the stroma contains 78% of water (5). This extracellular water provides a large volume for solutes such as electrolytes, metabolites like glucose and lactate, and low-molecular-weight proteins, including cytokines. The stromal cells, the keratocytes, form a syncytium, which was clearly perceived when confocal microscopy was introduced (6).

The Louis Emile Javal Lecture 1998, given at the XXVIth International Congress of Ophthalmology - Amsterdam, June 22, 1998

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TABLE I - LEVELS OF SOME METABOLITES IN THE CORNEAL EPITHELIUM (10, 11, 16, 47, 48)

Metabolite levels of rabbit corneal epithelium µMol/g wet weight, mean ± S.D.	
ATP	3.03 ± 0.10 (n=14)
ADP	0.26 ± 0.02 (n=14)
ATP/ADP	12.23 ± 0.89 (n=14)
AMP	0.93 ± 0.08 (n=14)
Glucose	2.02 ± 0.22 (n=14)
Lactate	9.89 ± 1.02 (n=14)
GSH	3.03 ± 0.43 (n=21)
GSSG	0.28 ± 0.05 (n=21)
ASC	11.55 ± 1.05 (n±24)

GSH: reduced glutathione, GSSG: oxidized glutathione, ASC: ascorbate

TABLE II - LEVELS OF SOME METABOLITES AND ELECTROLYTES IN THE CORNEAL STROMA. THE ELECTROLYTES ARE GIVEN IN µMol/g TISSUE WATER AND THE METABOLITES IN µMol/g WET WEIGHT (41, 48, 49)

Stroma of rabbit cornea mean ± SD, Hydration = 3.2 ± 0.4			
µMol/g H_2O (n = 20)		**µMol/g wet weight (n = 15)**	
Na	125 ± 42	Lactate	9.66 ± 1.2
Cl	123 ± 25	Glucose	3.63 ± 0.8
K	24 ± 4	ATP	0.20 ± 0.05
S	105 ± 21	ADP	0.07 ± 0.03
P	12 ± 4	ASC	4.72 ± 1.44

Electrolytes and metabolites in corneal stroma and epithelium

The electrolytes, sodium (Na), potassium (K), chlorine (Cl), calcium (Ca) and sulfate (S), form the ionic milieu for living cells (7, 8). Glucose is the major nutrient metabolite of corneal tissues (9). Lactate is the product of the anaerobic energy-producing metabolism. In the cornea, like in many other tissues, adenosine triphosphate (ATP) and adenosine diphosphate (ADP) are the major carriers of cellular energy. Therefore, ATP levels are considered to indicate the vitality of tissues and cells (10-1 2). Since ATP can be assayed with the bioluminescence method in tissue samples of a few milligrams (13), even in histological sections it can be analysed widely in clinical and experimental eye research. ATP/ADP ratios indicate the state of cellular energy independently of absolute metabolite levels (Tab. I).

Na, Cl and S are distributed mainly in the extracellular space of the stroma. The electron dispersive X-ray analysis method (EDXA) was recently adapted and calibrated to analyse quantitatively elements in corneal samples not larger than 10 micrometers. This procedure evaluates back-scattered X-ray spectra in the scanning electron microscope. The peaks obtained from the elements in the sample irradiated by the electron beam can be quantitatively evaluated, if the background scatter is eliminated by a special peak-background calculation procedure (7). In addition, a microtrephine was constructed to take corneal biopsies of 160 µm in diameter, allowing for minimally invasive diagnostic examination (14). This new technique makes it possible to investigate concentrations of electrolytes and elements in various parts of the cornea *in situ*.

While glucose remains mainly outside the cells, lactate is found in the intracellular and extracellular fluid. Both these metabolites can be assayed now with sensitive micromethods (15). Unlike glucose and lactate, ATP and ADP are restricted to cells (Tab. II). Glutathione also occurs mainly intracellularly. It is an important reducing metabolite and its generation is closely connected with the energy-producing metabolism (16). So is ascorbic acid which, in the cornea, can achieve an extraordinarily high concentration (17). In the epithelium, the ascorbic acid concentration was ten times higher than in the aqueous humour and 50 to 100 times higher than in plasma. It is assumed that in the anterior eye segment, ascorbic acid serves for scavenging oxygen and hydroxyl radicals, which are generated to a large extent in the light-exposed tissues of the eye (18, 19).

Trauma to the ocular surface

Trauma, defined as an adverse influence including mechanical, chemical or physical impact, is considered to be a quantitative process. In this context, quantity is understood as a function of adverse impact and

time. Severe trauma, like alkali burns, immediately causes severe damage (20). On the other hand, mild trauma, like badly fitted contact lenses, may induce only slight alterations to the tissue. But as the irritation becomes chronic with time, the stimulus may accumulate and cause more severe damage (6, 21). Small trauma and disease are easily overcome by the defence mechanisms of a healthy organism, but severe trauma or chronic disease may induce violent and exaggerated defences.

The ocular surface fluid is composed not only by tears, but may be influenced by eye drops and rinsing solutions, to say nothing about burning agents. As long as the corneal epithelium is intact, there is little interaction between the cornea and ocular surface fluid, but surface trauma may damage the epithelial barrier and induce interactions between surface fluid and corneal tissues. Erosions or complete defects of the epithelium involve the stroma. With its special anatomical structure and its swelling pressure, the stroma soaks up fluid from the surface like a sponge. Clinically, this appears as edema but, as revealed by EDXA and other analyses, corneal edema from surface lesions brings about dramatic changes in the chemical composition of the stroma.

Even contact lenses, gentle rubbing of the epithelial surface, or eye drops, which usually contain disinfectants, can produce lesions of the epithelium and pathological reactions, which may be detected by analysis of enzyme activities and metabolite levels. For example, after 6 to 8 hours of wearing contact lenses, ATP and glycogen storage in the epithelium were significantly decreased (Fig. 1), although the surface was clinically intact and showed no fluorescein staining (22).

The protective action of glutathione on the corneal epithelium was demonstrated when the ocular surface was rinsed for 10 minutes with hydrogen peroxide at the concentrations used for cleaning contact lenses. The hydrogen peroxide was eliminated and the epithelial surface remained intact, but the glutathione concentration in the epithelium was reduced (Fig. 2) (23). Even well-fitted contact lenses can irritate the cornea. This was assessed from the increase of hydration and lactate levels. Depending on the construction and the fit of contact lenses, the cornea swells slightly, its hydration increasing by 2-45% (Fig. 3).

The lesions to the epithelium became apparent from 1-200% increase in lactate levels in the epithelium

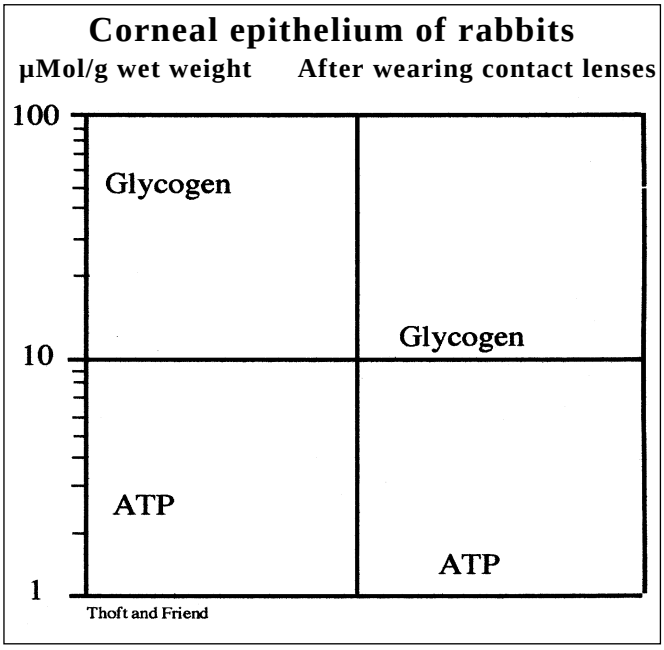

Fig. 1 - *ATP and glycogen levels in corneal epithelium of rabbits. As the concentrations vary over a wide range, the ordinate is on a logarithmic scale. The metabolites are indicated at the level of the individual concentrations. After wearing contact lenses, the levels markedly decreased (22).*

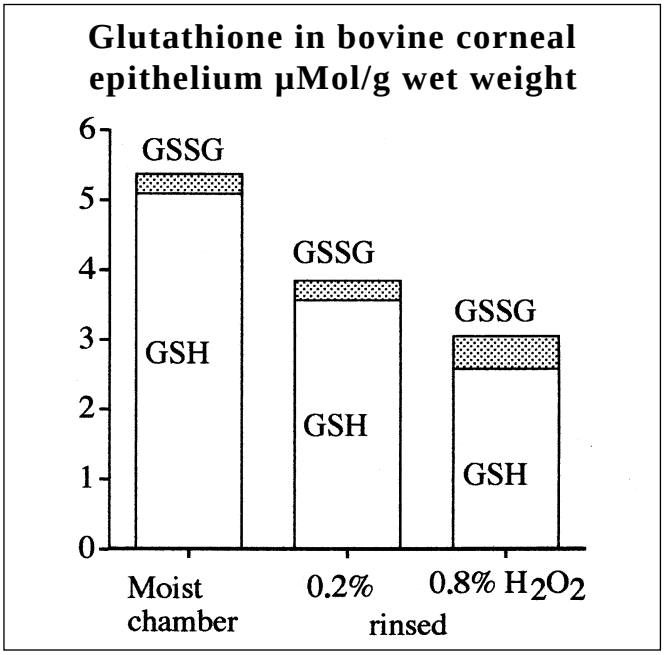

Fig. 2 - *Elimination of hydrogen peroxide (H_2O_2) from the corneal surface and glutathione levels in the corneal epithelium of isolated bovine eyes. The open columns represent reduced glutathione (GSH), the dark parts oxidized glutathione (GSSG). The ordinate gives the levels in µM/g wet weight. After rinsing the corneal surface with H_2O_2, glutathione levels decreased (23).*

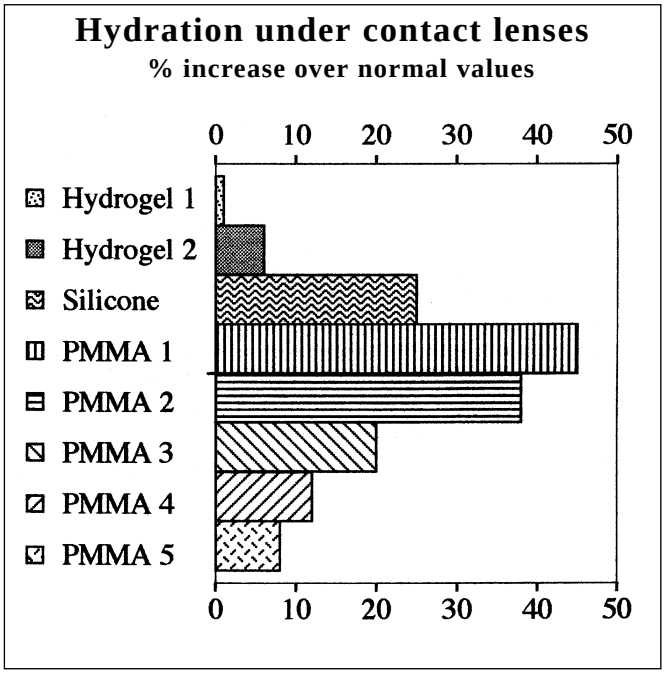

Fig. 3 - *Corneal hydration under contact lenses in rabbits. Different lens types and materials induced highly variable increases. Corneal hydration is defined as H = wet - dry weight/dry weight (21, 24-29).*

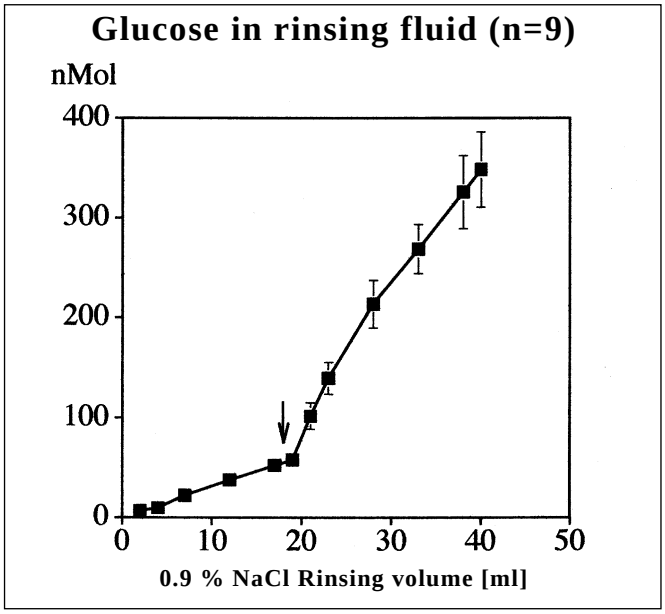

Fig. 5 - *Effect of rinsing the corneal surface in a rabbit experiment. The ordinate shows the accumulated amount of glucose (m ± s.e.m) in the rinsing fluid and the abscissa gives the rinsing volume. Very little glucose leaked out from the corneal surface as long as the epithelium was intact. The arrow indicates when the epithelium was abraded. The glucose rinsed out from the stromal surface then increased significantly (2, 25).*

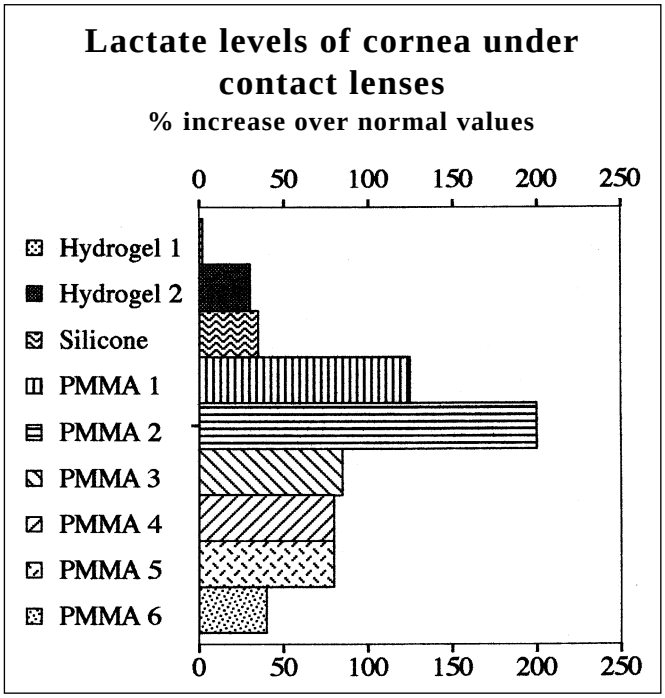

Fig. 4 - *Lactate levels in the cornea under various contact lenses. The levels increased during contact lens wear, like hydration shown in Figure 3 (21, 24-29).*

under the contact lenses (Fig. 4). These results indicated that minimal surface trauma may be assessed from these physiological and biochemical parameters (21, 24-29).

Another small alteration of the corneal surface was observed during simple rinsing with saline. Glucose appeared in the rinsing fluid, it was released from the intact epithelium. When the epithelium was removed, the glucose leaking out increased (Fig. 5). The same effect could be seen with lactate (Fig. 6) but the loss of lactate from the surface was already marked when the epithelium was intact. When it was removed, the amount of lactate rinsed out did not increase further (30). In clinical cases, slight surface traumata, like mild eye burns, corneal foreign bodies or scratches, also caused glucose and lactate to be released from the epithelium and raised the levels in tears. Lactate was elevated to twice, and glucose up to five times the normal values (Fig. 7) (31).

Surface epithelia of the eye also release lysosomal enzymes in response to trauma or disease. N-acetyl-glucose aminidase is a marker enzyme (32) that appears together with metalloproteinases. In cases of

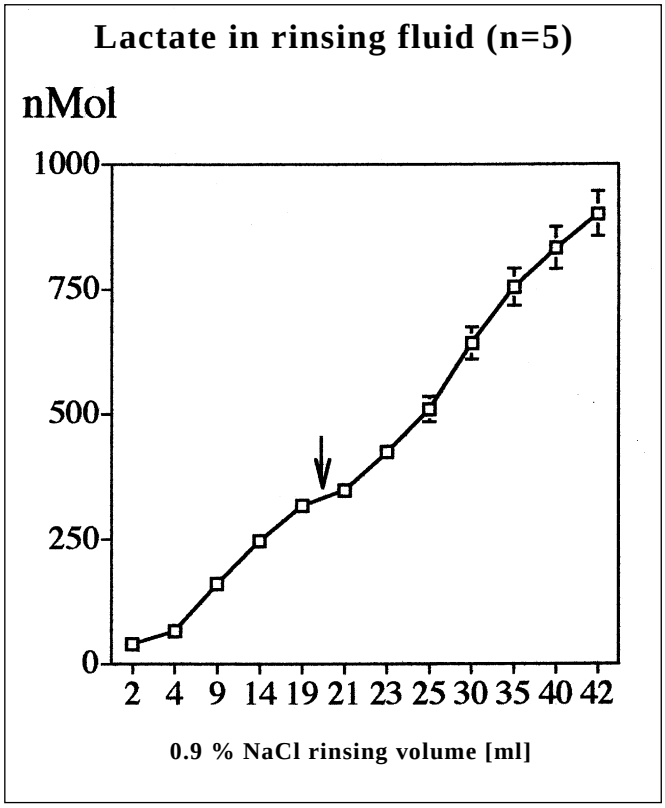

Fig. 6 - *Effect of rinsing the corneal surface in a rabbit experiment. The ordinate shows the amount of lactate found in the rinsing fluid (m ± s.e.m.). The ordinate gives higher values than Figure 5. The abscissa shows the rinsing volume. Large amounts of lactate leaked out from the intact corneal surface. The arrow indicates when the epithelium was abraded, after which, the lactate rinsed out from the stromal surface did not increase further (2, 25).*

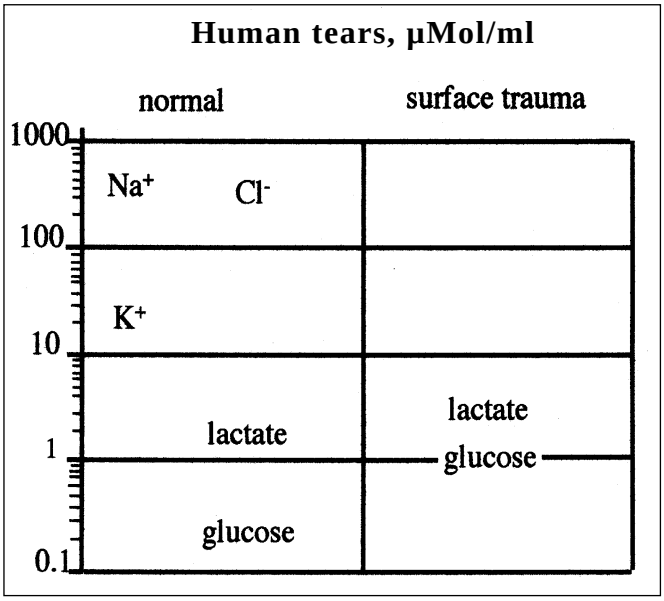

Fig. 7 - *Concentrations of Na, K, Cl, glucose and lactate in human tears values collected from 10 clinical cases. The abscissa is in a logarithmic scale. The range of concentrations is very wide. After surface trauma, lactate and glucose concentrations increased, although the tear flow volumes were much larger than in normal healthy eyes (31).*

mild and moderate eye burns and in atopic conjunctivitis, the release of this enzyme to the surface fluid may reach five to ten times normal (Fig. 8), (31).

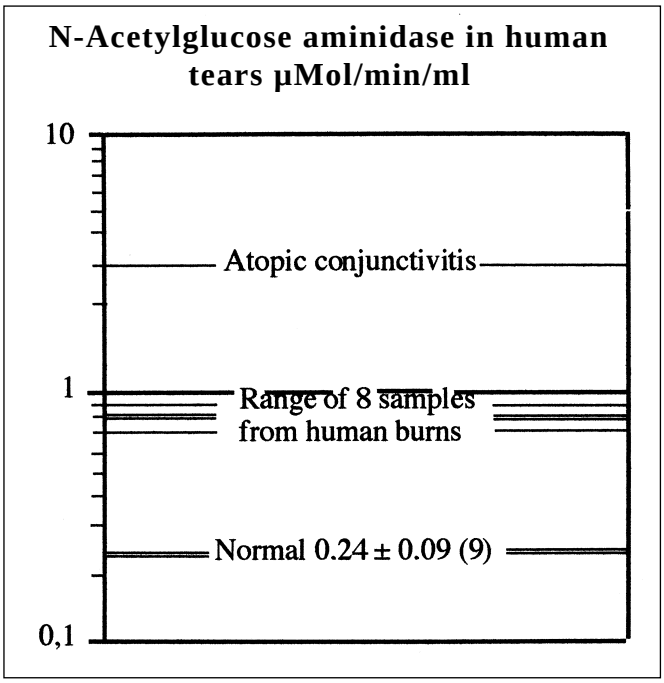

Fig. 8 - *Activity of N-acetylglucose aminidase (NAcGA, E.C. 3.2.1.50) in human tears collected from nine cases with eye burns and one atopic patient. The ordinate is on the logarithmic scale. The enzyme activity (µMol/min/ml) was considerably higher in the eye surface diseases (31).*

Cytokines and growth factors in cornea and tears

Cytokines and growth factors play an important part in health and disease.There are many reports of cytokines in ocular cells and tissues. Normal tears contain large amounts of two important growth factors: epidermal growth factor (EGF), which enhances the regeneration of the corneal epithelium and stimulates proliferation of epithelial cells and fibroblasts; transforming growth factor-beta (TGF-beta) is a kind of antagonist to EGF, inhibiting proliferation of the corneal epithelium. Presumably, these two are responsible for homeostasis of the surface epithelium (Tab. III), (33,

TABLE III - CYTOKINES AND GROWTH FACTORS IN TEARS INFLUENCING THE CORNEAL EPITHELIUM (33, 36, 50)

Cytokines in tears
• EGF ---------> regeneration of epithelium
• TGFbeta 2 ----> inhibits proliferation
• TNFalpha, in inflammation
• Many others
• but presumably released from damaged surface epithelia
Sotozono and Kinoshita, Kruse, Mishima et al

TABLE IV - RELATIONS BETWEEN CYTOKINES AND GROWTH FACTORS IN CORNEAL EPITHELIUM AND STROMA (37, 50, 51)

Organization of cytokines in cornea (from Li DQ and Tseng SCG; 1995)
Expressed by epithelium
TGFalpha IL-1beta PDGF.....receptors in fibroblasts
Expressed by keratocytes
KGF, HGF .....receptors in epithelial cells
Expressed by epithelium and fibroblasts
IGF-1, TGFbeta 1, TGFbeta 2 bFGF... and their receptors

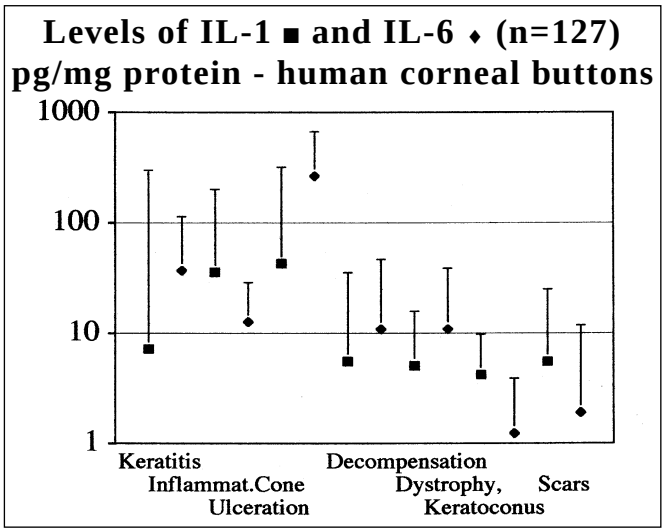

Fig. 9 - *Interleukin-1beta (IL-1) and interleukin-6 (IL-6) in human corneal buttons from keratoplasty (total number ot cases 127). The logarithmic ordinate shows the concentrations in pg/mg extractable protein. The symbols show the median; squares stand for IL-1, circles for IL-6. The error bars indicate the 75% percentiles. On the abscissa, the diagnoses correspond to the position of the symbols (39).*

34). By continuous secretion from the lacrimal gland, they are present on the ocular surface to fulfil this task. Tumor necrosis factor-alpha (TNF-alpha) is an Inflammatory cytokine released from macrophages and lymphocytes. Since both cells are constantly present in the lacrimal glands, its secretion with tears in response to adequate stimuli is observed in anterior segment diseases. Many other growth factors and cytokines are found in the ocular surface fluid, but they are mainly released from damaged or sick epithelial cells or leucocytes.

Recently, an organization of the action of cytokines was proposed: TGF-alpha, interleukin-1beta (IL-1beta) and platelet derived growth factor (PDGF) are synthesized in the epithelium, but their receptors are found in the stroma fibroblasts, not in the epithelium. In contrast keratocyte growth factor (KGF) and hepatocyte growth factor (HGF) are formed in stroma cells and have their receptors in the epithelium (Tab. IV), (35-37). This distribution suggests an interdependence of the surface epithelium and the stroma, which was observed many years ago (38), but no messenger of this interaction has yet been found.

As concluded from *in vitro* and *in vivo* experiments, interleukines were assumed to trigger and maintain inflammation. Recently, IL-1 and IL-6 were analysed in corneal buttons obtained from corneal grafts for various diseases (39). In the inflammatory corneas, high levels of IL-1 and IL-6 were found (Fig. 9). The relation of clinical diagnoses and cytokine levels in the buttons showed clearly how much the inflammatory disease had increased when interleukin levels were higher. Highest levels were in keratitis and corneal ulceration (Fig. 10). Clinical stages of individual cases suggested that early in the disease IL-1 was increased, but later IL-6 reached the highest levels. An unexpected result was the rather high levels of IL-1 and IL-6 in acute, inflammatory keratoconus. Quiet corneas with degenerative diseases, like Fuchs dystrophy, hereditary dystrophies, keratoconus and corneal scars, showed only mildly elevated interleukin levels. There were a few cases with corneal scars without visible irritation, with rather high IL-1 levels in buttons obtained on keratoplasty (Fig. 11). Interestingly, these eyes developed severe inflammatory reactions after keratoplasty.

Low concentrations of IL-1 and IL-6 were associated with non-inflammatory conditions. Thus the low con-

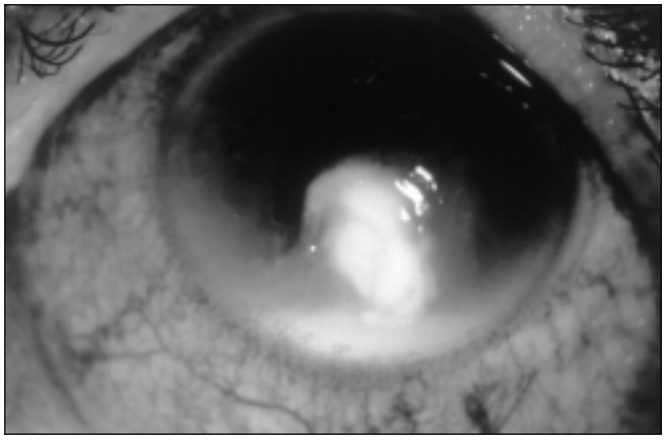

Fig. 10 - *Corneal ulcer in 57-year-old man. In the corneal button trephined at keratoplasty* à chaud*, there was 402 IL-1 and 745 pg/ mg protein IL-6 (Eye Clinic of Faculty of Medicine, Technical University Aachen, Germany).*

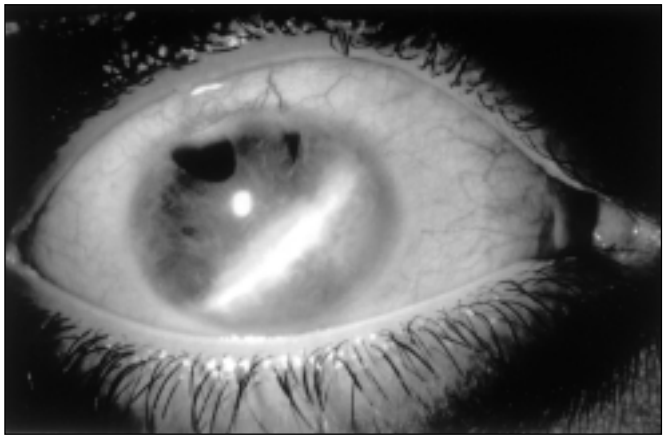

Fig. 11 - *Corneal scar in a young man of 16 years following severe ocular trauma several years earlier. The corneal button obtained on keratoplasty had high levels of IL-1 and IL-6, with 48 and 63 pg/mg protein, respectively. Although the eye looked quiet before surgery, as shown here, it developed an unexpected heavy inflammatory response afler keratoplasty (Eye Clinic of Faculty of Medicine, Technical University Aachen, Germany).*

centrations of IL-1 and IL-6 in corneal buttons trephined on keratoplasty might have been related to an intact epithelium on a not-inflamed stroma. However, it cannot be excluded that the low interleukin levels in clinically quiet corneal buttons were a response to the surgical trauma. Nevertheless, the clinical investigation of IL-1 and IL-6 levels in human corneas showed the significance of these cytokines for human diseases (39).

Electrolyte levels in corneal stroma pathology

The EDXA found dramatic alterations of the ionic equilibrium in the corneal stroma. The epithelial defect induced stromal edema, which is often considered a minor clinical problem. But underlying the clinical aspect, chemical analyses revealed important alterations of the electrolyte levels. Sodium, chlorine and sulfur were considerably decreased within 24 hours, sodium and sulfur to half the normal values, and chlorine by about one third (Tab. V), (8, 40). Lactate levels were also low, but the concentrations were generally ten times lower than the electrolytes. In alkali burns, which were frequently used as an experimental model to investigate acute and chronic inflammation, the epithelium was completely destroyed and the stroma denuded. Electrolyte levels of alkali burnt corneas, rinsed with saline four times daily for 16 days, are presented in Table VI. Under these conditions, the sodium deficit was still quite imposing, but chlorine and especially sulfur were largely diminished (41). Sodium, potassium, and chlorine are solute electrolytes, which make up the ionic milieu of the tissue fluid, while sulfur is mainly different.

The severe loss of sulfur from the corneal stroma was observed several times after epithelial defects, even with short rinses. Sulfur in the corneal stroma is bound almost equimolarly in collagen and glycosaminoglycan molecules (42). Looking into the chemical structure of glycosaminoglycans, sulfur is found

TABLE V - LEVELS OF SOME METABOLITES AND ELECTROLYTES IN THE CORNEAL STROMA 24 HOURS AFTER REMOVAL OF THE EPITHELIUM. Na, Cl, S AND LACTATE DECREASED (41, 52)

	Stroma of rabbit cornea	
	Normal	**24 hours denuded**
Na	125 ± 42 [20]	65 ± 22 [8] alkali burn
Cl	123 ± 25 [20]	130 ± 29 [8] alkali burn
S	105 ± 21 [20]	55 ± 0.7 [8] alkali burn
Lactate	14.9 ± 0.2 [14]	6.3 ± 0.4 [11] scraped
Glucose	3.6 ± 0.2 [14]	3.9 ± 0.2 [11] scraped

Hydration 3.1 ± 0.4 [20], 4.7 ± 0.3 [8] and 6.0 ± 0.2 [11] respectively. Index number of cases; lactate and glucose: µMol/g wet weight; Na Cl, S: µMol/g H_2O mean ± SD

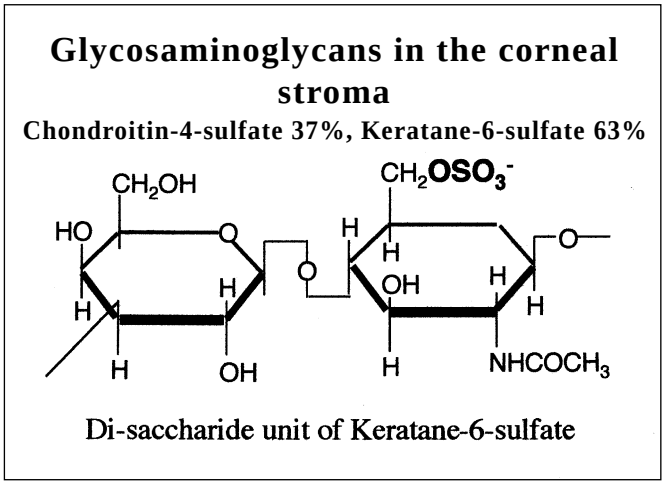

Fig. 12 - *Unit of keratane sulfate. The sulfate ion is bound to the N-acetyl glucosamine part, which is linked to the galactose in 1.4-position.*

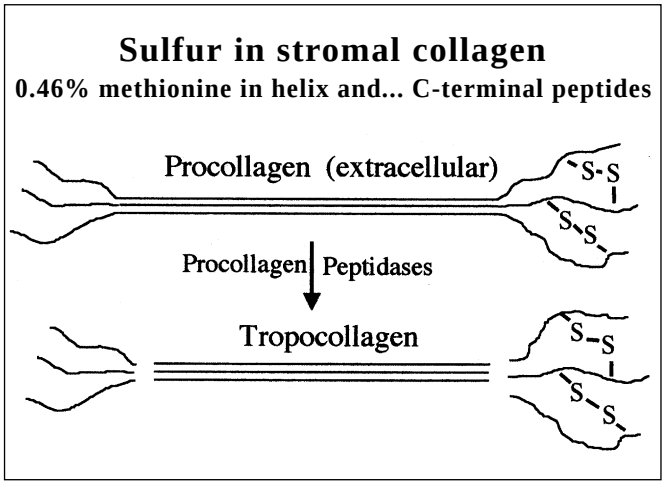

Fig. 13 - *Model of stromal collagen. The C-terminal peptides of the procollagen contain several disulfide groups. On condensation of fibrils, the C-terminal peptides were split off and dissolved in the extracellular space of the stroma.*

in the sulfate ion bound to the sugar moiety (Fig. 12). This part can be easily separated and washed out from its macromolecules. The sulfur component incorporated in the collagen triple helix is methionine, which makes up only a small proportion of the collagen. The major part of the sulfur in these molecules is found in the C-terminal peptides of procollagen, where it forms disulfide bridges. On condensation of the freshly synthesized procollagen to tropocollagen, the C-terminal peptides of the procollagen are separated from the triple helix (Fig. 13). Since this reaction takes place outside the keratocytes, in the extracellular matrix, the sulfur compound of the C-terminal peptides may be washed out as well.

TABLE VI - LEVELS OF SOME ELECTROLYTES IN THE CORNEAL STROMA AFTER ALKALI BURN. THE DENUDED STROMA WAS RINSED WITH SALINE FOUR TIMES DAILY FOR 16 DAYS. Na, Cl AND ESPECIALLY S DECREASED WHILE P INCREASED (41, 42, 53)

	Stroma of rabbit cornea µMol/g H_2O, mean ± SD	
EDXA	**Normal (n = 20)**	**Alkali burn, denuded, rinsed with 0.9% NaCl, 4x daily for 16 days (n = 8)**
Na	125 ± 42	90 ± 11
Cl	123 ± 25	65 ± 15
S	105 ± 21	24 ± 4
P	12 ± 4	22 ± 22
Ca	3 ± 3	1 ± 3

Hydration 3.1 ± 0.4 and 6.5 ± 0.9 respectively

When the eyes with the alkali burn denuded of epithelium were rinsed with phosphate buffer, the loss of sodium, chlorine and sulfur was the same as in the experiments before, but calcium and phosphorous increased tremendously (Tab. VII) to concentrations far exceeding the normal values of sodium and chlorine (43). In these corneas, calcification of the cornea was visible within a few days (2, 40, 44).

EDXA gave the ratio of calcium to phosphate levels as 0.51 ± 0.15 (n=10). This identified the precipitate as calcium phosphate, i.e. hydroxyl apatite, comparable to bone formation. Such calcifications were also observed in clinical patients with long-standing corneal epithelial defects, whose eyes were rinsed with isotonic phosphate buffer (Fig. 14). Visible calcifications also occurred in patients who were simply treated with eye drops containing phosphate buffer as solvent, or with compounds coupled to phosphate like prednisolone phosphate (2, 45, 46). Calcium is a component of cell membranes, but it also occurs in extra- and intracellular fluid. Phosphate is part of many organic compounds, such as sugar phosphates and adenosine or other nucleotide phosphates. While organic phosphates are mainly

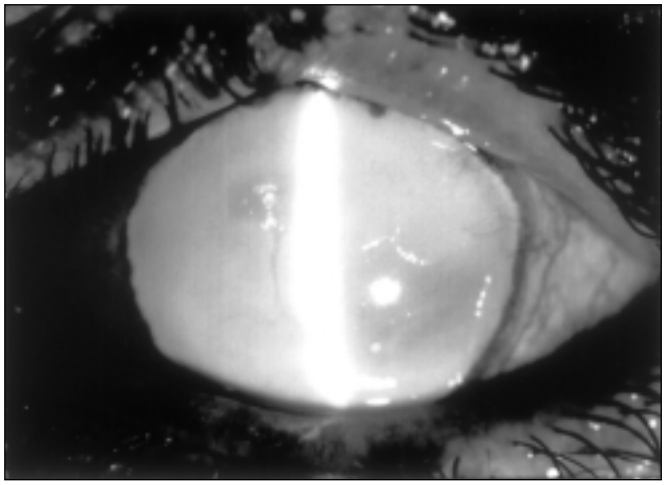

Fig. 14 - *Cornea of a 48-year-old patient six months after a burn with slaked lime. The eye was rinsed for first aid and subsequent therapy was with isotonic phosphate buffer. The severe calcification was later removed by excimer laser ablation.*

found inside the cells, inorganic phosphate is found in intra- and extracellular fluid as well, although in rather low concentrations. In the case of calcification by external application of large amounts of phosphate, local calcium may precipitate in the cornea and new calcium moves in from other cells or body fluids, in tears and aqueous humour. This was concluded from frequent observations that calcification of the cornea occurred not only from lime burns, but also from pure sodium, potassium, and various acid burns.

TABLE VII - LEVELS OF SOME ELECTROLYTES IN THE CORNEAL STROMA AFTER ALKALI BURN AND RINSING WITH ISOTONIC PHOSPHATE BUFFER FOUR TIMES DAILY FOR 16 DAYS. Na, Cl AND ESPECIALLY S DECREASED, BUT P AND Ca ROSE STEEPLY. CLINICALLY, CALCIFICATION OF THE CORNEA WAS OBSERVED (41, 43, 53)

	Stroma of rabbit cornea µMol/g H_2O, mean ± SD	
EDXA	**Normal (n=20)**	**Alkali burn, denuded, rinsed with phosphate buffer 4x daily for 16 days (n=8)**
Na	125 ± 42	105 ± 22
Cl	123 ± 25	88 ± 33
S	105 ± 21	28 ± 4
P	12 ± 4	623 ± 307
Ca	3 ± 3	435 ± 198

Hydration 3.1 ± 0.4 and 5.1 ± 1.2 respectively

CONCLUSIONS

Healthy corneal epithelium is well equipped for defence. But even minor trauma, which may not be visible with the slit lamp microscope, may alter the corneal metabolism. Therefore, analyses of metabolites such as ATP or lactate may be useful to investigate cell vitality of the cornea.

Cytokines play an important part in the function of corneal cells. Increased levels of cytokines in cornea and tears may indicate trauma or inflammatory responses. Investigation with EDXA, especially of the electrolytes in the corneal stroma, opened up new aspects of corneal and cell physiology. If the epithelium is defective, it is not only the clinically visible edema that hurts the cornea, but also rapid and severe changes in the concentrations of elements like sodium, potassium, chlorine, sulfur and phosphate.

These changes may impair the basic environment for keratocytes like the composition of ions, osmotic pressure, pH, and buffering properties. Such changes may harm the vitality of keratocytes and affect epithelial regeneration as well.

The finding that rinsing fluids and eye drop solvents influenced the concentrations of stromal electrolytes suggested a search for the best fluid compositions to maintain a physiological environment for the ocular surface and for the denuded stroma. Corneal calcification may be a dramatic side effect of drug vehicles and rinsing fluids containing phosphate. Finally, we found that in corneal epithelial defects rinsing fluids removed an important part of the sulfur from the stromal matrix. Therefore, rinsing fluids and eye drop solutions used on the denuded corneal surface should not only resemble stromal extracellular fluid, but should also prevent or compensate for the loss of sulfur from the stroma. The denuded corneal stroma is an exposed, unprotected tissue and needs special attention in trauma and disease.

ACKNOWLEDGEMENTS

Supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, Germany.

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REFERENCES

1. Thoft R, Friend J. Functional competence of the regenerating ocular surface epithelium. Invest Ophthalmol Vis Sci 1978; 17: 134-9.
2. Reim M, Kottek A, Schrage N. The cornea surface and wound healing. Prog Retin Eye Res 1997; 16: 183-225.
3. Rohen JW. Die Hornhaut. Der präcorneale Film. Francois J and Hollwich F, eds. Augenheilkunde in Klinik und Praxis. Stuttgart: G Thieme, 1977.
4. Gipson IK, Spurr-Michaud S, Tisdale A, Keough M. Reassembly of anchoring structures of corneal epithelium during wound repair in the rabbit. Invest Ophthalmol Vis Sci 1989; 30: 425-34.
5. Reim M. Chirurgische Anatomie, Physiologie, Biochemie sowie Fragen der Inlay-Technik. Ophthalmologe 1992; 89: 109-18.
6. Thaer AA, Geyer OC. Die Anwendung der konfokalen Spalt-Scanning Mikroskopie auf die *in vivo*-Untersuchung der Kornea-Mikrostruktur im Zusammenhang mit dem Tragen von Kontaktlinsen. Contactologica 1997; 19: 158-77.
7. Schrage NF, Benz K, Beaujean P, Burchard WG, Reim M. A simple empirical calibration of energy dispersive X-ray analyses (EDXA) on the cornea. Scanning Microsc 1993; 7: 881-8.
8. Schrage NF, Flick S, Redbrake C, Reim M. Electrolytes in the cornea: A therapeutic challenge. Graefes Arch Clin Exp Ophthalmol 1996; 94: 492-5.
9. Reim M. Die Bedeutung des Limbus für die Ernahrung der Cornea. In: W Doden ed. Limbusprobleme. Stuttgart: F Enke, 1982; 1-6.
10. Hennighausen U, Schmidt-Martens FW, Reim M. Metabilitspiegel und Enzymaktivitaten des Energie liefernden Stoffwechsels im regenerierenden Corneaepithel. Ber Dtsch Ophthalmol Ges 1972; 71: 95-9.
11. Reim M, Foerster KH, Cattepoel H. Some criteria of the metabolism in the donor cornea. XXI. Concil Ophthalmol Interna Mexico City. Amsterdam: Excerpta Medica 1970; 1: 728-33.
12. Reim M, Henninghausen U, Hildebrandt D, Maier R. Enzyme activities in the cornea epithelium and endothelium of different species. Ophtalmic Res 1971; 2: 171-82.
13. Salla S, Redbrake C, Frantz A. Employment of bioluminescence for the quantification, of adenosine phosphates in the human cornea. Graefes Arch Clin Exp Ophthalmol 1996; 234: 521-6.
14. Schrage NF, Lorenz U, von Fischern T, Reim M. The microtrephine - A new diagnostic tool for obtaining corneal biopsies. Acta Ophthalmol 1994; 72: 384-7.
15. Frantz A, Salla S, Redbrake C. A sensitive assay for the quantification of glucose and lactate in the human cornea using a modified bioluminscence technique. Graefes Arch Clin Exp Ophthalmol 1998; 236: 61-4.
16. Reim M, Beermann HR, Luthe PHC. The redox state of the glutathione in the bovine corneal epithelium. Albrecht Von Graefes Arch Klin Exp Ophthalmol 1976; 201: 143-8.
17. Reim M, Seidl M, Brucker K. Accumulation of ascorbic acid in the corneal epithelium. Ophthalmic Res 1978; 10: 135-9.
18. Reim M, Becker J. Cell protection in the anterior segment of the alkali burnt eye. In: KH Schmidt ed. Cell and tissue protection in ophthalmology. Stuttgart: Hippokrates Verlag, 1995; 49-55.
19. Ringvold A, Anderssen E, Kjønniksen I. Ascorbate in the corneal epithelium of diurnal and nocturnal species. Invest Ophthalmol Vis Sci 1998; 39: 2774-7.
20. Reim M, Kaufhold FM, Kehrer T, Kuckelkorn R, Kuwert T, Leber M. Zur Differenzierung der Therapie bei Veratzungen. Fortschr Ophthalmol 1984; 81: 583-7.
21. Kilp H, Heisig B. Veränderungen der Glukose- und Laktatkonzentrationen in Tranenflassigkeiten des Kaninchen auf mechanische Reizung und Kontaktlinsentragen. Graefes Arch Clin Exp Ophthalmol 1975; 193: 259-67.
22. Thoft RA, Friend J. Biochemical aspect of contact lens wear. Am J Ophthalmol 1975; 80: 139-45.
23. Reim M, Ashauer D. The glutathione of the cornea. Arch Ophtalmol (Paris) 1975; 35: 153-8.
24. Burns RP, Roberts H, Rich LF. Effect of silicone contact lenses on corneal epithelial metabolism. Am J Ophthalmol 1971; 71: 486-9.
25. Kilp H. Einfluss von Kontaktlinsen auf Metabolite und Hydratation der Kaninchenhornhaut. Graefes Arch Clin Exp Ophthalmol 1974; 190: 275-80.

26. Praus P, Brettschneider J, Dreifus M. Study of the effect of hydrophilic gel contact lenses on the cornea. Ophthalmologica 1969; 159: 398.
27. Morley N, McCulloch C. Corneal lactate and pyridine nucleotides with contact lenses. Arch Ophthalmol 1961; 66: 379-82.
28. Smelser GH. Relation of factors involved in maintenance of optical properties of cornea to contact lens wear. Arch Ophthalmol 1952; 47: 328-43.
29. Turss R, Schebitz H. Die Bedeutung des Kammerwassers und der Randschlingengefässe für die Ernährung der Hornhaut. Ber Dtsch Ophthalmol Ges 1972; 71: 87-91.
30. Reim M. Warum ist die Cornea durchsichtig? - Über Physiologie, Biochemie und Stoffwechsel der Cornea. Ber Dtsch Ophthalmol Ges 1972; 71: 58-77.
31. Reim M, Leber M, Kaufhold FM. Normale und pathologische Bestandteile der Tränen des Bindehautsekretes. Neues Optiker J 1982; 20: 375-82.
32. Reim M, Leber M. N-acetylglucose aminidase activity in corneo-scleral ulceration following severe eye burns related to individual patients. Cornea 1992; 12: 1-7.
33. Kruse F, Tseng S. Transformierender Wachstumsfaktor beta 1 und 2 hemmen die Proliferation von Limbus- und Hornhautepithel. Ophthalmologe 1994; 91: 617-23.
34. Mishima H, Nakamura M, Murakami J, Nishida T, Otori T. TGF-beta regulates actions of EGF, Interleukin-6 and fibronectin on the functions of corneal epithelial cells. Invest Ophthalmol Vis Sci 1991; 32 (suppl): S954.
35. Sotozono C, Inatomi T, Nakamura M, Kinoshita S. Keratinocyte growth factor accelerates corneal epithelial wound healing *in vivo*. Invest Ophthalmol Vis Sci 1995; 36: 1524-9.
36. Sotozono C, Kinoshita S. Growth factors and cytokines in corneal wound healing. In: Nishida T ed. Corneal healing responses to injuries and refractive surgeries. The Hague: Kugler Publications, 1998; 29-40.
37. Li DQ, Tseng SC. Three patterns of cytokine expression potentially involved in epithelial-fibroblast interaction of human ocular surface. J Cell Physiol 1995; 163: 61-79.
38. Dohlman C. The function of the corneal epithelium in health and disease. Invest Ophthalmol Vis Sci 1971; 10: 383-407.
39. Becker J, Salla S, Dohmen U, Redbrake C, Reim M. Explorative study of interleukin levels in the human cornea. Graefes Arch Clin Exp Ophthalmol 1995; 233: 766-71.
40. Reim M, Redbrake C, Schrage N. Chemical injuries, surgical and medical treatment based on clinical and pathophysiological findings. Arch Soc Esp Oftamol 2001; 76: 79-102.
41. Fischern TV, Lorenz U, Burchard WG, Reim M, Schrage NF. Changes in the mineral composition of the rabbit cornea after alkali burns. Graefes Arch Clin Exp Ophthalmol 1998; 236: 553-8.
42. Langefeld S, Reim M, Redbrake C, Schrage NF. The corneal stroma: an inhomogeneous structure. Graefes Arch Clin Exp Ophthalmol 1997; 235: 480-5.
43. Haller W. Therapeutische Beeinflussung der Hornhautelemente nach Verätzung. Doctoral Thesis. Faculty of Medicine. RWTH. Aachen 2000: 160.
44. Schrage NF, Reim M, Burchard WG. Untersuchungen an schwerstveratzten Corneae nach Langzeittherapie unter besonderer Beachtung von partikularen Ruckstanden aus Trauma und Therapeutika. Beitr El ektronenmikroskop Direktabb Oberfl 1988; 21: 465-72.
45. Schrage N, Reim M. Particulate matter contamination in the corneal stroma of severe eye burns in humans. Lens Eye Toxicity Res 1990; 7: 427-44.
46. Schrage NF, Flick S, Aschenbrenner W, Scheerer A, Reim M. Rinsing therapy in severe alkali burns of rabbit eyes. Vision Res 1996; 36 (suppl): S143.
47. Reim M, Weidenfeld E, Budi Santoso AW. Oxidized and reduced glutathione levels of the cornea *in vivo*. Graefes Arch Clin Exp Ophthalmol 1979; 211: 165-75.
48. Reim M, Beil KH, Kammerer G, Krehwinkel S. Influence of systemic ascorbic acid treatment on metabolite levels after regeneration of the corneal epithelium following mild alkali burns. Graefes Arch Clin Exp Ophthalmol 1982; 218: 99-102.
49. Reim M, Cattepoel H, Bittmann K, Kilp H. Methodische und physiologische Aspekte bei der statistischen Auswertung von Metabolitspiegeln in verschiedenen Kompartimenten der vorderen Augenabschnitte. Graefes Arch Clin Exp Ophthalmol 1969; 177: 355-68.
50. Mishima H, Nakamura M, Murakami J, Nishida T, Otori T. Transforming growth factor-beta modulates effects of epidermal growth factor on corneal epithelial cells. Curr Eye Res 1992; 11: 691-6.
51. Tseng SCG. Regulating of limbal epithelial stem cells. In: T Nishida ed. Corneal healing responses to injuries and refractive surgeries. The Hague: Kugler Publications, 1998; 59-77.
52. Reim M, Boeck H, Krug A, Venske G. Aqueous humour and cornea stroma metabolite levels under various conditions. Ophthalmic Res 1972; 3: 241-50.
53. Schrage N. Elemente in der Cornea. Habilitationsschrift. Faculty of Medicine. Technical University. Aachen 1997: 90.