The outer eye in chronic renal failure

Nynke Klaassen-Broekema

THE OUTER EYE IN CHRONIC RENAL FAILURE

Aandoeningen van het uitwendige oog bij chronisch nierlijden

NYNKE KLAASSEN-BROEKEMA

STELLINGEN

1. Het ontstaan van kalkneerslag in de limbus bij dialyse patienten berust op het verschijnsel calciphylaxis.

2. Het optreden van een acute diffuse conjunctivale en episclerale hyperaemie bij dialyse patienten heeft te maken met de axonreflex.

3. Door het niet herkennen van de latente vorm van conjunctivitis lacrimalis kan men voor onaangename verrassingen komen te staan.

4. Het is onjuist te veronderstellen dat pneumokokken door infectie van de traanafvoerwegen in virulentie toenemen.

5. De introductie van antibiotica bij de behandeling van septische shock heeft niet geleid tot een afname van de mortaliteit ten gevolge van deze aandoening.

6. Het vóórkomen van auto-antistoffen bij huisdieren van SLE-patienten wijst op de mogelijke rol van omgevingsfactoren in het ontstaan van deze ziekte.

7. De overstap van verticaal naar horizontaal schrift was het directe gevolg van de toegenomen organisatiegraad van de Sumerische beschaving.

8. De uitspraak van Dodonaeus over koriander, "een seer stinckende ende ghelijck die wantluysen rieckenden cruyt", is incompleet.

9. Het doel van de veldtocht in Vlaanderen werd niet bereikt; het gevolg was echter van grote betekenis.

10. Rendementsverbetering van energie-omzetters en distributiesystemen leidt eerder tot energiebesparing dan verbruiksbeperkingen voor de consument.

11. Niet het waarschijnlijk maken van waarheid maar het elimineren van onwaarheid is het kenmerk van wetenschap.

N. Klaassen-Broekema
Groningen, 14 december 1994

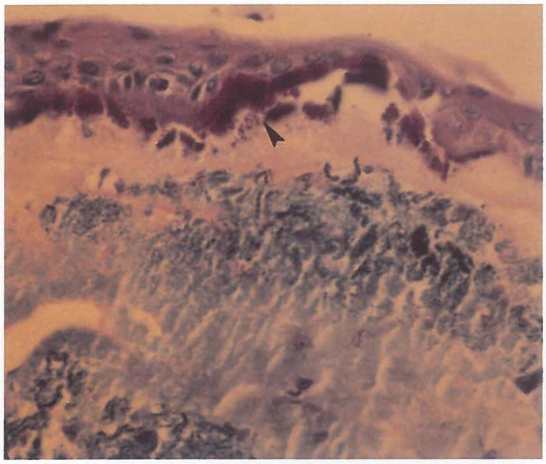

Figure a. Elastosis of the collagen fibers is visible as shaggy blue structures in the connective tissue. Calcification is visible under the conjunctival epithelium and to a mild degree in the basal cells (arrow). In the areas of elastotic degeneration of the collagen fibers no calcification is present. Giemsa stain, 520.*

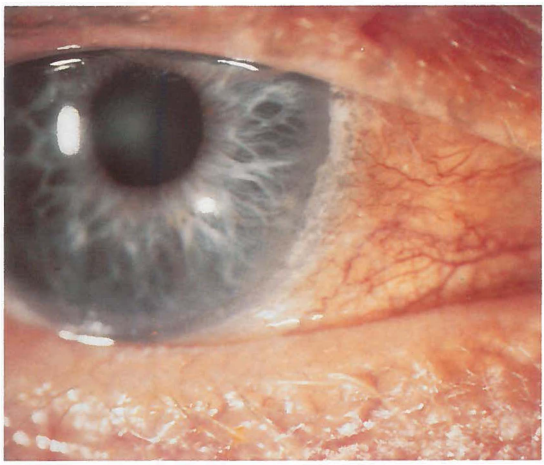

Figure b. Early band-shaped keratopathy. In this stage the calcium and phospate product is markedly elevated because of tertiary hyperparathyroidism. Note also the coarse white limbal calcification.

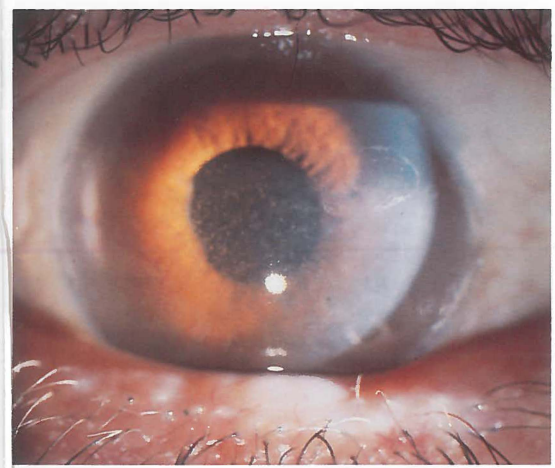

Figure c. Centrally located disc-shaped opacities in a band-like pattern in the interpalpebral area. Limbal calcification is visible.

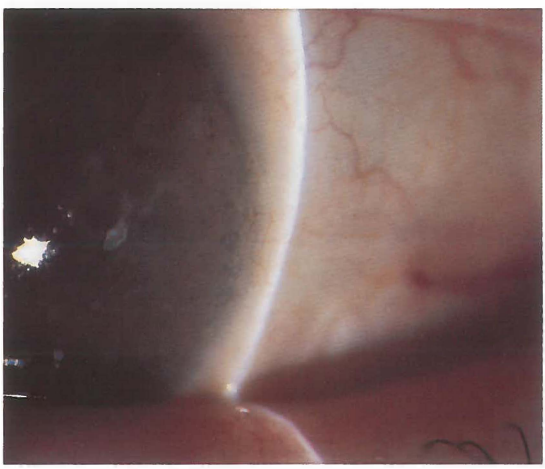

Figure d. Early band-shaped keratopathy. The calcific precipitation visible at the level of the membrane of Bowman shows the pathognomonic holes.

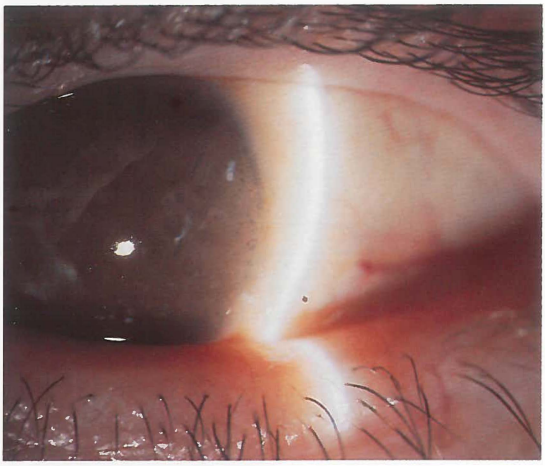

Figure e. The end-stage of the development of the band-shaped keratopathy. Visual acuity at this stage was 0.05.

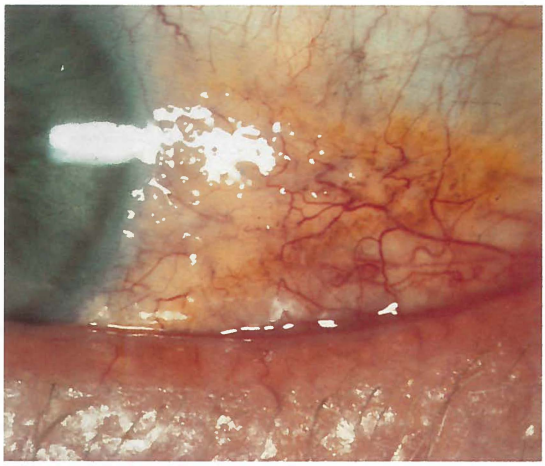

Figure f. Localized hyperaemia around a raised greyish area of the bulbar conjunctiva in chronic renal failure. Clinically these lesions are indistinguishable from inflamed pingueculae.

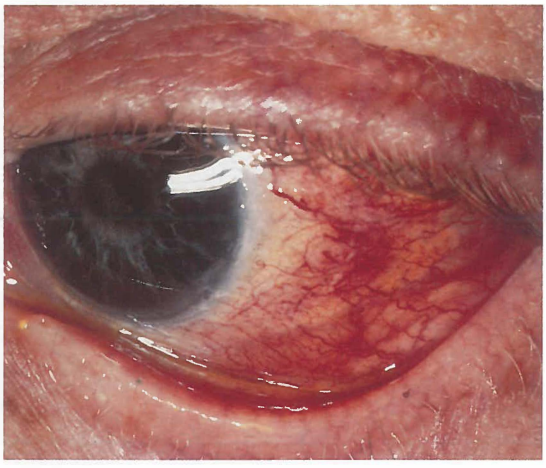

Figure g. More or less diffuse waxy red hyperaemia of the episcleral tissue and the conjunctiva over it extending beyond the palpebral fissure in patients with renal failure having strikinly high serum calcium concentrations.

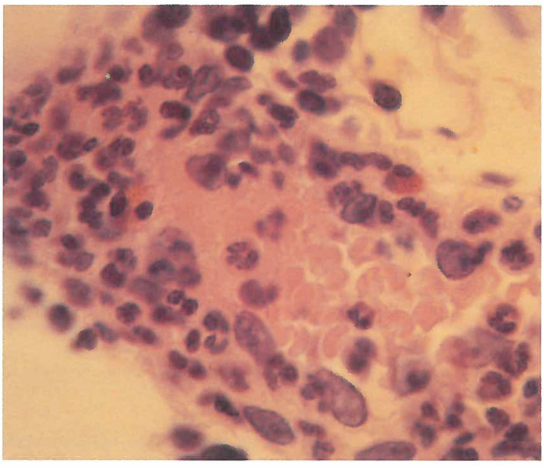

Figure h. Histopathology of a conjunctival biopsy of a patient from group C, clinically characterized by a waxy red episcleral hyperaemia extending beyond the palpebral aperture. Pavementing of the endothelium by polymorphonuclear leucocytes of the vascular wall is shown. Also shown are lymphocytes, eosinophilic cells, plasma cells, as well as perivascular infiltration of leucocytes. Haematoxylin and eosin stain, 900.*

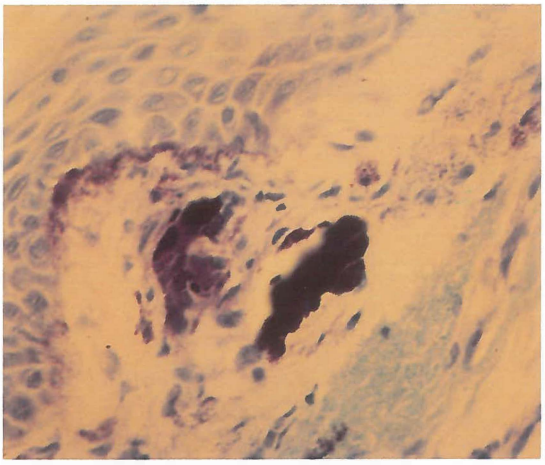

Figure i. Patient from group C: subepithelial calcific deposits as a large plaque adjacent to small calcific granules without any crystal phagocytosis. Giemsa stain, 520.*

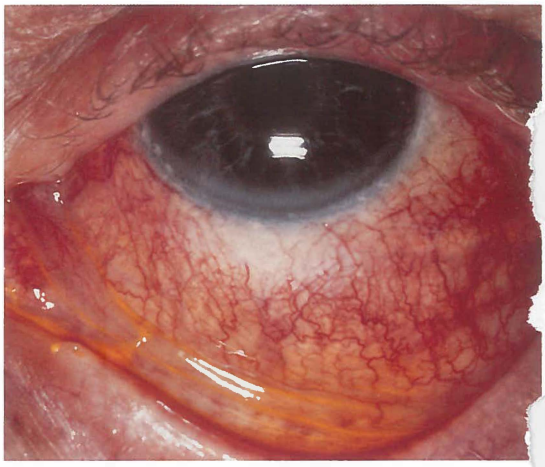

Figure j. Diffuse combined conjunctival and episcleral hyperaemia in a patient with acute shedding of calcium precipitates in the interpalpebral zone.

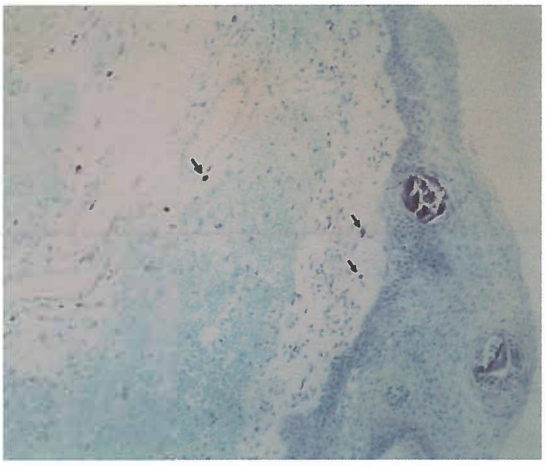

Figure k. Conjunctival biopsy of a patient with combined episcleral and conjunctival hypermaemia: a moderate amount of mastocytes (arrows) is present in the substantia propria: both in the nearing as at a certain distance from the calcific precipitates. Toluidine blue stain, 410.*

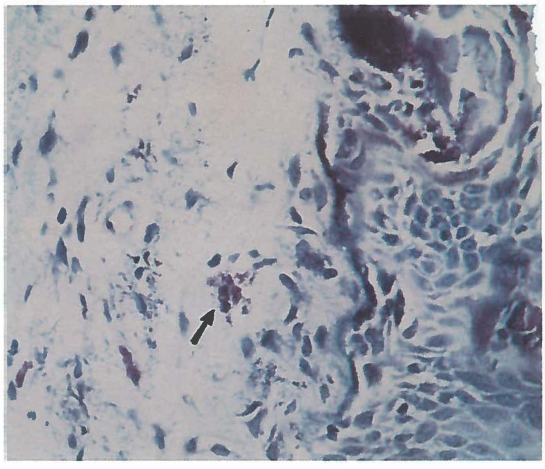

Figure l. Conjunctival biopsy of a patient with combined episcleral and conjunctival hyperaemia: a degranulating mastcell (arrow) is present next to the calcium precipitate. Giemsa stain, 900.*

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CONTENTS.

Key words

axon-reflex
body weight
blood osmolality
BUT (tearfilm break-up time)
band-shaped keratopathy
calciphylaxis
challenger
chronic renal failure
conjunctival, limbal and corneal calcification
conjunctival hyperaemia
crystal-induced inflammation
dialysis
dystrophic calcification
episcleral hyperaemia
haemodialysis
hydroxyapatite
intraocular pressure
lactoferrin test
Lissamin green staining test
local challenger
lysozyme test
metastatic calcification
peripheral corneal calcification
phagocytosis
pinguecula
pingueculitis
red eyes in/of renal failure
renal transplantation
Schirmer test
sensitizer
serum calcium concentration
serum phosphate concentration
serum calcium and phosphate product
tear flow
secondary hyperparathyroidism
tertiary hyperparathyroidism
white limbus girdle of Vogt type I and II

GENERAL INTRODUCTION

I. Historical background.

During centuries the life span of patients with serious renal disease was rather short due to lack of specific treatment modalities. In the last 70 years, however, there have been various major break-throughs in the treatment of renal failure which prolonged the life of patients with hitherto lethal kidney diseases. In the autumn of 1924 George Haas (Giessen, Germany) performed the first human dialysis in a patient with terminal kidney insufficiëncy.

Almost twenty years later, in 1943, the rotating dialyser was developed bij Kolff (1) in the department of internal medicine in Kampen, the Netherlands. This method proved to be suitable for human application in practice and over the past fifty years the dialysing technique of Kolff has been elaborated on and modified and is now applied worldwide. In 1993 the number of patients in the Netherlands with chronic renal failure that underwent dialysis treatment was approximately 3400 (i.e. one in 5 thousand inhabitants). About 2400 of these patients require haemodialysis and about 1000 peritoneal dialysis (Data supplied by the Foundation "Renine").

Although dialysis treatment results in an impressive prolonged life-expectancy of patients, it does not completely restore the metabolic consequences of renal failure and in fact it has to be stated that dialysis itself causes a certain degree of morbidity. Therefore, while the treatment of renal failure continued to improve, there has been a keen interest by many medical disciplines to study the complications of chronic renal failure and this is also true for the specialty of ophthalmology.

II. Metabolic consequences of chronic renal failure.

The kidneys play a pivotal role in the clearance of exogenous and endogenous substances from the blood via the production and excretion of urine. In addition, the kidneys have several endocrine activities. Clinically, chronic renal failure is characterized by the retention of fluid and sodium, leading to hypertension and accelerated atherosclerosis. Hyperkalaemia and acidosis are usually present as well as hyperuricaemia and uremia. Ureum is considered by some investigators to be directly toxic to cells and uremia is associated, for example, with pericarditis and thrombocytopathy. Anaemia is

commonly present as a result of the reduced production of erythropoietin by the diseased kidneys.

The loss of renal function leads to progressive disturbances of the calcium and phosphorus metabolism and is - as a rule - accompanied by the development of abnormalities in bone formation. Metabolic acidosis, as a consequence of renal failure, causes an increased bone resorption and a decrease of skeletal calcium. In addition, there will be hyperphosphataemia as a result of phosphate retention. Increased levels of ionized calcium, being released from the bone, in the presence of elevated phosphate levels, can lead to the formation of calcium-phosphate complexes and subsequently, under proper local conditions, to the deposition of calcium-phosphate salts in the tissues, i.e. soft tissue calcification or extraskeletal calcification.

The formation of calcium-phosphate complexes causes a transient decrease in the serum level of "free" calcium which is a main stimulus for the parathyroid glands to increase its production of parathyroid hormone (PTH). In most patients the serum calcium levels can be maintained within normal limits as a result of this adaptive hyperactivity of the parathyroid glands. However, as renal disease progresses the increased levels of PTH are responsible for the development of abnormalities in bone formation, a condition that is referred to as renal osteodystrophy.

Phosphate retention contributes even more to the maintenance of secondary hyperparathyroidism by blocking the enzyme 1-alpha-hydroxylase, an enzyme that is necessary for the final step in the transformation of vitamin D in its bioactive form, calcitriol. The resultant lower levels of calcitriol lead to hypocalcaemia by a decreased intestinal calcium resorption and the serum levels of PTH will continue to increase.

When dialysis treatment is initiated, the urinary production, and thus the excretion of phosphate, is usually minimal. No form of conventional dialysis is able to excrete the phosphate sufficiëntly. Also, the serum phosphate concentration cannot be sufficiëntly reduced by the use of a phosphorus restricted diet. Therefore, the administration of phosphate binders is necessary in almost all anuric patients.

Essentially, two different types of dialysis exist. In **haemodialysis**, peripheral blood is pumped along dialysis membranes with a counter-current flow of a dialysis-buffer. Those patients who require haemodialysis are usually treated between 9 and 12 hours per week in two or three equally divided

sessions. In **peritoneal dialysis** (CAPD = continuous ambulatory peritoneal dialysis) the dialysis buffer is instilled into the peritoneal cavity by means of a semipermanent catheter. Patients who are treated with CAPD receive this treatment continuously 24 hours a day.

III Ocular complications as a consequence of chronic renal failure and dialysis treatment.

III.1 Changes in the volume status during dialysis.

As a result of conventional dialysis treatment several more or less osmotic active substances, such as glucose, sodium and urea, are elininated from the blood. There will also be a substantial removal of body fluids that can be as much as two liters per haemodialysis session. Both these factors might act upon the production of aqueous fluid and, therefore, upon the intraocular pressure. Also, the tear fluid production might be affected by intermittent rapid changes in the volume status of the patient. In Chapter 1 several relevant clinical and laboratory tests for tear gland function are discussed.

The loss of body fluids tends to lower the intraocular pressure, whereas a reduction in blood osmolality will contribute to an increase in the intraocular pressure. There is a marked and unsolved controversy in the reports of both clinicians and researchers on the effect of dialysis on the intraocular pressure (2-15). Therefore, we have restudied this topic by measuring the intraocular pressure before and every hour during haemodialysis in a number of patients. The data of this study are presented in Chapter 2.

A prominent sign in patients undergoing dialysis is the development of "red eyes" (16-23). Porter and Crombie (22) believed this to be associated with a markedly decreased tear volume. It is entirely possible that during treatment, as a result of loss of body fluids, tear production is reduced to such a degree that it gives rise to the signs and symptoms of an acute and temporary keratoconjunctivitis sicca with a concommitant "red eye". In Chapter 3 the hypothesis of Porter and Crombie is tested.

III.2 Disregulation of the calcium and phosphate metabolism and the development of soft tissue calcification.

Even when patients are adequately treated with dialysis and conservative dietary measures, an elevated calcium-phosphate product is not uncommon. It is generally accepted that this elevated product is associated with the development of extraskeletal calcifications. Several tissues are involved in extraskeletal, or soft tissue calcification in dialysis-patients: arterial, subcutaneous, cutaneous and visceral calcifications as well as calcifications in the joints are frequently present (24). Corneal and conjunctival calcification, however, are the most common types reported in patients undergoing long-term dialysis (24) and it is the only type of soft tissue calcification seen in children with renal failure (25).

Virchow (26) believed rightly so that calcium salts dissolved from bone in uremia were carried in the blood and were deposited at some distant site. As this process resembles the dissemination of cells from a neoplasma, he coined the term metastatic calcification. The essential feature of metastatic calcification is its occurrence in previously normal tissue exposed to an abnormal chemical environment, in contrast to dystrophic calcification which occurs in abnormal tissue in a normal chemical environment.

It has been claimed that in uremia both metastatic and dystrophic factors may act together in a variable proportion in the pathogenesis of soft tissue calcification (19,21). In the strict sense, the term dystrophic calcification is not applicable to ocular calcification in dialysis patients. In a wider sense, however, the definition of dystrophic calcification in renal failure is in need of study as clinically and histopathologically the corneal and conjunctival changes in renal failure that precede calcification closely resemble the white limbus girdle of Vogt type II and pingueculae respectively. By studying the relationship between the degree of calcification and the magnitude of conjunctival degeneration as well as the association between the degree of calcification and the levels of serum calcium and phosphate and their product, we tried to clarify the proportion of metastatic to dystrophic calcification in the local pathogenesis of ocular calcification (Chapter 4).

Almost all patients on regular dialysis show limboconjunctival calcification. Doughman et al. (27) were able to induce corneal calcification in rabbits that were treated with dihydrotachysterol and subjected to various

wounding procedures and Fabian (28) demonstrated calcium precipitation in rat corneas after exposure. Dramatic was the clinical observation of Bloomfield et al. (29) of the development of an acute corneal calcification in a patient with renal failure due to a rapidly progressive glomerulonephritis with a high serum calcium and phosphate product in an eye that was accidently exposed for two hours.

Local injury of the outer eye in patients on chronic dialysis treatment is likely to develop as we demonstrated a marked decrease in tearflow after each dialysis session (Chapter 2). In Chapter 5 we measured the pre- and postdialytical teargland function in a larger group of patients. The latter study focusses on the role of tissue devitalisation - as a result of the chronic recurrent decrease of tearfluid production after each dialysis session - in the development of limboconjunctival calcification.

In the course of renal failure, some patients show a tendency to increasing, medicamentally incontrollable, levels of serum calcium and parathyroid hormone. This condition is by some clinicians referred to as tertiary hyperparathyroidism. In Chapter 6 we studied a group of dialysis patients with tertiary hyperparathyroidism and hypothesized that these patients, in analogy with patients with primary hyperparathyroidism without renal insufficiëncy, could possibly show a true corneal component in calcification in addition to limboconjunctival calcification.

Occasionally, true corneal calcification can develop in an unexpected and atypical way that rapidly progresses and finally results in a seriously decreased visual acuity (Chapter 7). Although the patient who is described in this chapter did not show the typical features of tertiary hyperparathyroidism, such as very high levels of parathormone, he did show a marked elevation of the calcium and phosphate concentrations. Peritoneal dialysis had to be changed into haemodialysis for a better control of these ion levels.

III.3 The disregulation of the calcium and phosphate metabolism, precipitation of lime salts and inflammatory reactions in the eye.

A serious disturbance of the calcium and phosphate levels, despite dietary measures and dialysis treatment, can be related to the use of calcium containing phosphorus binders. The use of calciumcarbonate, for example, can

lead to hypercalcaemia. In 1990 we observed a patient with diffuse conjunctival and episcleral hyperaemia that was associated with a sudden rise of the serum calcium concentration caused by an overdose of calciumcarbonate. This observation prompted a number of studies on inflammatory reactions in patients with chronic renal failure" (Chapter 8).

Calcium-phosphate salts are sparingly soluble and if their - local - concentrations are large enough to exceed the solubility product, lime salts readily precipitate in the form of microcrystalline hydroxyapatite (30). In 1966 Abrams (16) was the first to draw attention to the association of "irritable red eyes" and calcific corneal deposits in a patient with chronic renal failure. Subsequent clinicians (17,18,20,23) studied this phenomenon and the association with the deposition of calcium in the cornea and the conjunctiva was generally accepted.

In 1968 Berlyne (17) formulated his skilful theory of crystal-induced inflammation - in analogy to gout - to explain the "red eyes of renal failure". This concept suggested ingestion of non-metabolisable crystals by a polymorphonuclear leucocyte followed by binding of the crystal surface to the membrane of secondary phagolysosomes. Disruption of this membrane and release of lytic enzymes caused destruction of the surrounding tissue. It is known that hydroxyapatite crystals also have a marked phlogistic potential (31). Chapter 9 describes in detail the pathophysiology of crystal-induced inflammation.

In Chapter 10 we tested the hypothesis of Berlyne by histopathological examination of biopsies taken at the height of the inflammatory reactions in patients with renal failure. It is of interest, however, that a large proportion of eyes with considerable lime salt precipitation remained "white". We studied in Chapter 11 the conditions that can lead to the development of focal and diffuse conjunctival inflammatory reactions. This chapter also speculates on the possible role of neurogenic inflammation in the development of acute diffuse conjunctival and episcleral hyperaemia that was associated with a sudden rise of the serum calcium concentration.

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CHAPTER 1

CLINICAL AND LABORATORY TEAR FUNCTION TESTS

*Klaassen-Broekema N, Mackor AJ, Bijsterveld OP van. The diagnostic power of the tests for tear gland related keratoconjunctivitis sicca. Neth J Med 1992; **40**: 113-6.*

Introduction.

Laboratory tests for the diagnosis of the dry eye syndrome, such as the lysozyme- and the lactoferrin test reflect the acinar and tubular function of the tear gland and have an excellent discriminatory ability. They do not have an immediate relation to ocular discomfort. Clinical tests, on the other hand, such as the Rose bengal staining test and the tear film break-up time, do have an association with superficial eye disease, such as dessiccation of the epithelium, due to tear gland deficiency and, therefore, have a direct relation to ocular discomfort. In order to trace any decrease in the tear gland function, before and after dialysis sessions, we performed the Schirmer test, the lysozyme- and lactoferrin test as well as the Rose bengal staining test and the tear film break-up time. In the next paragraphs these clinical and laboratory tear function tests will be discussed separately.

Schirmer's test.

The most commonly used clinical tear gland function test was developed by Schirmer in 1903 (1). It measures the amount of tear fluid, collected in a filter paper strip, which is produced in a period of 5 minutes. The filter paper strip is 35mm by 5mm, at one end of which a piece of 5 mm is bent. The corners of the strip are cut off. The bent piece is hooked over the lid margin and allowed to remain there for five minutes. The wetted part is measured excluding the bent 5 mm piece.

The Schirmer test seemed to be an excellent method for quantifying tear gland degeneration but unfortunately the overlap between the distribution of the values measured in patients with Keratoconjunctivitis Sicca (KCS) and control persons is considerable (2). The probability of misclassification is balanced at 16% when the limit between normality and disease is choosen at 5.5 mm wetting of the filter paper strip after 5 minutes. This means that at this limit one in every six patients is wrongly classified as being normal, and one in every six healthy controls is misclassified as having KCS.

The theory of basic and reflex secretion was proposed by Jones (3). He suggested that the basic tear fluid secretion was affected by the accessory lacrimal glands of Wolffring and Krause, whereas reflex secretion was affected

by the main lacrimal gland. In his view the Schirmer test measured the reflex secretion as the filter paper strip stimulated the trigeminal nerve. Therefore, the basal secretion should be measured by using local anaesthesia.

The overlap between the frequency distributions of Schirmer values in patients with KCS and control patients with and without local anaesthesia was considerable in both tests, but slightly less for the Schirmer test without anaesthesia. It was, therefore, concluded that the Schirmer test without anaesthesia had a better discriminatory ability than with anaesthesia, but this difference is clinically of no consequence.

The Rose bengal staining test.

The study of some pathologic conditions of the outer eye is facilitated by vital staining, i.e. instilling a dye in the conjunctival sac. One of the most important vital stains is rose bengal that stains those cells that have a tendency to keratinisation. The Rose bengal (Rb) test is, therefore, a parameter of corneal epithelial damage.

For vital staining of the cornea and the conjunctiva a number of stains were used by many investigators including fluorescein, methylene blue, scarlet red, rose bengal, Victoria yellow, Alcian blue and many others (4). Sjögren (5) was the first to use rose bengal staining for the diagnosis of KCS. Norn introduced the vital stain Lissamin green in 1973 (6).

Rose bengal is a fluorescein derivative (tetrachloro- tetraiodo fluorescein sodium). Despite chemical similarity, the two dyes differ completely with regard to vital staining properties. Fluorescein does not actually stain structures and diffuses further or penetrate into the aqueous humour from a surface defect (4), whereas Rose bengal stains cells, and especially those that tend to keratinisation.

In KCS, rose bengal yields punctate staining both of the cornea and of the part of the conjunctiva that is uncovered by the eyelids (7). However, an increased Rose bengal score can also be found in patients with infectious conjunctivitis, allergic conjunctivitis or other forms of chronic irritative conjunctivitis. This makes the Rb score less specific as a diagnostic test for KCS.

The clinical procedure for performing the Rose bengal test was

discussed by many authors (4,8-10,11,12). Most investigators used a concentration of 1% rose bengal while Holm (8) used a 2-4% solution. Norn (4) compared the staining characters of rose bengal 1% and rose bengal 10%. He concluded that mildly degenerated cells are not stained by 1% rose bengal but may be stained by the 10% concentration. On the other hand, the 10% preparation also stains normal structures of no clinical interest.

Most investigators advise irrigation of the excess dye while Norn (4) has pointed out that irrigation is only needed if a drop of dye is directly instilled from the pipette bottle because, as he explained, the conjunctival cavity can hold no more than one-fifth of a normal drop. He found no need for irrigation if a glass rod or a wooden stick is employed to convey a smaller dye drop.

Van Bijsterveld (12) introduced numerical scoring for the intensity of staining of both medial and lateral bulbar conjunctiva and of the cornea. Each section was given a score up to three points, so that a maximum score of nine points could be obtained. If one compares the frequency distribution of this parameter in patients with KCS and in control persons the overlap is small. If the limit between normality and disease is set at 3.5 points, the probability of misclassification is 10%. In daily practice this means that one in every ten controls is mistakenly considered to have KCS.

Rose bengal may cause ocular discomfort. The discomfort seems to be roughly proportional to the amount of stained epithelium. Hence, many authors use a surface anaesthetic prior to the test. Forster (10) and Norn (13) stated that this did not interfere with the results of the test. On the other hand, Sjögren (9) advised against the use of local anaesthetics as he observed some false positive results due to hypersensitivity to certain local anaesthetics with subsequent death of epithelial cells.

Tear film Break-Up Time (BUT).

The knowledge of the presence of a precorneal layer dates back to the last century, and so is the knowledge that the cornea will dry up if blinking is prevented (14). Wolff (15) designated this layer "the precorneal tear film". He described it as composed of three layers, an outer lipid layer, an intermediate aqueous layer and an inner mucoid layer.

The corneal epithelial surface is composed largely of lipophilic material and is, therefore, relatively hydrophobic. It would be difficult to wet this surface with an aqeous solution unless the solution contained an effective surfactant which would lower its surface tension. Mucus (glycoprotein) was viewed by Lemp et al. (16) as the principal factor in spreading and maintaining the tear film over the cornea.

Holly (17) hypothesized that the closing of the eyelids distributes conjunctival mucus over the cornea. The superficial lipid layer becomes compressed between the two adjacent lid edges while the rest of the tear film becomes sandwitched between the cornea and the palpebral conjunctiva. The opening of the eye creates a new tear film - air interface which is immediately coated by the expanding lipid film. This is closely followed by mucin spreading at the lipid water interface. In other words, the tear film regains its three-layered structure everytime a blink is completed.

In Holly's view, the dry spot formation is due to the migration of superficial layer lipids - when the eye is open - to the epithelial surface, and thus, contaminating the adsorbed mucin layer and converting it again into an hydrophobic layer no longer able to support the aqueous layer. According to this view, the tear film is essentially unstable and the blinking action of the eyelids is necessary to reform the tear film layer. This requires that the blinking interval be shorter than the tear film break-up time (18).

Norn (1) defined the tear film break-up time (BUT) as the time in seconds, after a complete blink, for dry spots of tear film to become visible on the cornea. The test is performed by instilling fluorescein in the cul-the-sac of the eye. The eyelids are not to be supported as this would constitute an important source of error since the framing effect of the lids might be interferred with and thus reducing the wetting time. If the BUT is less than 10 seconds, it is considered to be pathologic.

As the BUT is dependent upon a number of variables, the test does not have a strong diagnostic value in KCS. It is known, for example, that the BUT decreases with advancing age. The relation between the BUT and the number of blinks in normal persons corresponds to a second degree curve: the BUT increases initially and then decreases rapidly.

It is important to realize that the BUT is related to changes and damage of the corneal epithelium as a result of tear gland deficiency and, therefore, is associated with ocular discomfort. The BUT is also related to the amount of

tear fluid secretion and to the tear protein concentration. Because of all these variables the BUT is not a particularly powerful test for the diagnosis of KCS. It is, however, the only test available for the diagnosis of tear film instability.

Tear fluid proteins.

Initial reports of the presence of tear proteins date back to 1922 (19). Gachon et al. (20) performed electrophoretic analysis of tear fluid and detected at least 60 proteins. Some of these proteins appeared to be present in tears in a much higher concentration than in serum and are now known to be synthesized by the lacrimal gland. Lysozyme, lactoferrin and tear-specific prealbumin, which are major components of the lacrimal fluid, are the most important examples (20,21).

Meyer (22) was the first to show that the tear lysozyme concentration was decreased in patients with KCS. His observation was confirmed by Regan (23) who found the lysozyme concentration to vary with the severity of the disease. The tear fluid proteins are synthesized in the acinar and tubular structures of the tear gland and it is evident that the degree of tear gland degeneration correlates strongly with the level of these tear fluid proteins.

The Lysozyme test.

Lysozyme is secreted both in the tubular and the acinar structures of the tear gland (24). The concentration of this protein in tear fluid can be assessed by an agar diffusion technique using Micrococcus lysodeikticus as substrate (12). In this agar-diffusion test filter paper discs of Whatman no. 3 are used. These discs have a diameter of 6 mm and are to be placed in the lower cul-de-sac of the eye. After the disc is entirely wet it is removed and excess fluid is eliminated by blotting it lightly on Whatman no. 3 filter paper. A meat infusion agar plate is flooded with a 24 hour broth culture of Micrococcus lysodeikticus and the excess fluid removed. The discs are then placed on the plate and the diameter of lysis is measured after incubation for 24 hours at 37 degree's Celsius. The level of tear fluid lysozyme is expressed in hen egg-white lysozyme (HEL). When the limit between normality and disease is set on

1425 microgram per milliliter HEL the probability of misclassification is 1.5%.

The Lactoferrin test.

Gillette and associates (24) demonstrated that lactoferrin was secreted in the acini but not in the tubuli of the tear gland. The concentration of lactoferrin in the tear fluid is assessed by an immunoprecipitation technique (25). This lactoferrin test does not require specific laboratory facilities and is commercially available ("Lactoplate", JDC Culemborg, the Netherlands). The test is performed by collecting tear samples using filter paper discs which are to be placed on previously prepared immunodiffusion plates. Precipitation rings are then allowed to develop for 24-48 hours at room temperature. From the ring diameters observed, the lactoferrin content can be calculated. If the limit between normality and disease is set on 950 microgram per milliliter (= 9.8 mm diameter of precipitation) the probability of misclassification is around 1.5%.

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CHAPTER 2

INTRAOCULAR PRESSURE DURING HAEMODIALYSIS

*Broekema N, Bijsterveld OP van, Bos Kuil RJC de. Intraocular pressure during haemodialysis. Ophthalmologica 1988; **197**: 60-4.*

Abstract.

In 14 patients undergoing haemodialysis, the intraocular pressure did not change significantly when measured at hourly intervals for 4h. There was a loss in body weight because of fluid loss; also the Schirmer values decreased significantly at the conclusion of treatment. Blood osmolality decreased markedly in a linear fashion. The fluctuations of intraocular pressure during haemodialysis were likely dependent on the dynamics of dehydration and hypo-osmolality. Regulatory forces stabilized the intraocular pressure to such a degree that statistical significance in these fluctuations was not reached.

Introduction.

Haemodialysis is a treatment for patients with severely compromised renal function. The principle of conventional haemodialysis is the elimination of osmotic active substances by diffusion, resulting in loss of body fluids and a decrease in blood osmolality. Both these factors have an effect on the intraocular pressure. Dehydration tends to lower the intraocular pressure whereas a reduction in blood osmolality will contribute to an increase in intraocular pressure.

In fact, marked changes in intraocular pressure have been observed both in animal experiments (1) as well as in patients under regular intermittent haemodialysis (2-4), but these were not considered significant by other authors (5-8). Ramsell et al. (9) found that the intraocular pressure at the beginning and at the end of haemodialysis was not significantly different, but they pointed out that if these pressures were studied hourly significant differences could be established. This claim, however, was challenged by Gutmann and Vaziri (10).

Dialysis seems to exert different and probably opposing influences on the intraocular pressure. Therefore, we have restudied the effect of haemodialysis on the intraocular pressure in patients to clarify the controversial views on this topic. The general volume status was defined by weight loss, preceding interdialysis weight gain, and the behavior of blood pressure. As a local indicator of volume, the Schirmer test was done, as these parameters could help to establish the ocular fluid dynamics in haemodialysis.

Table I. Number, age and sex of the individual patients with their clinical diagnosis, duration of haemodialysis treatment in years and the duration of haemodialysis per week.

No	Age	S	Diagnosis	D	Dur.
1	42	M	Polycystic disease	4	3x4.5
2	57	F	Polycystic disease	10	3x3
3	66	M	IgA nephropathy	4	1x3.5,2x3
4	60	F	Unknown	7	2x3
5	50	M	Neurogenic bladder	4	3x2.5
6	52	F	drug-induced	6	3x3
7	57	M	benign M. Kahler	2	3x3
8	33	F	Polycystic disease	7	2x4.5
9	60	F	Glomerulonephritis	2	2x3
10	42	F	Polycystic disease	3	3x4
11	58	M	Glomerulonephritis	10	3x4
12	56	M	Polycystic disease	11	3x4
13	40	F	Hypoplasia	4	3x4
14	43	M	Polycystic disease	10	1x4,2x3,5

S = sex
D = duration of haemodialysis in years
Dur. = duration of each dialysis in hours/week

Patients, materials and methods.

A total of 14 patients, 7 males ranging in age between 42 and 58 years and 7 females ranging in age between 33 and 60 years, who were undergoing haemodialysis for periods from 2 to 11 years, were studied. Eleven patients were dialyzed three times a week, with a mean duration of each dialysis of 3.6 h. Three patients were dialyzed only twice a week with a mean duration of 3.5 h. In Table I, the patient characteristics and haemodialysis details are shown. All dialyses were performed using the AK-10 Gambro system. Patients were allowed unlimited

intake of fluid during this period.

Intraocular pressure was measured in both eyes in an upright sitting position with the Haag-Streit applanation tonometer before and every hour during haemodialysis and at the termination of the treatment. Blood samples were taken in order to determine blood osmolality which was measured by freezing-point depression (Advanced Instruments, New Highlands, Mass.). Body weight was measured using the Seca scale, model 713. Blood pressure was measured before and after haemodialysis. Schirmer tests were performed before and after haemodialysis using filter paper strips of 5x35 mm. The wetting of the strip was recorded after 5 min. Variance analysis and regression analysis were used as statistical tests.

Results.

In Table II, the average intraocular pressure of the patients at hourly intervals during haemodialysis is shown. As we could not demonstrate statistically any difference in intraocular pressure between the right and the left eye, the values were averaged for a better estimate. The initial decrease and subsequent rise in intraocular pressure suggested nonlinearity. In Figure 1 the original data (●) and also the second degree curve fitted to these data (●) are presented.

In Table III, the average loss in body weight expressed in kilograms is shown. Male and female patients differed on the average by 20.5 kg in initial weight. Loss in body weight due to haemodialysis was statistically significant both in males and females ($p < 0.01$); but there was no difference in weight loss between male (on the average 1.54 kg) and female patients (on the average 1.51 kg). The interdialysis weight gain was statistically not significantly different from the loss. The average gain was 1.63 kg.

Blood pressure changes were observed during haemodialysis. The systolic and diastolic blood pressure decreases were statistically significant ($p < 0.05$). The average systolic blood pressure before haemodialysis was 157 mmHg and the diastolic pressure 85 mmHg. After dialysis the average systolic blood pressure was 126 mmHg and the average diastolic blood pressure 74 mmHg.

A statistically significant decrease in Schirmer values during haemodialysis was observed ($p < 0.01$). The average initial Schirmer value was 17.5 mm wetting of the filter paper strip; there was no difference between males and females. At the conclusion of the dialysis, the average Schirmer value was 12.1 mm, which represented a percentage loss of 31%.

Table II. Average intraocular pressure of 14 patients before and during haemodialysis, measured every hour (T1-4) after the beginning of the treatment.

	before	T1	T2	T3	T4
av.	14.75	14.21	14.38	13.33	14.42
sd(n-1)	2.20	2.49	2.40	2.05	2.87

av. = average
sd(n-1) = standard deviation (n-1)

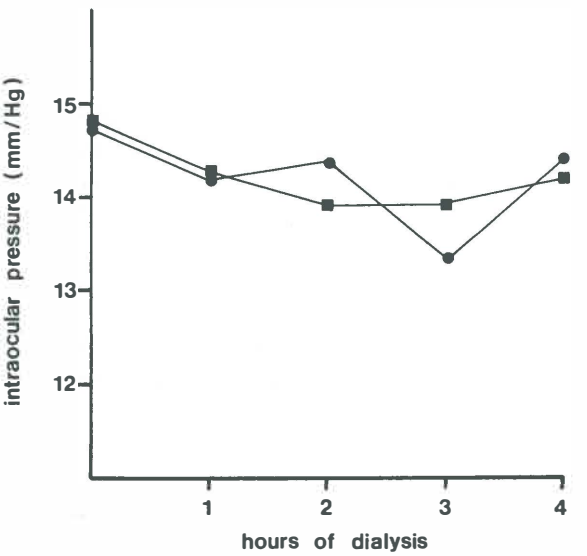

Figure 1. The average intraocular pressure before and during haemodialysis: a second-degree curve (■) is fitted to the original data (●).

Table III. Average loss in body weight, average decrease in systolic and diastolic bloodpressure and reduction of tear volume, standard deviations and levels of probability (p) in 14 patients undergoing haemodialysis.

	body weight	syst.b.p.	diast.b.p.	Schirmer test
average	1.53	31.0	11.1	5.39
sd(n-1)	0.75	26.66	12.43	5.70
p value	<0.01	<0.01	<0.05	<0.01

body weight loss expressed in kilogrammes
systolic and diastolic blood pressure expressed in mmHg
Schirmer test expressed in mm wetting after 5 minutes

No association was found between loss of body weight, decrease in systolic and diastolic blood pressure and reduction in tear volume on the one hand and the change in intraocular pressure on the other hand. A significant association was found between loss of body weight and decrease in systolic blood pressure. Loss in body weight and decrease in diastolic blood pressure or changes in tear volume were not correlated.

The regression of blood urea osmolality on the stated examination periods is linear ($r=-0.98$) and statistically significant ($p<0.001$).

Discussion.

All reports on intraocular pressure during haemodialysis, with the exception of that by Wizemann et al. (3), show an initial decrease in pressure,

which is also our experience. From the dynamics of haemodialysis one can expect an overall dehydration of the body because of loss of fluid. We found the body weight loss to be significant. It is known that in cases of dehydration such as is observed in diabetic coma, cholera and malnutrition ocular hypotony occurs (11,12), which, depending on the cause, can be of an acute nature. The initial decrease in intraocular pressure could be the result of dehydration.

The subsequent rise in intraocular pressure may have been the result of a disequilibrium between blood and aqeous osmolality. During treatment blood osmolality decreases. If the blood becomes hypotonic to the intraocular fluid, osmotic forces cause a transfer of fluids into the eye (13). As there is a steady fall in blood osmolality that shows a strong linear relationship with time, osmotic disequilibrium between blood and aqueous could be prolonged.

Therefore, one can expect a tendency to a progressive increase in intraocular pressure between 10 minutes (14) or later (15) after beginning of the treatment. In our patients, the fluctuations did not reach significance and this could indicate that other factors may have been operative to regulate intraocular pressure such as changes in the blood-aqueous barrier or changes in the facility of outflow.

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CHAPTER 3

REDUCTION OF TEAR FLOW DURING HAEMODIALYSIS AND THE RED EYE OF RENAL FAILURE

Klaassen-Broekema N, Bijsterveld OP van. Reduction of tear flow during haemodialysis and the red eye of renal failure. In: The Lacrimal System. O.P. van Bijsterveld, M.A. Lemp, D. Spinelli (eds.), 57-62, 1990.

Abstract.

A prominent sign in patients requiring dialysis is the development of hyperaemia of the outer eye. This study tested the hypothesis that a marked reduction of tearflow as a result of dialysis is responsible for the development of these "red eyes in renal failure". In spite of the dramatic decrease in Schirmer values in some patients, or even a decrease below 5 mm in others, after one haemodialysis session, in none of our patients a red eye developed.

Introduction.

Dialysis is a treatment for patients with severely compromised renal function. The principle is, on the one hand, elimination of more or less osmotic substances from the blood, and, on the other, removal of body fluids. Many patients on haemodialysis treatment complain of dry eyes. Strempel et al. (1) found that half of a group of patients with severe renal disease and/or kidney transplants had typical dry-eye symptoms and in over two-thirds of the patients a reduction of Schirmer values was measured.

A prominent sign in patients requiring dialysis is the development of the red eyes of renal failure. Porter and Crombie (2) found this to be associated with a marked reduction of tear flow in the interdialysis period. It is entirely possible that during treatment, as a result of loss of body fluids, tear production is reduced to a low level resulting in an acute and temporary keratoconjunctivitis sicca and that the "red eye" is an immediate irritative result of this decrease in tear flow.

On the assumption that haemodialysis does mediate an acute decrease in tear production, we measured the Schirmer values and tear protein concentrations before and after one haemodialysis session in a group of 14 patients on regular treatment. Also, the relation between the tear function and the changes in conjunctival and episcleral injection was studied, as well as the relation between the changes in the tear function profile and the changes in markers of the general volume status, such as body weight, systolic and diastolic blood pressure.

Table I. Predialysis tear function of the group of patients; values of the right and left eye were averaged for a better estimate.

	Lysozyme	Lactoferrin	Schirmer	Tear function
a	1700	11	6	
b	1400	10	12.5	KCS
c	1400	10	6.5	KCS
d	2300	13	26	
e	5000	15.5	11.5	
f	4900	8	1	
g	1650	10.5	30	marg.
h	1300	12.5	30	KCS
i	1750	9	10.5	marg.
j	3900	14	13	
k	2950	15	29.5	
l	2000	11.5	12.5	
m	1700	10	26.5	marg.
n	1550	12	30	marg.

KCS = keratoconjunctivitis sicca
marg. = marginal tearfunction
The lysozyme concentration is expressed as microgram per ml hen eggwhite lysozyme (HEL).
The lactoferrin concentration is expressed as mm diameter precipitation.

Materials and methods.

A total of 14 patients, 7 males ranging in age between 42 and 58 years and 7 females ranging in age between 33 and 60 years, who were undergoing hemodialysis for periods from 2 to 11 years, were studied. Eleven patients were dialyzed three times a week, with a mean duration of each dialysis of 3.6 hours. Three patients were dialyzed only twice a week with a mean duration of 3.5 hours. All dialyses were performed using the AK-10 Gambro system. Patients were allowed unlimited intake of fluid during this period.

Table II. Average loss in body weight and average decrease in systolic and diastolic blood pressure and the standard deviations (sd) and levels of probability (p) in 14 patients undergoing haemodialysis.

	Body weight	Syst.b.p.	Diast.b.p.
average	1.53	31.0	11.1
sd(n-1)	0.75	26.66	5.70
p value	<0.01	<0.01	<0.01

Body weight loss expressed in kilogrammes
Systolic (syst.b.p.) and diastolic blood pressure (diast.b.p.) expressed in mmHg

Tear function parameters were estimated in both eyes before and immediately after treatment. For the measurement of tear flow the Schirmer test was carried out, using filter-paper strips of 5 x 35 mm (Whatman no. 41). The wetting of the strip was recorded after 5 minutes (3).

Biomicroscopy, using the Haag-Streit slitlamp, was performed for grading conjunctival and episcleral hyperaemia before and after haemodialysis.

Tear samples to estimate the lysozyme concentration, which was determined by the agar diffusion method, were obtained by inserting a sterile Whatman no. 3 filter paper disc of 6 mm diameter into the cul-de-sac of the eye. The lysozyme concentration was expressed as microgram per ml hen eggwhite lysozyme (HEL) (4).

The lactoferrin concentration was determined by the radial immunodiffusion method, using the lactoplate (JDC, Culemborg, the Netherlands). The diameters were read after 72 hours, the concentration expressed in mm diameter precipitation (5).

The general volume status before and immediately after treatment was estimated by body weight, systolic and diastolic blood pressure. To compare differences in body weight, systolic and diastolic blood pressure and Schirmer values, as a result of haemodialysis, the non-parametric related sample test of Walsh was used. The Spearman-Rank correlation and partial regression analysis were used to analyze any associations.

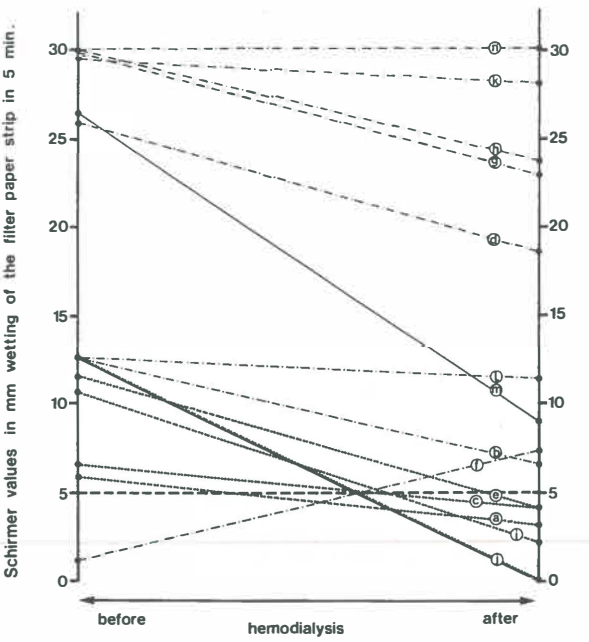

Figure 1. Schirmer values of each patient before and immediately after haemodialysis.

Results.

In Table I the predialysis tear function of each patient is shown. In Table II all the markers of the general volume status during haemodialysis are summarized. Weight loss, as a result of haemodialysis, was found in all 14 patients examined and this was statistically significant. On the average, 1.53 kg was lost and this represented a 2% weight loss of the initial value.

As we could not demonstrate any difference in Schirmer values between the right and left eye, the values were averaged for a better estimate. A statistically significant decrease in Schirmer values during haemodialysis was observed. The average initial value was 17.5 mm wetting of the filter paper

Table III. Correlation between the amounts of decrease of the components of the general volume status and the Schirmer values.

Components compared	Corr.coëff. r=	Significance
Body weight vs. Schirmer	+0.18	NS
Syst.b.p. vs. Schirmer	+0.37	NS
Diast.b.p. vs. Schirmer	+0.05	NS
Syst. vs. diast.b.p.	+0.33	NS
Body weight vs. syst.b.p.	+0.60	Sig., $p<0.01$
Body weight vs. diast.b.p.	+0.17	NS

syst.b.p. = systolic blood pressure expressed in mmHg
diast.b.p. = diastolic blood pressure expressed in mmHg
Corr.coëff. = correlation coëfficiënt
NS = not significant
Sig. = significant

strip and after haemodialysis the average Schirmer value was 12.1 mm wetting of the filter paper strip. The reduction represents a 31% loss of the initial values.

In Figure 1 the reduction of tear flow in each patient is graphically shown. There is a marked difference between the patients. In patients J and M a prominent reduction of 12.5 and 17.5 mm, respectively, is observed, whereas patients A, C, E, I and J even showed a decrease in tear flow below 5 mm. In one patient (F), however, an increase in tear flow was measured. In spite of the dramatic decrease in Schirmer values in some patients, or even a decrease below 5 mm in others, in none of our patients a red eye developed during haemodialysis.

The concentration of tear fluid lysozyme and lactoferrin did not change

statistically significantly during haemodialysis. The average values and the standard deviation before haemodialysis for lysozyme was 2393 microgram/ml HEL, sd (n-1) 1290 microgram/ml HEL, and after haemodialysis these values were 2764 and 1218, respectively. For lactoferrin the pre- and postdialysis values were 11.6 mm diameter precipitation with sd(n-1) 2.2 mm and 12.9 with sd(n-1) 3.9.

As could be expected, the systolic and diastolic blood pressure decreased during haemodialysis. The average percentage loss of systolic and diastolic blood pressure was 20 and 13%, respectively (Table II).

It appeared that the amount of loss in body weight was significantly associated with the amount of loss in systolic blood pressure but not with the amount of reduction of diastolic blood pressure, nor with the amount of reduction in tear flow (Table III). Both the amount of loss in body weight and in systolic blood pressure, when analyzed separately, were not associated with the amount of reduced tear flow. Analysis together, as in a partial regression analysis, also failed to show any association with the amount of reduction of tear flow.

In Figure 2 the relation between expected decrease in Schirmer values, calculated on the basis of the amount of reduction both in body weight and systolic blood pressure, and the observed decrease of Schirmer values are graphically shown. This figure shows that there was no association.

Discussion.

Patients in our study showed on the average normal initial Schirmer values and normal tear protein concentrations before haemodialysis. Nevertheless, three patients could be classified formally as having keratoconjunctivitis sicca and four other patients had marginal tear function, based on tear flow, lysozyme- and lactoferrin concentration, indicating that in some of our patients pre-existing tear function abnormalities were present. During haemodialysis the tear flow decreased significantly, which means that the tear volume is reduced to a low level shortly before and shortly after the termination of each treatment. After each haemodialysis procedure we found that the decreased tear flow returned to the level of their initial predialysis values.

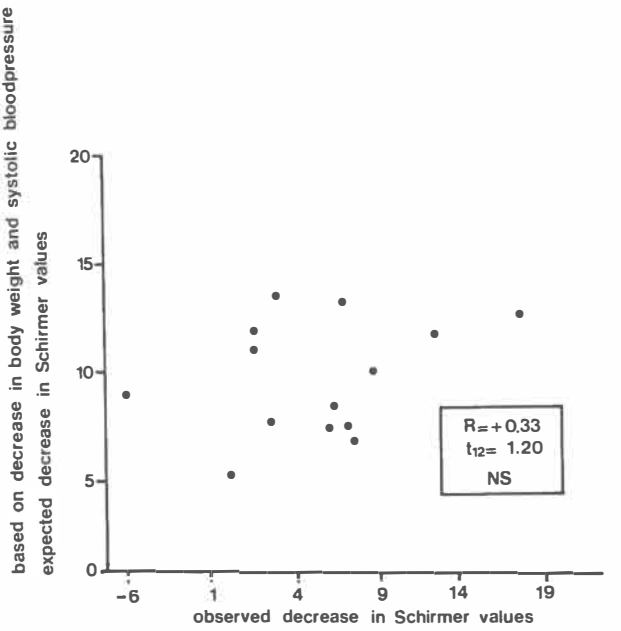

Figure 2. The relation between the expected decrease in Schirmer values, calculated on the basis of the amount of reduction both in body weight and systolic bloodpressure, and the observed decrease in Schirmer values.

We could not demonstrate any association between the marked decreasein tear flow during haemodialysis and increase in conjunctival or episcleral injection. Porter and Crombie (2) observed in 18% of their patients Schirmer values of 5 mm or less wetting of the filter paper strip and demonstrated that these low values were associated in slightly less than half of the cases with red eyes of renal failure. It is entirely possible that Porter et al. had in their group patients with pre-existing keratoconjunctivitis sicca, as we had in our group, and that the association with red eyes was coïncidental. Therefore, the red eye of renal failure is probably dependent on factors other

than tear function.

Our data indicate that the loss of body weight and the decrease in systolic blood pressure on the one hand, and the observed tear flow reduction on the other, are a consequence of different mechanisms, as no associations whatsoever were found between these parameters, analyzed separately or combined. Moreover, in spite of the observed reduction in tear flow we did not find a statisticallly significant change in the tear protein concentrations, suggesting that active regulatory mechanisms of tear function must be operative during haemodialysis.

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CHAPTER 4

METASTATIC OR DYSTROPHIC CONJUNCTIVAL CALCIFICATION IN RENAL FAILURE?

Klaassen-Broekema N, Landesz M, Krul B, Bijsterveld OP van. Metastatic or dystrophic conjunctival calcification in renal failure? Eur J Ophthalmol 1992; 2: 150-4.

Abstract.

In renal failure the incidence of pingueculae is significantly higher than in a comparable control group but there is no evidence that the calcific precipitation in renal failure is of a dystrophic nature. The lime salts are not located within the area of elastotic degeneration, a prerequisite for the definition of dystrophic calcification. Moreover, there is no association between the magnitude of the conjunctival degeneration and the degree of calcification. We, therefore, suggest that the calcium precipitates are more likely to represent metastatic calcification even though admittedly support for this assumption is tenuous.

Introduction.

In patients with renal failure undergoing regular intermittent haemodialysis the most common location of soft tissue calcification is the limbus and conjunctiva. The calcifications are visible as subepithelial whitish deposits in the limbus arranged as one or more whitish lines of crystals close to the peripheral cornea and concentric to it. In the conjunctiva the calcific deposits are located at the palpebral aperture, mostly close to the limbus and with their centre on the horizontal meridian (1-3).

Local factors may contribute to the precipitation of calcium-phosphate salts. It is believed that because of the loss of carbon dioxide from the surface cells in the interpalpebral area secondary to the high carbon dioxide gradient between these cells and the atmosphere to which they are exposed, the pH rises so that in the presence of a high serum calcium and phosphate product calcium salts are deposited preferentially in this area (4).

Clinically and histopathologically the limbal changes in renal failure resemble the white limbus girdle of Vogt type II while the conjunctival changes are very similar to pingueculae with whitish calcific deposits (5). The white limbus girdle and the pingueculae are considered age-dependent degenerative changes. This led Ehlers et al. (5) to speculate that the ocular complications in renal failure were none other than accelerated age changes.

In patients with renal failure on chronic intermittent haemodialysis the calcium and phosphate product is increased as compared to healthy persons.

Therefore, calcium deposits are considered to be of the metastatic type. However, in renal failure pingueculae are often present (2, 5-8), although their precise incidence is not known. The calcium precipitates are clinically visible in the areas of the pingueculae or in flat conjunctival degeneration. Therefore, it is questioned whether the calcific deposits in renal failure represent metastatic or dystrophic calcification, or both (9).

In this study we have established the incidence of pingueculae in patients with renal failure and compared it with the incidence of these conjunctival degenerations in control persons. We have studied the relationship between the degree of calcification and the magnitude of the degeneration and the association between the degree of calcification and the serum calcium and phosphate product.

Materials and methods.

Incidence of pingueculae.

Fifty-seven dialysis patients were evaluated for the presence and location of pingueculae. Of these patients, 46 did not experience any inflammatory reaction of the outer eye during the observation period of six years (group A); eight patients had a period of an inflammatory reaction associated with pingueculae (group B), and three patients (group C) had a waxy red episcleral and conjunctival inflammatory reaction that extended beyond the palpebral aperture during the observation period. At the time of examination, however, all patients were in a quiescent stage with regard to any inflammatory condition of the outer eye they may have had. As controls, 150 persons without renal failure from the outpatient department were selected, with the same male to female ratio as the patients, 20 in each of the same age groups as the patients.

Calcification and conjunctival degeneration.

The relationship between the surface area of nasal and temporal degeneration of the conjunctiva, including the surface area of the pingueculae if present, and the degree of calcium deposits expressed as the "Porter and Crombie" score (3), was studied in 36 patients who were willing to pose long enough to have drawings made by an experience illustrator of the areas of conjunctival degeneration, under identical conditions and all on the same scale (1:150).

The degree of calcification and the Ca and P product.

To study the relationship between the degree of calcification and the product of the serum calcium and phosphate concentrations, only patients from group A were used. The calcium and phosphate products of patients of groups B and C were or had at one time or another been prone to marked fluctuations. In order to minimize the effect of fluctuations of the serum calcium and serum phosphate concentrations, the average values for the product of

both parameters were calculated from ten predialysis serum calcium and phosphate values collected over a period of three years, or a shorter period if dialysis did not exceed this time.

Histopathology.

Conjunctival biopsies were taken from six patients with large pingueculae and clinically visible calcific deposits. The tissues were fixed in phosphate buffered neutral formalin (10%). Histological sections were processed by standard methods. The histochemical stains used were hematoxylin-eosin to reveal the general cell structure, Giemsa stain and toluidine blue for the study of polymorphonuclear leucocytes. The van Gieson stain was used to study elastotic degeneration of connective tissue. Calcium deposits were shown by the von Kossa stain. The sections were examined by light microscopy. The linear regression method, the Mann-Whitney U test and the Chi-squared test were used for statistical analysis.

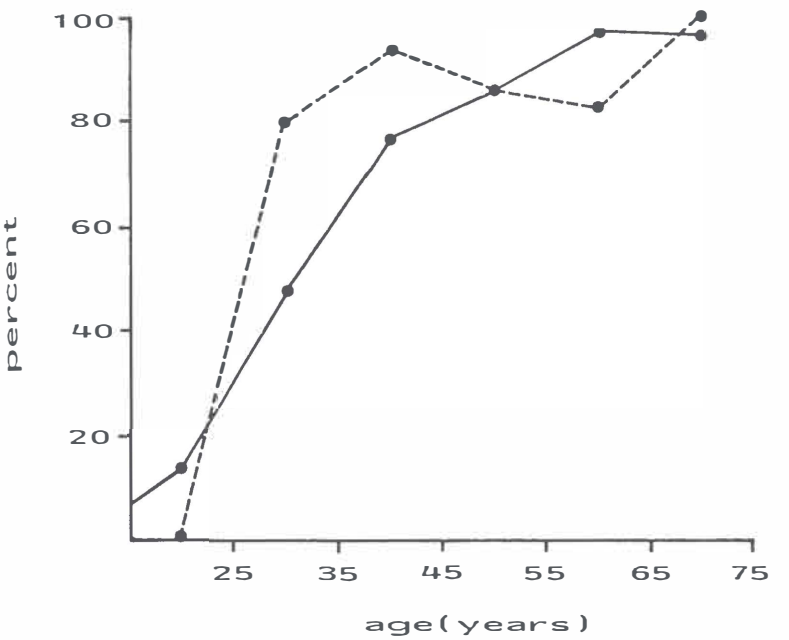

Figure 1. Distribution of pingueculae in the stated age categories for patients with renal failure (dotted line) and controls (solid line). There is a moderate but significantly higher incidence of pingueculae in patients with renal failure.

Table I. The percentage pingueculae in patients with renal failure and controls.

	present	absent
patients	86	14
controls	71	29

Results.

Figure 1 shows the percentages of patients and controls with pingueculae in the various age classes. Although there is a difference, on the whole it is small. A significant difference between both groups could only be established by analysing the number of pingueculae of all patients, versus all controls (Table I). The Chi-square value (Yates correction for continuity and calculated for the actual numbers) is 4.36, $p<0.05$. Table II shows the average serum calcium and phosphate concentrations and their standard deviations, as well as their products with the standard deviations.

The unit surface areas of conjunctival degeneration did not differ significantly between the right and left eyes. The unit surface area of conjunctival degeneration and the degree of calcification, the latter being averaged over both eyes ($r=0.01$). Also no significant association was present between the average serum calcium and phosphate product and the Porter and Crombie score but there was a trend: the higher the product the greater the degree of calcification (Figure 2).

Subepithelial and modest epithelial calcium deposits were found in all six biopsies. The epithelial deposits were observed in the basal cells adjacent to the subepithelial calcium deposits. Elastosis of the connective tissue was

Table II. Average serum concentration of calcium (mmol/l) and phosphate (mmol/l) with the standard deviations and the calcium and phosphate product of the 3 groups examined.

	Group A n=46		Group B n=8		Group C n=3	
	av.	sd	av.	sd	av.	sd
calcium	2.49	0.28	2.61	0.27	3.53	0.65
phosphate	1.85	0.65	1.92	0.53	1.92	0.64
Ca*P	4.52	1.33	5.02	1.41	6.62	1.62

av. = average
sd = standard deviation sd(n-1)
Ca*P = calcium and phosphate product

prominent in these biopsies. Although calcium deposits were usually found in the subepithelial layers above the degenerated areas, no calcium infiltration was found within the area of elastotic degeneration of the conjunctiva itself (Figure a, see addendum).

Discussion.

In addition to pingueculae, conjunctival degeneration can also present itself as flat opaque yellowish-white areas. Histopathologically they do not differ from pingueculae as they are also characterized by thickened epithelium, atrophy of the subepithelial layes, hyaline degenerations and early elastosis.

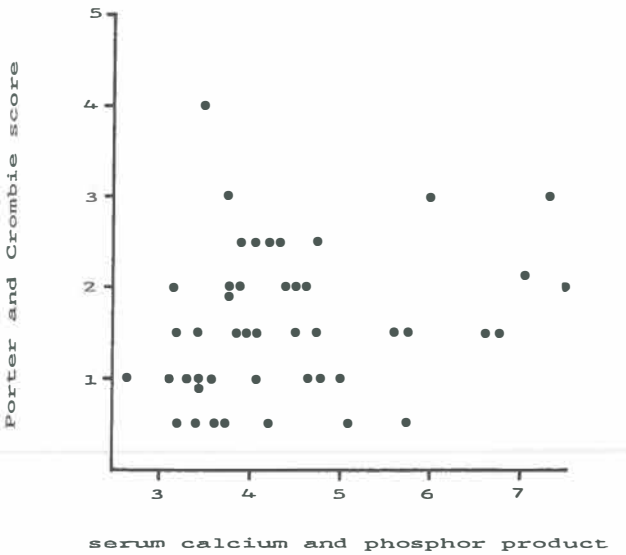

Figure 2. Regression of the serum calcium and phosphate product against the degree of conjunctival calcification expressed in the Porter and Crombie score. The association is not statistically significant (correlation coëfficiënt r=0.20 and t=1.31).

We could not find calcium deposits within the degenerated conjunctiva; this was also the finding of Ehlers et al. (5). Moreover, there was no correlation whatsoever between the size of the degenerated area and the degree of calcification.

When the calcium and phosphate solubility product is exceeded locally, it is assumed that deposition of lime salts will result, and this is referred to as metastatic calcification. In renal failure the serum calcium and phosphate product is very often elevated and the local solubility product is, therefore, likely to be exceeded.

Also in healthy persons the interstitial fluids are supersaturated with respect to calcium and phosphate ions (10). However, the liquid to solid transformation of calcium and phosphate ions will occur only under proper local conditions, such as ion concentrations, ion complex formation and pH. Differences in these local conditions between the patients on dialysis treatment may be responsible for the poor correlation between the calcium and phosphate product and the degree of calcium precipitation in the tissues.

In conclusion, on the basis of our clinical examination and the suggestive histopathological findings, we feel that soft tissue calcification in patients with renal failure is not of the dystrophic type but is most likely of the metastatic type, although we were unable to demonstrate an association between the degree of calcification as expressed in the Porter and Crombie score and the serum calcium and phosphate product.

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CHAPTER 5

A LOCAL CHALLENGER OF OCULAR CALCIPHYLAXIS IN PATIENTS WITH CHRONIC RENAL FAILURE: A HYPOTHESIS

Submitted for publication

Abstract.

During each dialysis session there is a considerable loss of body fluid and a decrease in tearflow. In some patients these changes may lead to damage of the corneal and conjunctival epithelial cells which is reflected as an increase in vital staining postdialytically as compared to the predialysis score. In our group of patients with chronic renal failure the degree of limboconjunctival calcification was found to be independent on the serum calcium and phosphorus product. There was, however, a statistical significant association between the degree of limboconjunctival calcification, as expressed in the Porter and Crombie grading system, on the one hand, and the degree of epithelial damage and the total number of dialyses on the other hand.

Limboconjunctival calcification in patients with chronic renal failure can be understood by the concept of local calciphylaxis: a condition of induced systemic hypersensitivity in which tissues respond to an appropriate challenger with local calcification. Renal failure acts as an indirect sensitizing calcifier, in which increasing levels of parathyroid hormone may occur and an increased serum calcium and phosphate product. A markedly decreased tearflow, as expressed in low Schirmer values, that develops during each dialysis session, and exposure lead to tissue dessiccation and this acts as a local challenger.

In patients that underwent renal transplantation there is a regression of the limbal calcification. Here, the induced systemic hypersensitivity decreases markedly as the systemic sensitizer is removed when renal function is restored. Parathyroid hormone will gradually return to normal levels as do the serum calcium and phosphate concentrations when dialysis treatment is suspended. Instead of the repeated deposition of lime salts, calcium precipitates are being resorbed slowly.

Introduction.

In patients with renal failure lime salts can be deposited in the conjunctiva and in the limbal area, usually overlying areas with elastotic degeneration, and under certain circumstances also in the cornea (1). It is of interest, however, that the main factors for soft tissue calcification, i.e. raised levels of serum calcium and/or phosphate, have not been demonstrated to have an

association with the degree of limboconjunctival calcification (2-5), with the exception of one study (6).

Several studies pointed to the role of tissue damage in the development of soft tissue calcification. Doughman et al. (7) were able to produce corneal calcification by the combination of administering toxic doses of calciferol and inducing immunogenic uveitis. Corneal calcification was prevented by lid closure. Fabian et al. (8) demonstrated calcium precipitation in rat corneas due to exposure.

Dramatic in this respect was the clinical observation of Bloomfield et al. (9) of the development of an acute right central corneal calcification in a patient with renal failure due to a rapidly progressive glomerulonephritis with a high serum calcium and phosphate product. This patient was successfully resuscitated from a cardiopulmonary arrest during which period the right eye was accidently exposed for two hours.

Local injury of the outer eye in patients on chronic dialysis treatment is likely to develop, as we demonstrated a marked decrease of tearflow during each dialysis session in earlier studies (10,11). Our present study is directed to the role of exposure, which can be measured by comparing the pre- and post-dialytical staining score of the outer eye, as a result of the chronic recurrent decrease of tear fluid production in the development of limboconjunctival calcifications expressed in the "Porter and Crombie" score (12).

Persons and methods.

The lacrimal gland function and its effect on the outer eye in 38 patients with renal failure were compared to 38 sex and age matched healthy persons. The values of both eyes were averaged for a better estimate. The tear fluid concentration of lysozyme was estimated by the agar diffusion technique (13), the tear fluid lactoferrin concentration was determined by radial immunodiffusion (14). Determination of the tear fluid lysozyme and lactoferrin concentration was done once predialytically and postdialytically.

For the assessment of the effect of dialysis on the Schirmer test, on the tearfilm break-up time and on the corneal and conjunctival epithelium of the exposed parts of the outer eye (the Lissamin green test) average values of three assessments of each test in the pre- and postdialysis period were used. To reduce bias, the predialysis measurements were always carried out by the same person and postdialysis measurements by another person. However, whether the person examined was a patient or a control person could not be masked.

The Schirmer test was carried out in a routine fashion. The tear film break-up time was done according to the method proposed by Norn (15). Instead of the usual Rose bengal

scoring (13), Lissamin green in a 1% solution was used, as this vital stain is virtually painless, to indicate devitalized corneal and conjunctival cells. The same scoring system was used as for Rose bengal. The serum calcium and phosphorus concentrations were determined in all patients and in 38 control persons and their product was calculated. Serum parathyroid hormone (PTH) levels were measured in the patient group by the radio-immuno assay technique (intact PTH). No PTH levels were measured in the control persons.

Another group of 11 patients with chronic renal failure underwent renal transplantation. The limboconjunctival calcification before the operation was graded according to the system of Porter and Crombie ("P&C" score) (12) and reassessed in a variable time after the operation. Eleven patients with renal failure but without transplantation served as controls. These controls were selected for an identical P&C score of limboconjunctival calcification and were adequately sex and age matched. The period between the initial assessment and the final assessment was identical in the patients with renal transplants and their respective controls.

For statistical analysis the Mann-Whitney U-test was used for independent samples and the Wilcoxon matched-pairs signed-ranks test was used for related samples. To assess associations linear correlation tests and partial regression tests were used. The Chi-square test was used for independent samples for nominal levels of measurements. All probabilities are two-tailed.

Results.

PTH, Ca, P and the Ca*P product.

The levels of PTH in all patients were elevated as a result of secondary hyperparathyroidism and showed marked fluctuations. In the patient group the serum levels of calcium, phosphate and their product were significantly raised in comparison to these levels in a control group (Table I). There was no association between the calcium and phosphate product in the patient group and the degree of limboconjunctival calcification. This confirms the results of our previous findings and reports by others.

Predialysis tearfunction in all patients and controls.

The tearfluid lysozyme and lactoferrin concentration and the Schirmer test values were lower in the patient group as compared to the values of the persons in the control group, but not significantly so. The Lissamin green vital staining score and also the tearfilm break-up time differed statistically significantly between the two groups. In Table II the data are listed. The difference in mean values of the Lissamin green scores between patients and controls is only 0.5: it is small but statistically significant.

Table I. The serum PTH, Ca en P levels and the calculated Ca*P product in 38 patients and 38 control persons. PTH was not determined in the control group.

	controls median (range)	patients median (range)	Sig.
PTH	-	16.1 (0.4-110)	-
Ca	2.44 (2.22-2.59)	2.51 (2.07-4.25)	$p<0.02$
P	1.26 (1.02-1.59)	1.77 (1.04-3.28)	$p<0.001$
Ca*P	3.11 (2.53-4.12)	4.46 (2.35-8.17)	$p<0.001$

PTH = serum parathormone concentration in picomol/l
Ca = serum calcium concentration in mmol/l
P = serum phosphate concentration in mmol/l
Mann-Whitney U-test was used as a statistical test.

Predialysis versus postdialysis tearfunction values in all patients.

After dialysis the Schirmer test values overall changed significantly. In two patients the values increased and in two other patients there was no change on the average in tearflow, but in all other patients the Schirmer test values were markedly lower. Also the Lissamin green score overall changed statistically significantly as did the tearfilm break-up time. The tear fluid lysozyme and lactoferrin concentrations did not differ statistically significantly in patients after dialysis compared to the predialysis values (Table III).

In 14 patients (ED = exposure demonstrable) the Lissamin green score increased consistently and statistically significantly ($p<0.01$) but modestly after dialysis. In the other 24 patients an increase in vital staining could not be demonstrated (END = exposure not demonstrable). None of the patients showed a decrease of the Lissamin green staining score after dialysis. In 4 patients of the ED group an increase of one half scoring point, averaged over both eyes, was found; in 7 patients a consistent increase of a full scoring point was found and in 3 patients an increase of one and a half scoring point was observed; only in the 3 latter patients grittiness of the eyes as a symptom was

Table II. The predialysis tear function parameters in 38 patients with renal failure and in an equal number of sex and age matched control persons.

	patients median (range)	controls median (range)	Sig.
Lysozyme	1900 (1050-5000)	2300 (620-6200)	NS
Lactoferrin	1740 (650-4180)	2000 (700-3000)	NS
Schirmer	15 (1-30)	20 (1-30)	NS
Break-up time	6 (2-15)	15 (8-40)	$p<0.001$
Lissamin green	1.5 (0.5-7.5)	1 (1-4)	$p<0.005$

Sig. = significance.
Tearfluid lysozyme and lactoferrin concentration in mcg per ml.
Schirmer values in mm wetting of the filter paper strip in 5 minutes.
Break-up time in seconds.
Lissamin green test in score points.
Mann-Whitney U-test was used as a statistical test.

volunteered. In Table IV the individual patient data are shown together with the degree of limboconjunctival calcification and the total number of dialysis treatments.

The ED group (n=14): the relation between the degree of calcification, the exposure and the number of times of exposure.

The regression of the degree of limboconjunctival calcification on the tissue exposure expressed as the difference between the pre- and postdialysis Lissamin green score shows a trend but is statistically not significant: ($t12=1.58$); it has a product moment correlation coëfficiënt of 0.41. There is also a suggestive association, but statistically not significant, between the

Table III. The predialysis tear function parameters in 38 patients with renal failure and in an equal number of sex and age matched control persons.

	predialysis median (range)	postdialysis median (range)	Sig.
Lysozyme	1900 (1050-5000)	2100 (1000-6200)	NS
Lactoferrin	1740 (650-4180)	1850 (700-4300)	NS
Schirmer	15 (1-30)	9.5 (0-30)	$p<0.01$
Break-up time	6 (2-15)	5 (1-11)	$p<0.01$
Lissamin green	1.5 (0.5-7.5)	2 (0.5-7.5)	$p<0.01$

Tearfluid concentrations of lysozyme and lactoferrin in mcg per ml.
Schirmer values in mm wetting of the filter paper strip in 5 minutes.
Break-up time in seconds.
Lissamin green test in score points.
The Wilcoxon matched-pairs signed-ranks test was used as a statistical test.

degree of calcification and the number of times of exposure, i.e. the total number of haemodialysis sessions ($r=0.46$, $t12=1.82$). A reliable relation, however, can be established if both the degree of exposure and the number of times patients were exposed are included in the analysis.

This partial regression of the degree of calcification expressed in the P&C score on both the difference in Lissamin green score pre- and postdialytically and the total number of dialyses was statistically significant ($r=0.67$, $t12=3.17$, $p<0.01$), and is shown in Figure 1 as the relation between the expected and the actual P&C score. There seems thus to be circumstantial evidence that the degree of limbal calcification is dependent on both the degree of exposure and the number of times a patient had this exposure. It is interesting to note that, when the ED group and the END group

were combined, a significant association between the degree of calcification and the total number of dialyses was present ($r=0.39$, $t36=2.58$, $p<0.02$).

Renal transplantation.

In Table V the data on the change in the degree of calcification in 11 patients that underwent renal transplantation and the control patients are listed. Chi-square analysis demonstrated the decrease of limbal calcification in patients with renal transplants compared to the control group to be significant. The derivation of the Chi-square distribution as an approximation for the distribution of the statistic Chi is made under the assumption that the expected value in each cell is in excess of five, which in our contingency table is not fulfilled. However, Lewontin and Felsensted (16) pointed out that this rule is too restrictive.

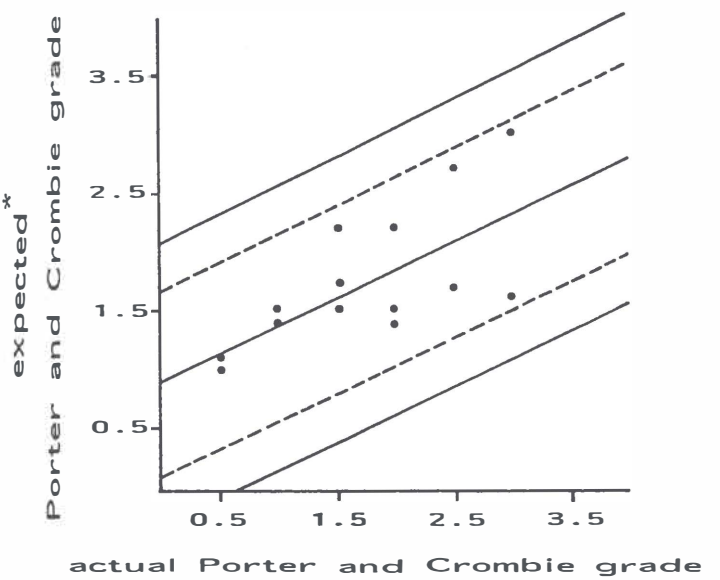

*Figure 1. The relation between the actual and expected P&C score with the 95% (—) and the 99% (——) confidence limits. The expected P&C score was calculated from the partial regression of the data of the actual score on the degree of exposure expressed as difference in Lissamin green score pre- and postdialytically (dlg) and the number of dialyses (tnd) with the formula: expected P&C score = 1.2485 * dlg + 0.0006 * tnd + 0.0529. This association is statistically significant: $r=0.67$, $t12=3.17$, $p<0.01$.*

Table IV. The effect of dialysis on the conjunctival and corneal epithelium expressed in the Lissamin green score in 14 patients (ED group) as well as the Porter and Crombie score and the number of dialysis sessions. In 24 patients of the END group no difference between pre- and postdialysis Lissamin green staining scores could be established (data of the END group are not shown in this table).

pat.	P&C	Lissamin green score			No. dial.
		pre	post	diff	
1	0.5	2.5	3.0	0.5	780
2	0.5	2.0	2.5	0.5	468
3	1.0	3.0	4.0	1.0	312
4	1.5	1.5	2.5	1.0	718
5	2.0	2.0	3.0	1.0	234
6	1.5	1.0	2.0	1.0	260
7	3.0	1.0	2.0	1.0	468
8	2.0	1.0	2.0	1.0	312
9	2.0	3.0	3.5	0.5	2496
10	1.5	1.0	2.5	1.5	468
11	2.5	4.0	4.5	0.5	1716
12	1.0	1.0	2.0	1.0	156
13	2.5	1.0	2.5	1.5	1248
14	3.0	1.0	2.5	1.5	1872

pre = predialysis staining score
post = postdialysis staining score
diff = difference between post- and predialysis staining score
No. dial. = total number of dialysis sessions.

Table V. The difference in degree of calcification between the first and final assessment in patients with renal transplants in a variable timespan.

	Degree of calcification					
	Decr.	%	Unch.	%	Incr.	%
Pts rt	6	54.5	3	27.3	2	18.2
Pts ct	0	0	5	45.5	6	54.5

Decr. = decrease, Unch. = unchanged, Incr. = increased.
Pts rt = patients with renal transplantation.
Pts ct = sex and age matched (within 5 years) control patients, with the same degree of limbal calcification.
Chi-square (df2) = 8.5, $p < 0.025$.
Fisher exact probability test $p < 0.01$.

Discussion.

It is a rather curious phenomenon that there is no association between the serum calcium and phosphate product and the degree of limboconjunctival calcification as an increase in the serum calcium and phosphate product is a prerequisite for the deposition of lime salts (17). On the other hand, we could demonstrate in 38 patients that the degree of the calcification was associated with the duration of dialysis which is in accordance with reports of previous investigators (6,12). This confirms the clinical observation that, although the association between soft tissue calcification and renal failure had been recognized for more than a century, it was only since haemodialysis was used as a routine procedure, that soft tissue calcification has become a major problem (18).

Haemodialysis leads to loss of body fluids and to a marked decrease in

tear flow and tearfilm stability that was related to demonstrable exposure of the conjunctival and corneal epithelium in some patients of our group. This was shown by the difference in pre- and postdialysis staining scores of the conjunctiva and cornea: a rather unsensitive method. For the ED group of patients the relation between the degree of tissue dessiccation and the degree of calcification was suggestive. If the number of times this traumatic event took place, i.e. the total number of dialyses, that as a single factor in the ED group was also not significantly associated with the degree of calcification, was included in the analysis, the association was significant.

The events that had led in some patients to a demonstrable tissue dessiccation because of a significant decrease in tear flow and tearfilm stability that was at least partially associated with the degree of calcification in these patients, can best be understood by the unifying theory of soft tissue calcification as proposed by Selye, i.e. calciphylaxis (19). This is a condition of induced systemic hypersensitivity by an indirect sensitizer such as renal failure with its subsequent rise in serum PTH levels and the mobilisation of calcium and phosphate. Tissues then respond to an appropriate challenger such as trauma or exposure (20) with local calcification that may evanescent.

One can speculate that dessiccation of the epithelium as a result of a decrease in tear flow and tearfilm stability might be an appropriate challenger for ocular calciphylaxis and although we were unable to demonstrate tissue exposure by the vital staining method for the majority of patients, it is most likely operative as there is a distinct association between the total number of dialyses as a single factor and the degree of calcification if the data of both the ED group and the END group were combined.

In patients with renal transplants the degree of calcification decreases. This can also be explained by the theory of calciphylaxis, as the effects of the indirect sensitizer, i.e. renal failure, are disappearing. If the transplantation proves to be successful, the levels of the serum PTH, Ca and P will return to normal. When the haemodialysis sessions are discontinued, there are no recurrent decreases in tear flow anymore and, therefore, no precipitous tissue devitalisation, i.e. the challenger disappears. The deposited calcium can then be slowly resorbed.

The mode of action of tissue dessiccation as a local challenger in renal failure might be explained by the release of pyrophosphatases in the interstitial fluid from the injured cells. These enzymes hydrolyze pyrophosphate to

inorganic phosphate, thereby increasing the local phosphate concentration and raising the calcium and phosphate solubility product locally. Enhancement of local tissue calcification can be the result of the loss of carbon dioxide from the surface of the epithelial cells, creating a local increase in pH that predisposes to precipitation of calcium salts.

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CHAPTER 6

LIMBAL AND CORNEAL CALCIFICATION IN PATIENTS WITH CHRONIC RENAL FAILURE

Klaassen-Broekema N, Bijsterveld OP van. Limbal and corneal calcification in patients with chronic renal failure. Brit J Ophthalmol 1993; 77: 569-71.

Abstract.

In patients with chronic renal failure on regular dialysis treatment, limboconjunctival degenerations and calcifications are commonly observed. In this study three groups of patients were followed over a period of 6 years. The first group consisted of 47 patients with renal failure, the second group of 17 patients with renal failure and hyperparathyroidism that was medicamentally uncontrollable (tertiary hyperparathyroidism) and the third group of 7 patients with primary hyperparathyroidism without renal failure. The aim of this study was to determine the progression of the limboconjunctival changes over time. The hypothesis that an increase in serum calcium and phosphate concentrations, as a result of tertiary hyperparathyroidism, could possibly add a corneal component to the limbal calcification was also tested. All patients with renal failure, in as much as the degenerative limbal features were not obscured by deposits of lime salts, had a type II white limbus girdle of Vogt. This limbal degeneration was observed in only 45% of controls. In all 47 patients with renal failure conjunctival calcification was observed; 26 of them also had limbal calcification. After 6 years 41 patients had developed limbal calcification. This progression was statistically significant. In 15 out of 17 patients with tertiary hyperparathyroidism a band-shaped keratopathy developed in addition to the limboconjunctival calcification.

Introduction.

In patients with renal failure on regular dialysis, chalky white lesions can be observed clinically at the limbus (1). These have been subsequently identified as hydroxyapatite (2). The precipitation of hydroxyapatite is assumed to occur when the product of the local serum concentrations of calcium and phosphate exceeds the in-vivo solubility product; i.e. in a markedly supersaturated concentration (3) under proper local conditions.

In renal failure, with its complexity of chemical and metabolic disturbances and because of the changes brought about by therapy, one can expect ever changing serum calcium and phosphate concentrations with a tendency, however, to low serum calcium concentrations early in renal failure and increasing serum calcium concentrations as the disease progresses and

tertiary hyperparathyroidism sets in. Tertiary hyperparathyroidism is a secondary hyperparathyroidism that is medicamentally uncontrollable and is characterized by high levels of serum calcium, phosphate and parathyroid hormone (PTH).

Certain constitutional diseases such as primary hyperparathyroidism (4), and toxic states such as vitamin D poisoning (5), are also characterized by high serum calcium levels over a prolonged period of time. In these cases a band-shaped corneal degeneration and calcification develops at the level of Bowman's membrane in the exposed part of the cornea.

The aim of this study was to determine the progression of the limbal degenerations and calcifications in patients with chronic renal failure over time. We also tested the hypothesis that an increase of the serum calcium and phosphate concentrations, as a result of tertiary hyperparathyroidism, could possibly add a corneal component to the limbal calcification.

Persons and methods.

A total of 64 patients with renal failure, 47 with controllable secondary hyperparathyroidism and 17 patients with tertiary hyperparathyroidism that were scheduled to undergo surgical intervention, as well as 7 patients with primary hyperparathyroidism but without intrinsic renal disease were evaluated for the presence and morphology of limbal and peripheral corneal degeneration and calcification.

One hundred and fifty healthy persons with an identical male to female ratio as in the patients, 25 in each of the same age categories as the patients, served as controls for limbal degeneration. Fifty of these patients served as controls for the serum calcium and phosphate levels.

Biomicroscopic examinations were performed using the direct and indirect illumination and scleral scatter techniques to study the difference in incidence of limbal and corneal degeneration and clinically visible calcification. Grading of the limbal calcification was done according to the system proposed by Porter and Crombie, i.e. to establish the degree of calcification in patients by comparing it to drawings depicting 5 stages of calcification intensity (6).

Grading of the limboconjunctival lesions was performed at the first visit and after respectively 3 and 6 years. The calcification scores of both eyes were averaged. Serum calcium and phosphate concentrations were determined in all patients and in 50 control persons. The control persons and the patients with primary hyperparathyroidism were not available for the second and final assessment. Linear regression, the Chi-square test, and analysis of variance were used as statistical tests.

Table I. Limboconjunctival degeneration (LCD) and calcification (LCC) in patients with renal failure (RFSH), in patients with renal failure and tertiary hyperparathyroidism (RFTH), in patients with primary hyperparathyroidism (PH) and in healthy control persons (Co).

	RFSH (n=47)	RFTH (n=17)	PH (n=7)	Co (n=150)
LCD	+	+	+	+
LCC	+	+	-	-
BSK	-	+	+	-

BSK = band-shaped keratopathy
+ = present
- = absent

Results.

Limbal degeneration.

In Table I the limboconjunctival and corneal changes in the patients of all groups are summarised. All patients with a "Porter and Crombie" (P&C) score of maximally 2 showed limbal degenerations that were seen as white incomplete arcs situated concentrically with the limbus in the interpalpebral zone. In patients with a higher P&C score, the limbal degeneration was masked to a varying degree by the calcification of the limbal area but it was assumed that also in these patients an accompanying limbal degeneration was present. In a healthy control group of 150 patients only 45% showed these degenerations.

The degenerative opacities were located immediately beneath the epithelial surface, usually without a clear interval with the corneoscleral

Table II. Limbal calcification in 47 patients with renal failure and on regular dialysis but without tertiary hyperparathyroidism, at the initial evaluation, and after 3 and 6 years, graded according to the system proposed by Porter and Crombie. The scores were averaged over the nasal and temporal section.

	Grade of calcification				
	1	2	3	4	5
Initial	21	15	8	3	0
3 years	10	18	12	5	2
6 years*	5	14	15	8	4

*One patient died before the final assessment.
Chi-square[8]=20, $p<0.025$.

border. On the central edge radiating curved, off-white lines were present. with a chalky aspect that appeared to be related to the terminal parts of the pallisades and limbal capillaries. In these deposits no holes were observed. These lesions are indistinguishable from the type II white limbus girdle of Vogt.

Limbal calcification.

In addition to the limbal degeneration, in all patients a mild or moderate calcification was present, even in the early stages of renal disease. This calcification was located in or rather over the area of degeneration. Limbal calcium salts were visible as a semilunar arc of delicate crystalline deposits adjacent to the scleral border. As the disease progressed, the degree of limbal calcification increased with the formation of coarse to very coarse amorphous plaques in some cases. Table II gives the degree of limbal calcification at the

calcification at the initial assessment, after 3 and after 6 years for patients.

In two cases of marked limbal calcification, the amorphous plaques extended onto the corneal tissue just beyond the central limbal rim. These calcium precipitates were shown biomicroscopically to be situated between the basal layers of the epithelium and the membrane of Bowman. The concretions occasionally flake, leaving painful corneal erosions that stained with fluorescein and a localized area of hyperaemia (7).

Degree of calcification.

There is a clinical impression that the intensity and the coarseness of the calcification was related to the calcium and phosphate product. However, no association could be demonstrated between the degree of calcification expressed in the P&C grading system and the serum calcium levels ($r=+0.02$), the serum phosphate levels ($r=+0.17$) or with the serum calcium and phosphate product ($r=+0.20$) in 47 patients with renal failure without tertiary hyperparathyroidism.

Progression of the limbal calcification.

The progression of the limbal calcification was on the average slow but statistically significant ($p<0.025$). Three years after the initial assessment, the limbal calcification had increased by slightly more than half grading point on the average. However, in some patients a rather rapid progression was noted that could be as much as three grading points in the final assessment; conversely in some other patients the progression was almost imperceptible. The association between the degree of calcification and the duration of dialysis (years) is statistically significant ($p<0.05$), but this association is weak: the product moment correlation coëfficiënt is $+0.30$.

Band-shaped keratopathy.

In 15 of 17 patients with renal failure and tertiary hyperparathyroidism, in addition to calcium deposits in the limbus, calcification of a different type and localization was observed. In 2 of these 15 patients the opacity was unilateral. This type of calcification presented itself as opacities with a soft grey colour showing biomicroscopically a subtle granular structure. They were visible approximately 1 mm from the corneoscleral junction on both the nasal and temporal side of the cornea and extended axially with an ill defined central

Table III. The average values and the standard deviations of serum calcium and the serum phosphate concentration as well as their product in patients with renal failure and secondary hyperparathyroidism (RFSH), with renal failure complicated by tertiary hyperparathyroidism (RFTH), with primary hyperparathyroidism (PH) and in controls (Co).

	No	calcium		phosphate		Ca*P	
		av.	sd(n-1)	av.	sd(n-1)	av.	sd(n-1)
RFSH	47	2.46	0.23	1.87	0.64	4.51	1.32
RFTH	17	2.70	0.28	2.17	0.68	5.82	1.78
PH	7	3.33	0.48	0.96	0.17	3.19	0.77
Co	50	2.41	0.10	1.30	0.17	3.13	0.46

av. = average
sd(n-1) = standard deviation (n-1)

border. In the greyish area dark round holes were distinguishable (Figure b, see addendum).

These axial corneal degenerations were indistinguishable from band-shaped keratopathy. Also all seven patients with primary hyperparathyroidism showed identical corneal calcifications. In only two of the 47 patients with secondary hyperparathyroidism and renal failure was also band-shaped keratopathy observed. One of these 2 patients suffered from long-standing renal insufficiëncy complicated by amyloidosis and the other patient had a serious diabetic nephropathy.

The serum calcium concentration differed significantly between the patients of the various groups but not between those with renal failure complicated by secondary hyperparathyroidism and the control persons. The serum phosphate concentrations also differed significantly between the patients

of the groups and control persons but not between the patients with secondary and tertiary hyperparathyroidism. Only between patients with primary hyperparathyroidism and control persons could no difference be demonstrated between the calcium and phosphate product (Table III).

Discussion.

The limbus is the transitional zone between the sclera and covering conjunctiva on the one hand and the cornea on the other. The cornea ends at a line that connects the membrane of Bowman with that of Descemet. As the membrane of Bowman is about one millimeter shorter than Descemet, the translucent conjunctival and subconjunctival tissue extends to the Bowman's membrane. Clinically this area resembles the cornea and is, therefore, usually regarded as such, but in its superficial layers it is more related to the conjunctiva and it is prone to develop the same degenerations as the conjunctiva.

Degenerations of the conjunctival, limbal and corneal tissues are frequently located in the interpalpebral area. This may be due to the exposed position of the globe to environmental influences. The development of a white limbus girdle, histopathologically characterized by elastotic degeneration of collagen fibers (8), in patients with renal failure may be also related to some general effect of uremia on connective tissue. Such an effect is suggested by the frequent occurrence of poor wound healing and investigation of this phenomenon showed marked elastotic degeneration at the expense of normal collagen fibres in various tissues including those of the conjunctiva and the limbal area (9).

There have been different interpretations on the similarity and the relationship between Vogt's white limbus girdle type II and the limbal calcification in renal failure (1, 10-12). In patients in which the effects of renal failure in one eye or in one part of the eye preceded the contralateral side, the sequence of events we observed was the development of degeneration, indistinguishable from the white limbus girdle type II, followed by calcification, initially as the deposition of a semicircular zone of delicate crystals and later as coarse amorphous calcific plaques as the metabolic situation deteriorated. Thus, the predilection of the deposition of lime salts in

the conjunctiva and the limbus may be related to the ability of these tissues to react with elastosis to uremia.

Calcium salts can be deposited in a suitable matrix if the concentration of calcium and phosphate exceeds the in-vivo solubility product. This form of calcification in undamaged tissues is referred to as metastatic calcification. There is an ever increasing body of experimental evidence, however, summarized and amplified by Scarpelli (13), that demonstrates that tissue damage always precedes calcification. Also clinically (14) it has to be remembered, that in healthy persons there is no calcium deposition inspite of the fact that the interstitial fluids are supersaturated with calcium and phosphate (15).

The production of a calcifiable matrix is necessary for the deposition of calcium salts (16). Elastosis which precedes calcification, could induce the formation of a calcifiable matrix but it is not in itself a matrix, as calcification has never been reported within the elastotic tissue itself (12,14). Calcification of the outer eye has a predilection for the immediate subepithelial site as it is believed that loss of carbon dioxide to the atmosphere occurs rapidly on the surface of the eye in the interpalpebral area (10). The fall in pCO2 results in a rise of pH. Hydroxyapatite is less soluble in an alkaline environment so that these lime salts are precipitated in areas with a relative high alkalinity.

In renal failure there is a rise in the serum phosphate concentration because of decreased elimination of phosphate in the kidneys, and relatively low or normal serum calcium concentration concentrations. The net result, however, is an increase of the product of the serum calcium and phosphate concentrations. Under these conditions there is a chalky white precipitation of lime salts in the sites of predilection, the conjunctiva and limbus. As the disease progresses there is an increased activity of the parathyroid glands that can result in an increase in the serum calcium levels leading to a different type of calcification. This corneal calcification is in no way different from the band-shaped keratopathy of primary hyperparathyroidism.

In primary hyperparathyroidism band-shaped keratopathy seems to be associated, amongst other things, with high serum calcium concentrations. In patients with renal failure complicated by tertiary hyperparathyroidism the serum calcium concentrations are significantly higher than in patients with renal failure and secondary hyperparathyroidism and this may be responsible for the development of band-shaped keratopathy in patients with tertiary

hyperparathyroidism.

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CHAPTER 7

RAPID PROGRESSION OF BAND-SHAPED KERATOPATHY WITH EARLY CENTRAL LOCALISATION IN A PATIENT ON CHRONIC DIALYSIS TREATMENT

Klaassen-Broekema N, Bijsterveld OP van. Rapid progression of band-shaped keratopathy with early central localisation in a patient on chronic dialysis treatment. Eur J Ophthalmol 1994; 2: 126-9.

Abstract.

This clinical study reports on an unusual start of a band-shaped keratopathy in a patient with diabetic nephropathy on dialysis treatment. The earliest corneal manifestations were centrally located small greyish-white disc-shaped lesions evenly distributed in the interpalpebral area in the left eye. Later a typical peripheral band-shaped keratopathy developed. In the course of the observation period the peripheral keratopathy rapidly spread towards the centre, finally resulting in a complete band-shaped keratopathy in which only the most central original disc-shaped lesions could be identified.

Band-shaped keratopathy most commonly develops in degenerated eyes, such as those eyes with chronic uveitis or with complications from diabetes mellitus. Band-shaped keratopathy is also likely to develop in patients with sustained high serum calcium levels in various metabolic diseases. Both factors most certainly have contributed to the rapid progression of the band-shaped keratopathy in the patient presented in this report.

Introduction.

Degenerative and calcific changes of the conjunctiva and the limbus are common in patients with chronic renal failure on dialysis treatment. These degenerations are clinically characterized by pingueculae and the white limbus girdle of Vogt type II and histologically by elastosis (1). Most patients on regular dialysis present conjunctival and limbal calcifications. Usually the cornea is not affected. However, if tertiary hyperparathyroidism sets in, as a complication of renal disease, a band-shaped keratopathy can be present.

We describe here a patient with diabetic nephropathy on peritoneal dialysis, who developed multiple small soft white-greyish disc-shaped lesions located in the interpalpebral zone in his left cornea. In the course of the disease peripheral corneal opacities also appeared that later developed into a typical band-shaped keratopathy. Tertiary hyperparathyroidism, however, was not detected.

Case report.

A 46-year-old white male had a seven month history of decreasing visual acuity in the left eye because of multiple, centrally located, small disc-shaped corneal opacities. The patient suffered from diabetes mellitus for 30 years. Serious proliferative diabetic retinopathy developed 18 years after the onset of the disease and the patient underwent extensive panretinal coagulation treatment in both eyes. At that time an early diabetic nephropathy appeared to be present.

Two years after coagulation treatment there were vitreous hemorrhages in the left eye and a pars plana vitrectomy was carried out. Later, the right eye also developed vitreous hemorrhages, but vitrectomy could not be performed because of cardiovascular problems. Traction retinal detachment and neovascular glaucoma finally resulted in blindness of the right eye. The left eye, at that time, showed a quiet non-progressive retinopathy which did not require coagulation treatment. Visual acuity was 0.25.

In August 1991 dialysis treatment was started. At that time both eyes had thin arc-shaped limbal opacities nasally and temporally in the interpalpebral area. There was no clear interval between the opacities and the sclera. Radiating curved white lines but no "holes" were observed. These arc-shaped opacities represented Vogt's white limbus girdle type II. This limbal degeneration was partly covered by calcium precipitates, arranged in a double arc concentric to the limbus.

In February 1992 multiple small whitish opacities developed in the left eye, located centrally in the cornea and distributed in a band-like pattern (Figure c, see addendum). Slit-lamp examination showed small white disc-shaped lesions in a regular pattern in the interpalpebral zone at the level of Bowman's membrane. The number of nummular infiltrates was estimated at 80. They had a dense white centre with a soft greyish-white zone around it. The dense centre measured 0.1 millimeters, the total diameter being 0.3 millimeters. The opacities showed no autofluorescence with Wood's light. Although the dots itself did not stain with fluorescein there was a diffuse, light punctate staining of the cornea. Six months later the soft surrounding zone had become almost as dense as the central zone.

Bacterial and viral cultures were negative. Impression cytology of the central and peripheral cornea and the conjunctiva adjacent to the limbus in the

interpalpebral area did not reveal any abnormalities.

At that time another arc-shaped opacity developed in the peripheral cornea, not connected to the limbal opacities. This was diagnosed as the white limbus girdle of Vogt type I. The opacity had a soft lace-like pattern and "holes" (Figure d, see addendum). Later this peripheral corneal opacity became considerably larger and spread towards the centre of the cornea. It was now clear that the peripheral corneal opacities represented a band-shaped keratopathy which had completely formed within three months. Only a few of the original central lesions could be identified (Figure e, see addendum). The end-stage visual acuity was 0.05.

At the moment of the development of the disc-shaped lesions the serum calcium and phosphate product, that previously had remained relatively low, increased markedly, mainly due to a high serum phosphate concentration (between 1.75 and 2.04 mmol/l). When the serum calcium concentration also increased (between 2.64 and 3.06 mmol/l) the soft white peripheral zone of the nummular lesions became as dense as the centre. No tertiary hyperparathyroidism could be detected in this period. Because of the impossibility of reducing the serum calcium and phosphate product, a penetrating keratoplasty or treatment of the cornea with calcium chelating agents was not indicated.

Discussion.

Our patient provides clinical evidence that the small, centrally located, disc-shaped corneal lesions are calcium precipitates that, in the course of the disease, appeared to be an early manifestation of a band-shaped keratopathy. Early peripheral corneal changes were initially diagnosed as the white limbus girdle of Vogt type I. However, after rapid central progression it was evident that the opacity was in fact a band-shaped keratopathy. This clinical observation confirms the suggestion of Franceschetti et al. (2) that the white limbus girdle type I can be regarded as an abortive form of band-shaped keratopathy as both conditions have essentially the same histopathological substrate.

In the majority of cases the initial changes in band-shaped keratopathy occur in the peripheral cornea near the limbus. Once the band is completed the

peripheral ends are wider than the central part. In our patient the initial changes were central, although at the same time the earliest changes of what later appeared to be a band-shaped keratopathy were already present peripherally. Very rarely band-shaped keratopathy is observed starting centrally but in such cases the opacity never quite reaches the limbus and remains widest at the centre. In our patient the central opacities were not progressive, with the exception of the increasing density of the surrounding zone. The peripheral opacities, on the other hand, showed a rapid course towards the centre of the cornea. The peripheral ends of the band were therefore wider than the central part and the opacity fully reached the limbus.

Characteristically the opacity spreads towards the centre over a number of years until the two segments meet. In our patient, however, the band reached completion in only three months. This rapid progression was also described in a patient with infantile hypercalcaemia (3). As band-shaped keratopathy can develop in degenerated eyes, in our patient both the presence of severe complications of diabetic retinopathy and the sudden and sustained rise in serum calcium and phosphate levels were considered to be responsible for the rapid progression of the band keratopathy.

Only one of our patient's eyes showed a central start and a rapid course of the band-shaped keratopathy. The main difference between the two eyes was the neovascular glaucoma of the right eye that had been treated by cyclocryocoagulation. Cryocoagulation can have opposite effects on the composition of the aqueous humour which is considered a modified blood filtrate. Smith et al. (4) showed that in effective cyclocryocoagulation, the blood-aqueous barrier is markedly raised because of vascular stasis in the ciliary body.

Quigley (5), on the other hand, demonstrated histopathologically in human eyes a loss of intercellular junctions in the ciliary epithelium after cryotherapy, which was assumed to be responsible for the long-term post-operative flare in the anterior chamber in some patients. In our patient the cyclocryotherapy was highly effective and, except for a few post-operative days, there was no aqueous flare and thus no serum leakage. Therefore, this patient could have had a raised blood-aqueous barrier that prevented calcium and phosphate reaching critical levels in the aqueous humour.

Histopathologically band-shaped keratopathy can be calcific or non-calcific; in the latter case elastosis is the histological substrate (6). Mixed cases

exist too. As we were unable to study the corneal changes histopathologically - a penetrating keratoplasty was rejected because of the risk of accelerated vitreoretinal complications and because of the inability to normalize the serum phosphate concentration - the differential diagnosis of the lesion had to be made clinically.

Our patient presented clinical evidence that the small central lesions were calcific and not elastotic as there was a complete absence of autofluorescence, a greyish-white colour rather than a yellowish colour of the central opacities and an exceedingly high serum calcium and phosphate product.

Non-calcific lesions in a band-like pattern have been recognized for many years and were known under a variety of names (7-10). Some investigators showed their substrate was elastosis (11-13). However, the clinical descriptions and the figures in these studies, in spite of the presence of a band-shaped pattern, were unlike that of our patient. Fraunfelder et al. (14-16) gave a detailed description of what they called spheroid degeneration of the cornea; clinically this was characterized by yellow spherules, mainly present near the limbus in the 3 and 9 o' clock positions and showing autofluorescence that was lacking in our patient.

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CHAPTER 8

RED EYES IN RENAL FAILURE

*Klaassen-Broekema N, Bijsterveld OP van. Red eyes in renal failure. Brit J Ophthalmol 1992; **76**: 268-71.*

Abstract.

In 57 patients with chronic renal failure that all had deposition of calcium salts in the conjunctival tissue, 2 patients developed a brief episode of painful irritation and redness of the conjunctiva and subconjunctiva. This hyperaemia was adjacent to erosions of the limbal epithelium of the eye as a consequence of exfoliation of calcium concrements from the superficial limbal epithelium. Eight patients showed inflammatory reactions of the conjunctivae that were clinically identical to inflamed pingueculae.

Three patients showed an inflammatory reaction of the eye that was characterized by a waxy red, more or less diffuse episcleral and conjunctival hyperaemia extending beyond the palpebral fissure. The average value of the serum calcium concentration in these patients was particularly high and statistically significantly higher than in patients with calcification but without inflammatory signs and also higher than in patients that showed "pingueculitis". We propose to reserve the term "red eye **of** renal failure" for the latter group of patients.

Introduction.

In 1966 Abrams (1) drew attention to the association of irritable red eyes and renal failure in a patient with calcific deposits in the corneae close to the limbus. These deposits were extremely superficial and some had flaked off, leaving small eroded areas that stained with fluorescein. The conjunctivae opposite the erosions was hyperaemic. The symptoms and signs were soon relieved by padding of the eye.

A year later Berlyne and Shaw (2) reported on 15 patients with red eyes and severe renal failure associated with raised serum phosphate concentrations and normal or low serum calcium. The illustration of a typical case showed bilateral temporal conjunctival hyperaemia in the interpalpebral area, extending from the corneal limbus to the canthi. Their illustration gave the impression of a small elevated focus in the centre of the temporal conjunctiva.

In 1968 (3) Berlyne described another 13 patients with renal failure, three of whom showed conjunctival irritation and injection, that were also associated with high serum phosphate levels and normal or even low serum

calcium levels. There was no further description of the conjunctival hyperaemia. The authors suggested that there was a relationship between the conjunctival redness and conjunctival calcium phosphate salts deposited as microcrystals.

Subsequent studies of Caldeira et al. (4), Ehlers et al. (5) and de Graaf et al. (6) identified the redness of the conjunctiva in patients with severe renal failure either by description or by diagnosis as conjunctival inflammatory reactions associated with pingueculae. It seems therefore, that there are at least two types of red eyes **in** renal failure; but what is the red eye **of** renal failure?

Patients and methods.

During a six year period a total of 57 patients, 36 males, ranging in age from 23 to 66 years, and 21 females, ranging in age from 33 to 68 years, with terminal kidney insufficiëncy and on regular dialysis were followed for the occurrence of inflammatory complications of the anterior surface of the eye.

The age distribution of the patients is shown in Fig. 1. The average age was 49 years and the average weight was 69.5 kg. In all patients a complete routine ophthalmological examination was carried out and repeated at periodic intervals. The limboconjunctival depositions were graded according to the criteria of Porter and Crombie (7).

Levels of serum calcium and phosphate were measured every three months in each patient and at the beginning of the development of inflammatory reactions of the outer surface of the eye. Fifty healthy persons, matched in sex and comparable in age and weight, were used as controls. The statistical test used was analysis of variance.

Results.

Types of red eyes.

Three distinct types of inflammatory reactions were observed in the patients during this period. In 2 patients irritation and local hyperaemia developed, with pain and photophobia, adjacent to erosions of the corneal epithelium that developed as a result of exfoliation of very superficially deposited calcium salts in the limbal epithelium in the palpebral fissure. These erosions stained with fluorescein. Signs and symptoms disappeared rapidly after dressing of the eye. These lesions were similar to those described by Abrams (1).

In 8 patients a more or less localized redness of the conjunctival vessels

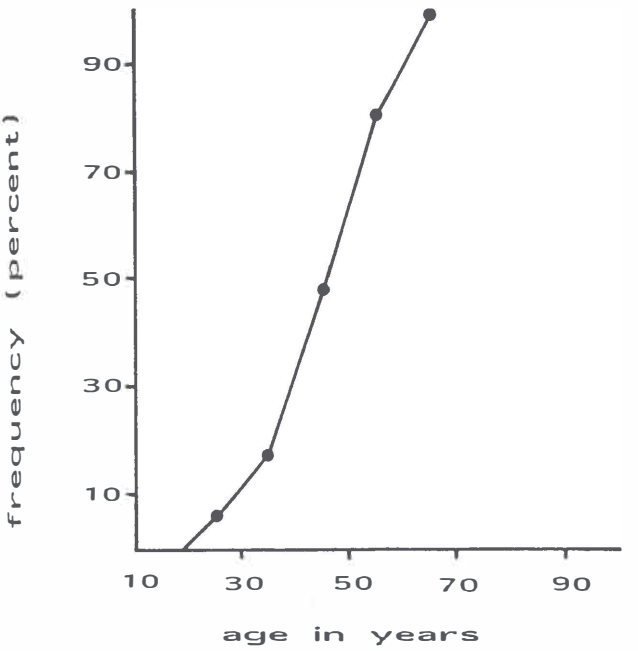

Figure 1. Cumulative frequency distribution of the age in 57 patients with chronic renal failure.

of varying intensity, and occasionally mild congestion of the episceral vessels, was observed. This local hyperaemia of the conjunctival vessels developed gradually around a greyish triangular area situated in the bulbar conjunctiva in the interpalpebral fissure, usually on either side of the cornea. The hyperaemia was characterized by exacerbations and remissions. Bacterial cultures were negative.

The greyish triangular lesions were studded with yellowish white spots that were occasionally confluing and more conspicuous at the periphery. In all patients both eyes were affected on the nasal as well as on the temporal side of

Table I. The number of patients (expressed as a percentage) in each grade at their first visit according to the criteria of Porter and Crombie.

P&C	0	1	2	3	4	5
A (n=46)	0	35	41	20	2	2
B (n=8)	0	37	25	25	13	0
C (n=3)	0	0	0	67	0	33

P&C = Degree of calcification according to the criteria of Porter and Crombie. The values of the nasal and temporal parts and of both eyes were averaged for a better estimate.

the conjunctiva. All lesions did fluoresce in ultra-violet light (8). Clinically these lesions are indistinguishable from inflamed pingueculae (Fig.f, see addendum).

In 3 patients the hyperaemic inflammatory reaction was of a decidedly different nature. In these patients there was congestion of the vessels of the episcleral tissue and of the conjunctiva over it. The congestion presented as a more or less diffuse, episcleral and conjunctival somewhat waxy hyperaemia of the bulbar region extending beyond the palpebral fissure (Fig. g, see addendum). Discharge, if at all present, was scanty. No pingueculae were observed. The symptoms, - itching and burning -, were mild. Bacterial cultures were negative. The inflammatory reaction subsided in 4 weeks on the average.

Groups of patients.

From all patients four groups can be constructed. Group A consisted of 46 patients with calcium deposits but without any inflammatory reaction of the conjunctiva. This group included two patients with a brief episode of limbal

Table II. The average values and standard deviations of the serum calcium and phosphate concentrations and the serum calcium and phosphate product in 50 healthy persons.

	Calcium	Phosphate	Ca*P
average	2.41	1.30	3.13
sd(n-1)	0.10	0.17	0.46

Calcium concentration expressed in mmol/l
Phosphate concentration expressed in mmol/l
Ca*P = serum calcium and phosphate product
sd(n-1) = standard deviation (n-1)

erosions and reflex irritation and hyperaemia of the conjunctiva. Group B consisted of 8 patients with calcification and inflammatory reactions associated with pingueculae. Group C consisted of 3 patients with an inflammatory reaction resembling diffuse episcleritis and associated conjunctivitis. The 3 patients from the last group, after the inflammatory reactions subsided, were placed in group D.

Calcium deposits.

In all patients a deposition of calcium salts in the subconjunctival and limbal tissue was present. The degree of the limboconjunctival calcifications for the four groups is shown in Table I. From this table it is apparent that patients who experienced one or the other type of inflammatory reaction had rather dense limboconjunctival calcium deposits.

Serum calcium concentration.

Table II shows the average values and standard deviations of serum calcium and serum phosphate and also the serum calcium and phosphate

Table III. The serum concentrations of calcium, phosphate and the calcium and phosphate product in the patient groups.

	A (n=46)		B (n=8)		C (n=3)		D (n=3)	
	av.	sd	av.	sd	av.	sd	av.	sd
calcium	2.49	0.28	2.61	0.27	3.53	0.65	2.41	0.39
phosphate	1.85	0.65	1.92	0.53	1.92	0.64	1.63	0.44
Ca*P	4.52	1.33	5.02	1.41	6.62	1.62	3.96	1.28

av. = average
SD = standard deviation (n-1)
Ca*P = calcium and phosphorus product
sd = standard deviation (n-1)

product in the control group. Data on serum calcium concentration in the several patient groups presented in Table III indicated that patients of groups A, B and C had statistically significantly elevated serum calcium concentrations compared to the control group. This is graphically shown in Fig. 2.

In 8 patients from group B, with the localized type of redness, associated with pingueculae, the average serum calcium concentration was not significantly different from the average value of group A, that showed no inflammatory reaction of the conjunctiva. In the small group of patients presenting with a more or less diffuse type of episcleral hyperaemia, there were strikingly high serum calcium concentrations in comparison to the other groups and these differences were statistically significant. Patients were treated with aluminum oxide hydrate in a dose of between 4.5 and 6 gr. daily and the systemic administration of dihydrotachysterole was discontinued. As soon as the serum calcium concentration returned to normal the inflammatory reaction

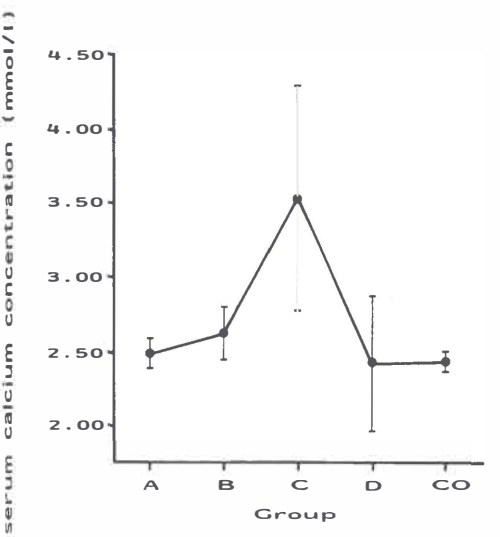

Figure 2. The average serum calcium concentrations with the 95% confidence limits, in the four groups and in the control group (Co). The average serum calcium values of groups A, B and C were statistically significantly higher than that of the control group. The average serum calcium of group C is markedly elevated and significantly higher than that of group A and B. The average values of group A and B did not differ significantly.

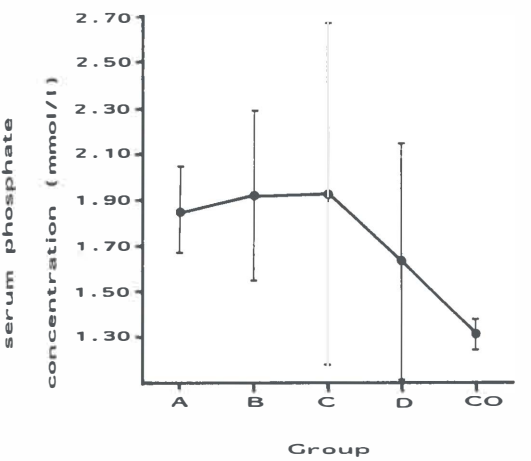

Figure 3. The average serum phosphate concentrations with the 95% confidence limits, in the four groups and in the control group (Co). The average serum phosphate concentration in all groups was significantly higher than in the control group, but statistically no difference was found between group A, B and C.

subsided.

Serum phosphate concentration.

In all groups the serum phosphate concentration was statistically significantly higher than in the control group (Fig. 3). The serum phosphate concentration between group A, B and C, however, did not differ statistically significantly. The serum calcium and phosphate product was statistically significantly higher in group C and this was because of the markedly elevated serum calcium concentration.

Discussion.

The first case on the association of irritable red eyes and renal failure was a patient in which calcium salts deposited in the corneal epithelium had flaked off and resulted in superficial corneal erosions, causing painful irritable red eyes that were soon relieved by padding of the eye (1). This case represented a reflex irritation and hyperaemia and, therefore, hardly qualifies as a specific ocular disease entity associated with renal failure.

The description of Berlyne and Shaw (2,3) on the type of the red eye, initially not having a slit-lamp available, was in rather general terms, such as "conjunctival hyperaemia" and "conjunctival irritation", and the illustration presented in a typical case seemed suggestive of inflamed pingueculae at the lateral aspects of the bulbus in both eyes. From the description and the illustration of Caldeira et al. (4) the red eye of renal failure seems identical with inflamed pingueculae, as were the lesions reported by Ehlers (5) and de Graaf et al. (6) and therefore, cannot be considered a specific disease entity.

These inflamed swellings close to the limbus associated with renal failure cannot be differentiated clinically from inflamed pingueculae not associated with renal failure. The formation of pingueculae are essentially the result of a combination of an age change and of exposure due to the prominent position of the globe in the palpebral aperture. Ehlers considered pingueculae in patients with chronic renal failure as an accelerated age change, a view shared by others (6) and this is also our opinion.

Therefore, we propose to reserve the term of "red eyes of renal failure" for those rare but specific inflammatory conditions of the anterior surface of the eyes that are characterized by a more or less diffuse waxy red episcleral and conjunctival hyperaemia, extending beyond the palpebral fissure, with little or no exudate. The lesion is quite unlike the nodular episcleritis or diffuse localized episcleritis periodica fugax. This red eye of renal failure is associated with a disturbance of the calcium metabolism, resulting in a high serum calcium concentration. Once the serum calcium concentration returns to normal the inflammatory reaction subsides.

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CHAPTER 9

PATHOPHYSIOLOGY OF CRYSTAL-INDUCED INFLAMMATION

* neutrophil-related mechanisms
* neurogenic-induced inflammation

Neutrophil-related mechanisms.

Introduction.

As early as in 1876 it was observed by Garrod that crystals that were deposited in tissues could cause an acute inflammatory reaction (1). Almost hundred years later the terms "crystal deposition disease" and "crystal-induced inflammation" were introduced. This disease was then characterized as a dose-related, completely reversible, and non-specific reaction (2) that could be applied to arthropathies and possibly also to ocular disease. Up until now, however, the exact mechanism of the inflammatory host reaction to microcrystals is not clear.

The presence of microcrystals in several human tissues is mainly associated with rheumatic diseases, such as gout and pseudogout, but can also occur during haemodialysis treatment in patients with chronic renal failure. In the latter case the tissues involved are not only the joints but also the eyes. The term "apatite deposition disease" was suggested by Dieppe et al. (3) in patients on regular haemodialysis treatment, as in these patients the crystalline precipitate consists of hydroxyapatite (4,5).

Several crystals are implicated in various diseases. The inflammatory response to monosodium urate (MSU) crystals in joint disease, such as gout, is one of the best understood types of inflammation. Less is known about the inflammatory mechanism induced by calcium pyrophosphate dihydrate (CPPD) crystals, which are responsible for the development of pseudogout, and inflammation caused by hydroxyapatite (HA) crystals. The phlogistic potential of cystine crystals is controversial (6). Diamond (7) and cholesterol crystals are probably not phlogistic.

The ability of microcrystalline material to perturb or disrupt cell membranes became a central concept in early formation of the pathophysiology of crystal-induced inflammation. This concept suggested ingestion of a non-metabolisable crystal by a polymorphonuclear leucocyte (PMN) followed by binding of the crystal surface to the membrane of the secondary phagolysosome. Disruption of this membrane and release of lytic enzymes within the cell finally resulted in cell death and disruption of the cell membrane of the leucocyte, causing further destruction of the surrounding tissue.

Alison (8) was the first to describe this "membranolysis theory" in the context of pulmonary disease produced by silicate crystals. Wallingford and McCarty (9) extrapolated this "rupture from within" hypothesis to crystal-induced arthritis. Uptil now this theory is common.

The PMN appears to be the absolute requirement of the host response to crystals, and the interaction between PMN and several crystals is, therefore, the major event in the development of crystal-induced inflammation (10, 11, 12). However, there is no invariant host response to a given crystal, and the simple presence of MSU crystals, for example, and PMN's within the joint is not a sufficiënt condition for acute inflammation. It appears, therefore, that the host response to the crystal must be modulated by one or more factors and it is likely that one of the major determinants of the biological activity of different crystals is the interaction of the crystal with serum proteins and other proteins.

It has been demonstrated in vitro that this activation of PMN's in crystal deposition disease can be the result of the presence of a number of inflammatory mediators (13). Furthermore, it has been stated that individual crystals can use several pathways to activate PMN's. This process of activation is called initiation. In the following paragraphs the mechanisms by which crystals activate PMN's will be discussed.

Interleukins.

In acute gouty arthritis, caused by MSU crystals, a possible role of interleukin-1 is suggested. Duff et al. (14) demonstrated in vitro that MSU crystals could stimulate "resident" monocytes to produce interleukin 1. Interleukin-1 is chemotactic for PMN's (15) and initiates therefore, the infiltration of these inflammatory cells (10). Once the PMN's are present interleukin-1 can activate them directly to produce a respiratory burst (16), degranulation (17) and thus the generation of other chemotactic substances, thereby initiating and perhaps amplifying the results of crystal-PMN interaction in the propagation of inflammation.

Acting on a variety of targets interleukin-1 is not only a chemotactic factor, but it accounts also for several aspects of the acute phase reaction, and its persistent elaboration may be responsible for some of the clinical hallmarks of chronic inflammatory disease (18). However, Malawista et al. (19) could

not show that HA and CPPD stimulated interleukin-1 production.

Immunoglobulins.

The first group to call attention to the binding of protein to the surface of crystals was Scheel et al. (20) in 1954. The most intense interest in crystal protein interaction, however, is focussed on immunoglobulin binding. Hasselbacher et al. (21,22) speculated that MSU crystals might be capable of preferential adsorption of the immunoglobulins G and M, the ionic charge of the protein and the crystal surface being the dominant force allowing adsorption (23,24). These authors also stated that surface immunoglobulin potentiated the acute inflammatory response to the crystals when they were released in the synovial cavity (22) and not by the novo precipitation of crystals within the joint cavity.

Immunoglobulins are a class of molecules produced by plasmacells evolved from lymphocytes. It should be emphasized that immunoglobulins are effectively bifunctional molecules. One part, which is extremely variable between different immunoglobulins, is called the Fab-fragment. The second part, the constant portion, is known as Fc-fragment and can bind to Fc-receptors of the membranes of neutrophils and monocytes.

If surface charge interactions play a role in the orientation of the IgG molecules on the crystal-surface, than the relatively more positively charged Fab-end of the molecule may be adjacent to the crystal surface whereas the relatively more negatively charged Fc portion is exposed to the surrounding environment. The Fc-fragment is, therefore, free to interact with neutrophils and monocytes.

The interactions at the Fc site account for a considerable proportion of the biological effects of crystal-protein interaction (25), such as particle phagocytosis. Some authors believe that adsorbed immunoglobulin G promotes complement activation (26) and increase neutrophil lysosomal enzyme release (27). It should be emphasized however, that the interaction of crystals with immunoglobulins may operate not only to enhance the inflammatory reaction (28) but also to protect against membranolysis (29).

Complement.

Complement may be one of the mediators of crystal induced inflammation. Naff et al. (30) were the first to note that phagocytosis of MSU crystals by neutrophils was markedly stimulated in the presence of opsonizing complement protein. Also HA and CPPD crystals can activate complement (26,31,32,33). MSU crystals seem to be the most powerful activators of complement; CPPD and HA are moderately active when compared to MSU on a weight basis (26,33).

The complement (C) system consists of at least 20 serum proteins, many of which are proteases, all capable of interacting with each other but also with antibodies and with cell membranes. These interactions lead to the generation of biologic activity. The biologic sequelae of activation of this system range from lysis of a spectrum of different kinds of cells, bacteria and viruses to direct mediation of inflammatory processes.

The individual proteins of this system are normally present in the circulation as functionally inactive precursor molecules. Each complement component must be activated sequentially under appropriate conditions in order for a complement reaction to progress. In particular, low molecular weight peptides, released during firing of the complement cascade (the sequential activation of complement enzymes), have powerful effects on inflammatory cells. Two major biological activities of the complement system are 1) the attraction of different types of leucocytes, 2) the formation of a cytotoxic protein complex, i.e. the membrane attack complex.

The complement system is usually triggerd by the interaction with antigen-antibody complexes. The first component, C1, is activated by the immune complex to form C1 esterase which, in turn, acts upon C4 and then C2. This produces C3 convertase, which cleaves C3 into C3a fragments (released into the medium) and C3b fragments. The C3b fragments bind to the surface of the cell, forming C423 enzyme. This interacts with C5 (cleaving off C5a fragments) followed by C6 and C7. C3a and C5a are strongly chemo-attractive for PMN's and monocytes. Finally there is binding of C8 and C9, and this leads to membrane damage and lysis of the cell via the membrane attack complex.

There are two parallel but entirely independent mechanisms or pathways leading to activation of the terminal portion of the complement

sequence. These mechanisms of activation are two enzyme cascades, termed the classical and the alternative pathway. Each involves several reaction steps. The two activation pathways converge at the midpoint of the complement system, and the remainder of the reaction sequence is common to both pathways.

The alternative pathway provides non-specific "innate" immunity, whereas the classical pathway represents a more recently evolved mechanism, which confers specific "adaptive" immunity. This classical pathway, involving C1, C4 and C2, with the production of C3 convertase, is activated by most antigen-antibody complexes and by certain non-immunologic agents such as plasmin and trypsin.

The mechanism of complement activation in crystal-induced inflammation is not clear (34). Kozin (27) stressed the importance of adsorbed immunoglobulin to the crystal surface as did Hasselbacher (26), whereas others suggest that activation arises as a direct effect of the crystal surface (32).
Doherty et al. (33) considered the immunoglobulin binding to the crystal surface as non-specific and supported the hypothesis that the crystal surface alone may be sufficiënt to confer complement activity. This was also the opinion of Naff et al. (31).

Naff et al. (31) could demonstrate complement activation via the classical nor via the alternative pathway and raised the possibility of a unique and unknown method of activation. Hasselbacher (26) has shown calcium dependent activation of serum C3 by MSU, HA and CPPD crystals and concluded that C3 activation occurs via the classical pathway. Also Ginsberg (35) demonstrated the classic pathway to be the operative mechanism.

Doherty et al. (33) suggested that complement activation by MSU, CPPD and HA at least in part is mediated via the alternative pathway. They postulated that crystals, with their rigid repetitive structure would be well suited for alternative pathway activation and showed that loss of the ordered crystal morphology (i.e. the amorphous state) resulted in inability to activate complement.

The general phlogistic potential of the crystals studied so far corrrelates roughly with the ability to activate complement. Thus, urate crystals seem to be most potent, with CPPD and HA intermediate. These three crystal types are the most clearly associated with acute inflammation in humans. Crystals such as silica, asbestos and diamond dust are not as clearly associated with acute

inflammatory syndromes and activate human complement little or not at all (2).

Polymorphonuclear neutrophilic leucocytes (neutrophils) and monocytes.

There is rather overwhelming experimental evidence pointing to the importance of the polymorphonuclear leucocyte in the pathogenesis of crystal-induced inflammation. MSU and CPPD crystals are both potent activators of neutrophils (36) and phagocytosis of MSU or CPPD crystals correlates strongly with the severity of inflammation (37,38). However, several studies on crystal induced **arthritis** pay attention to the lack of correlation between the number of crystals in joint fluid and the number of polymorphonuclear leucocytes (39,40,41,42).

In vitro studies of Maurer et al. (43) showed that HA crystals were readily phagocytosed by human PMN's. Although much of the work on the interaction of neutrophils and crystals has been centred on the release of lysosomal enzymes (44,45,46), the first reaction, chronologically, of the neutrophil to crystals is the production of oxygen radicals (47,48). These toxic oxygen derived products include superoxide, peroxide, and hydroxyl radicals and their release is triggered within 30 seconds of contact between the neutrophil membrane and crystals. Also, crystal-PMN interactions have been shown to lead to stimulate the synthesis of a low-molecular weight glycoprotein referred to as crystal-induced chemotactic factor (49).

Wallingford (9) introduced the hypothesis of the "suicide sac" or the "rupture from within", and this hypothesis was actually an extension of the concept of the inflammatory mechanism in pulmonal disease (8). After ingestion of a non-metabolisable crystal by a neutrophil it was suggested that the crystal binds to and disrupts the membrane of a secondary phagolysosome, which results in release of lytic enzymes within the cytoplasma of the cell. This would cause cell death of the PMN and membranolysis with release of the contents of the cell, including the crystal, thereby inducing a chemical inflammatory reaction in the surrounding tissues.

Although PMN's predominate in acute attacks of, for example, crystal induced synovitis, the PMN does not seem to be the sole cell responsible. Phagocytosis of both MSU and CPPD by monocytes (37) and synovial cells (50) occurs frequently. However, it would appear that not all cells are equally

susceptible to membranolysis by crystals. The cell membrane of murine peritoneal macrophages, for instance, is much more resistent to release of lysosomal enzymes than are neutrophils (51). Also not all crystals are equally capable to induce membranolysis. Cystine crystals, for example, after being phagocytosed do not result in rupture of the membrane of the neutrophil (6).

Cheung et al. (52) observed phagocytosis of HA and CPPD crystals when added to cultured synovial cells. Phagocytosis of both HA crystals and CPPD crystals by synovial cells appeared to be associated with the release of collagenase, neutral protease and prostaglandins into the surrounding medium. Also monocytes are known to release collagenase and neutral proteases as a consequence of the phagocytosis of particles and this will result in tissue damage.

Hirsch et al. (53) studied the interaction of HA crystals and monocytes in vitro. Although rapid uptake of the crystals by the monocytes occurred, they found no morphological evidence that the monocytes were actively engaged in crystal dissolution. Early discharge of lysosomes did occur after phagocytosis, but HA crystals remain in the cells without attracting a phagolysosomal response or resulting in tissue damage.

Crystal induced tissue destruction and inflammation have been recorded in patients at a time when there could have been only a very few neutrophils present (41,42,54). Phagocytosis of crystals is expedited when certain proteins are adsorbed to the surface of the crystal (55) and inhibited, on the other hand, if crystals are coated by competing proteins. Hyaluronate, for example, appears to interfere with crystal phagocytosis (54) and alpha-2-HS glycoprotein was demonstrated to be an active inhibitor of HA-induced PMN-stimulation (56).

Neurogenic-induced inflammation.

The axon-reflex.

Evidence is accumulating that sensory nerves play a role in the generation of certain types of inflammation. It is likely that neurogenic inflammation is also important in the development of crystal-induced inflammation. In the next paragraphs the pathophysiology of neurogenic-

induced inflammation and the role of mastcells will be described.

Inflammatory reactions, such as flare and weal, are present in allergic conditions but can also be the result of trauma. Lewis et al. (57) demonstrated the responses to intradermal histamine-injection and injury to be similar and it became apparent that mastcells, being the source of histamine, could be prompted to release their contents not only by an IgE-mediated process but also by the nervous system.

Golz et al. (58) showed already in 1874 the presence of vasodilator fibres in peripheral nerves and Stricker et al. (59) showed 2 years later that such fibres left the spinal cord in the dorsal roots. Bayliss (60) confirmed the presence of vasodilator fibres in peripheral sensory nerves and demonstrated that, when these nerves were stimulated antidromically, blood vessels in the skin dilated.

Langley et al. (61) formulated the concept of the axon-reflex. Lewis et al. (57) developed this hypothesis in the skin and showed that vasodilation, which clinically is referred to as flare, can be mediated by a neurogenic mechanism in which axonreflexes are involved. Additional work demonstrated the neurons carrying vasodilator fibres to be unmyelinated vasodilator C fibers originating in the dorsal root ganglion (62).

Celander and Folkow (63) suggested that one, peripheral, branch of such a C fibre is associated with a "nociceptor" (the input), whereas another peripheral branch forms a neuro-effector junction with some target cell. Stimulation of these nociceptors, that can be achieved by heat, firm pressure or by some chemicals and possibly also by microcrystals, initiates impulse transmission in the afferent neurone towards the spinal cord and, at the same time, these impulses can pass antidromically into other peripheral branches of the C fiber. These other branches then can release neuropeptides that are directed to mediate inflammation: either directly or indirectly by releasing mastcells.

Neuropeptides.

Neuropeptides, such as substance P, somatostatin, neurokinin A (substance K), calcitonin gene-related peptide (CGRP), have been localized in the nerves which are involved in the axon-reflex and they are considered to be

the transmitters for neurogenic inflammation. An intradermal injection of, for example, substance P produces a flare and weal reaction comparable to that seen following a similar injection of histamine. However, the flare induced by substance P is prevented by pretreatment with an antagonist of histamine at H1 receptors (64,65,66) whereas the weal is only partially inhibited. There are two possible explanations for these observations.

Firstly, when injected intradermally, substance P may release histamine from mastcells local to the site of injection. The histamine so released, in turn, may then stimulate the nociceptors to activate an axon-reflex which produces a flare distant from the site of injection. In this case, the flare would actually be produced by neuropeptides released from the sensory nerve through the axon-reflex, and not by the injected substance P.

A second possibility is that the neuropeptide released from the sensory nerve by the axon-reflex does not itself act directly to produce the vasodilation but acts indirectly by the release of histamine from mastcells. In this case, it is histamine which is the direct mediator of flare and there are several pieces of evidence which suggest that this is likely.

The mastcell.

Skotfisch et al. (67) showed in 1985 that fibres containing substance P form neuro-effector junctions with mastcells around blood vessels. It has also been shown that antidromic stimulation of sensory nerves induces mast cell degranulation (68). There is now good evidence that the development of flare in inflammation can be neurogenic, being the consequence of substance P-induced histamine release from mastcells. The development of an increased vascular permeability, which is clinically recognized by weal, on the other hand, appears to be a direct effect of substance P on vascular permeability with no contribution from histamine released by mastcells (66,69).

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CHAPTER 10

THE RED EYE OF RENAL FAILURE: A CRYSTAL-INDUCED INFLAMMATION?

*Klaassen-Broekema N, Bijsterveld OP van. The red eye of renal failure: a crystal-induced inflammation? Brit J Ophthalmol 1992; **76**: 578-81.*

Abstract.

Of 57 patients with chronic renal failure and calcification of the anterior membranes of the eye, 8 patients developed inflammatory reactions, clinically indistinguishable from pingueculitis. In 3 patients an acute inflammatory reaction of the episcleral tissue and the conjunctiva over it developed that we considered to represent the red eye **of** renal failure. In these patients, massive shedding of calcium phosphate salts was clinically evident.

Histopathologically in all these patients calcium deposits were observed. Although sporadically polymorphonuclear leucocytes were present in the inflamed tissues we could not demonstrate crystal phagocytosis. We believe that in the red eyes **in** renal failure and more specifically in the red eyes **of** renal failure, a crystal-induced inflammatory mechanism is not operative.

Introduction.

In patients with renal failure requiring regular dialysis, calcium deposits in the conjunctiva and limbus are common. As calcium phosphate salts are sparingly soluble and as for the precipitation of the salt the product of the concentration of the positively and negatively charged part of the salt is constant, an increase either in serum calcium or serum phosphate concentration, large enough to exceed the in-vivo solubility product, will lead to the deposition of calcium-phosphate salts in the form of microcrystalline hydroxyapatite (1).

An acute inflammatory response in the joints associated with the presence and phagocytosis of urate crystals was described in 1962 (2). Hydroxyapatite crystals also have a marked phlogistic potential (3). After being ingested by polymorphonuclear leucocytes they lead to cell death and to the release of large quantities of enzymes all capable of initiating inflammatory reactions. The crystals extruded in the extracellular tissue set in motion a new cycle of phagocytosis, cell death and the release of inflammatory mediators continuing the inflammatory response (4).

Berlyne (5) formulated the skilful theory of crystal-induced inflammation, a dose related and reversible inflammatory reaction (6), for the explanation of the mechanism of the red eyes of renal failure. The

histopathological study of Berlyne demonstrated the presence of subconjunctival polymorphonuclear leucocytes and calcium phosphate crystals small enough to be phagocytosed, a prerequisite for the crystal induced inflammation theory. However, no mention was made on crystal phagocytosis and he did not persue the crystal induced inflammation concept to explain the red eyes in renal failure.

Berlyne and Shaw (7) took a conjunctival biopsy of a patient they considered to present with the red eye in renal failure. However, their biopsy may have been taken from an inflamed pinguecula as demonstrated by the figure shown in their report. We, therefore studied biopsies from patients with a distinctly different type of inflammatory reaction, clinically characterized by a waxy-red hyperaemia of the episclera and the conjunctiva over it, patients which were biochemically characterized by a high calcium over phosphate ratio (8). In addition we examined biopsies that were taken from patients without any inflammatory reactions of the outer membranes of the eye and also from patients with inflammatory reactions clinically indistinguishable from pingueculitis.

Patients and methods.

The patient groups were identified both clinically and biochemically: group A consisted of 46 patients with calcium deposits but without the occurrence of an inflammatory reaction during the observation period, which was six years on the average. Group B consisted of 8 patients with calcification and inflammatory reactions associated with pingueculae. We considered these patients to represent the red eyes in renal failure. Group C consisted of three patients with an inflammatory reaction resembling diffuse episcleritis, well extending beyond the palpebral aperture, and an associated conjunctivitis, that we considered the red eye of renal failure. Biochemically these patient groups were characterized on the basis of the relationship between the serum calcium and phosphate concentration. Fifty healthy persons, matched in sex and comparable in age and weight, were used as controls.

Conjunctival biopsies were taken from patients of the three main groups and were examined by light microscopy. The tissues were fixed in phosphate buffered neutral formalin (10%). The histologic sections were processed according to standard methods. The histochemical stains used were hematoxilin-eosin, to reveal the general cell structure, Giemsa stain and toluidine blue for the study of polymorphonuclear leucocytes. The van Gieson stain was used to study elastotic degeneration of connective tissue. Calcium deposits were shown by the von Kóssa stain. Fresh tissue biopsies were taken and examined with immunofluorescence using anti-C1q and C3c to reveal tissue complement activation.

Table I. The average calcium and phosphate product and the number of persons in groups A, B, C and the control group (Co) are shown with the standard deviations.

	A	B	C	Co
average	4.52	5.02	6.62	3.13
number	46	8	3	50
SD(n-1)	1.33	1.41	1.62	0.46

Results.

Serum calcium and phosphate product.

In Table I the average values of the serum calcium-phosphate product of patient groups A, B, C and the control group is shown. Statistically there was a significant difference between the patient groups and the control group. The patient groups all showed higher values than the control group. This is shown graphically in Figure 1. From this figure it is apparent that between the patient groups there was also a difference; statistically this difference was significant ($p < 0.05$).

The highest calcium-phosphate product was observed in patients of group C. These patients had inflammatory reactions characterized by a diffuse waxy red epislceral and conjunctival hyperaemia. Only in this group the average value of the serum calcium-phosphate product exceeded the empirically observed serum calcium-phosphate product value that as of old was associated with the red eye of renal failure (7).

Serum calcium over phosphate ratio.

In Table II the average serum calcium-phosphate ratios are shown for

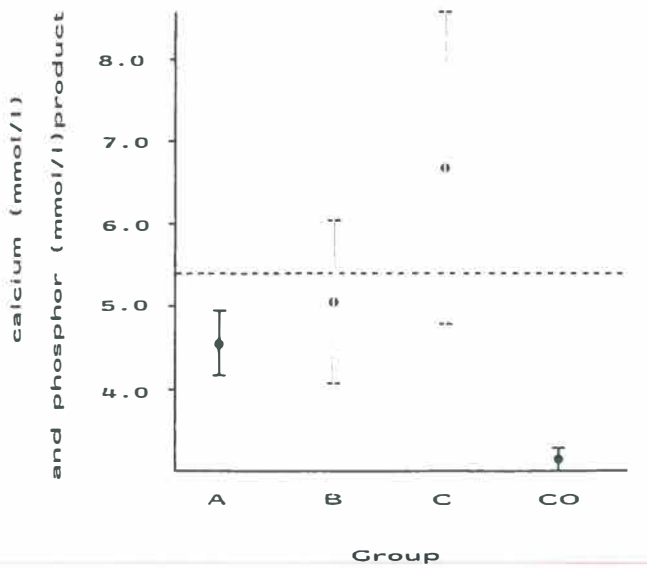

Figure 1. The average serum calcium (mmol/l) and phosphate (mmol/l) product with the 95% confidence interval of 46 patients (A) with calcific deposits but without inflammatory reactions, of 8 patients (B) associated with pingueculae and of 3 patients (C) characterized by a waxy red diffuse episcleral inflammatory reaction and the conjunctiva over it, well extending beyond the palpebral fissure, at the height of the inflammatory reaction. The calcium over phosphate product of the control group (Co) is also shown. The dotted line shows the empirical calcium and phosphate product above which the red eyes in renal failure were said to be associated.

the patient groups A, B, C and the control group. The control group and group C had the highest values. There was a statistically significant difference in the serum calcium over phosphorus ratio between the patient groups and the control group but not between the patient groups. The inability to demonstrate a difference between group A and B on one hand and group C on the other hand was the consequence of the small number of patients in group C. The difference between the patient groups we observed, however, was found to be dependent on the contribution of the average serum calcium concentration to the ratio; the serum phosphate concentration was rather constant, as shown in Table III.

Table II. The average serum calcium/phosphate ratio, the number of patients and the standard deviation in patients of groups A, B, C and the control group (Co).

	A	B	C	Co
average	1.51	1.44	2.00	1.90
number	46	8	3	50
SD(n-1)	0.56	0.39	0.75	0.24

Histopathology.

A conjunctival biopsy of a representative patient from group A showed marked subepithelial calcium deposits. There was a certain amount of elastotic degenerated connective tissue. No inflammatory cells in or around the calcific deposits were observed, but perivascularly some polymorphonuclear leucocytes were seen. There was no distension of the conjunctival or episcleral vessels.

In the biopsies of patients from group B also marked deposits of subepithelial calcium were seen. The collagen fibers were degenerated. A localized, moderate increase in polymorphonuclear leucocyte infiltration was seen in almost half of the cases. Sporadic leucocytes were observed in 14% of the biopsies, however these inflammatory cells may be completely absent. No crystal phagocytosis of calcium material was observed. The histopathology of this group is compatible with that of pingueculitis.

In the biopsies of patients of group C both conjunctival and episcleral vessels were markedly dilated. There was a marked degree of elastotic degeneration of collagen fibers. Calcium salts were present subepithelially. A few degranulating mastcells near the calcium deposits were observed. A moderate inflammatory reaction was shown in all patients, characterized by pavementing of the endothelium of the vascular walls by polymorphonuclear

Table III. Percentage increase of the serum calcium and phosphate concentrations in patient groups A, B and C in comparison with these values of the control group.

	A	B	C
calcium	3.2	7.7	31.7
phosphate	30.0	32.6	32.6

leucocytes; these cells were also visible perivascularly. A few eosinophilic cells, lymphocytes and plasmacells were among the inflammatory cells. The inflammatory reaction was diffuse and non-granulomatous (Fig. h, see addendum). Polymorphonuclear cells were not located in the areas of calcification (Fig. i, see addendum). No crystal phagocytosis was observed. The histopathology of this group is compatible with primary, simple episcleritis. By immunofluorescence no complement activation was detected.

Discussion.

After being phagocytosed microcrystalline hydroxyapatite is very toxic to leucocytes (9) because these crystals are membranolytic. It is therefore not surprising that for Berlyne the development of the red eye in renal failure is none other than the mechanism of crystal induced arthritis. He looked at the conjunctival sac as a specialized joint in which the moving surfaces are the lids and eyeball; the conjunctiva being analogous to the synovial membrane. In fact the presence of polymorphonuclear cells, mononuclear cells as well as crystals small enough to be phagocytosed, being the conditions for the induction of a crystal induced inflammation, seems to favour such a mechanism.

Hydroxyapatite crystal deposition in soft tissues will occur when the serum concentrations of calcium and phosphate locally exceed the solubility product; that is, in a marked supersaturated solution. In vitro the serum calcium over phosphate ratio determines the type of salt formed. In a high calcium over phosphate ratio of 1.67 or more, salts are deposited in a crystalline form, while a low ratio results in amorphous deposits.

If the concentration of calcium and phosphate ions increases homogeneically, large crystals are formed; small crystals are deposited if the ion concentrations increase inhomogeneically. In a situation that favours the precipitation of calcium salts of a crystalline nature and in view of the pathophysiology of renal failure one can expect the deposition of predominantly microcrystalline deposits of hydroxyapatite, which was demonstrated (5).

Particularly in the rather acute and pronounced diffuse episcleral and conjunctival inflammatory reactions of the patients of group C, the average serum calcium-phosphate ratio was 2.00, the result of the rather marked increase of the serum calcium concentration. Here, one can expect massive shedding of crystals, which we observed clinically, one of the prerequisites of crystal-induced inflammation.

Hydroxyapatite crystals most of which are less than 0.5 microns long cannot be seen by light microscopy and crystal phagocytosis could be difficult to demonstrate in a routine histopathological examination. Hydroxyapatite crystals have, however, a marked tendency to clump and to arrange themselves within a connective tissue matrix (10, 11). These clumps of crystal and matrix can be seen by light microscopy, free or intraleucocytic, as in periarthritis.

In spite of the presence of subconjunctival extracellular hydroxyapatite crystals in an acutely inflamed episcleral and conjunctival tissue, we were not able to observe crystal phagocytosis. In group C and also in the majority of the patients in group B there is an increased polymorphonuclear leucocyte infiltration, but they are not in the vicinity of calcium deposits. We did not observe chemotaxis exerted by hydroxyapatite crystals in the conjunctiva.

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CHAPTER 11

THE RED EYE OF RENAL FAILURE: A NEUROGENIC-DRIVEN INFLAMMATION

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Abstract.

In patients with chronic renal failure treated by long-term dialysis, inflammatory reactions occasionally develop in the bulbar conjunctiva; only rarely the episcleral tissue is also involved. Diffuse congestion of both the conjunctiva and episclera was present in 5.3% of our patients and is associated with a sudden and marked rise in the serum calcium concentration. Histopathological examination suggests that this form of hyperaemia, clinically being preceded by a marked shedding of calcium precipitates, is the result of a neurogenic driven inflammatory reaction in which mast cell degranulation is mediated by the axonreflex.

Focal hyperaemia associated with elastosis ("pingueculitis") was present in 6.7% of the patients. This type of hyperaemia was observed after an extended period of increasing levels of serum ureum and seemed to be independent on both calcium and phosphate serum levels. Diffuse hyperaemia of the conjunctiva, clinically being distinctly different from the combined diffuse conjunctival and episcleral hyperaemia, was also observed in 6.7%. Diffuse conjunctival hyperaemia seemed to be associated with low serum ureum concentrations. Here, also, an association with calcium and phosphate was not observed.

Introduction.

Inflammatory reactions can develop occasionally in the conjunctiva and epislceral tissue in patients with chronic renal failure on routine intermittent dialysis treatment. These "red eyes in renal failure" were assumed to be related to calcium precipitates in the limboconjunctival area (1,2). In some patients this relation is obvious: exfoliation of calcium concrements leave painful erosions of the limbal epithelium adjacent to focal hyperaemia of the conjunctiva.

In other patients the relation between calcium precipitates and hyperaemia is not evident. In patients with "pingueculitis" and renal failure (2-6), for instance, the occurrence of focal inflammatory reactions seems erratic and is clinically in no way different from that seen in healthy individuals. In patients with "diffuse conjunctivitis" or "pink eyes" (7), in whom a diffuse conjunctival

hyperaemia without exudate is present, there also seems to be no direct relation to calcium precipitates.

Acute diffuse conjunctival and episcleral hyperaemia, on the other hand, coïncides clinically with visible shedding of crystals in the interpalpebral zone of the conjunctiva (8). The present study speculates on the pathophysiology of the latter form of hyperaemia based upon histopathological examinations of conjunctival tissue removed at the height of the inflammation. We also investigated in a long-term follow-up study the conditions that can lead to the development of focal and diffuse conjunctival inflammatory reactions.

Patients and methods.

A total of 75 patients with chronic renal failure and on regular dialysis, 49 males and 26 females, were followed for ocular complications during a period up to 7 years. Grading the limbo-conjunctival calcification was done according to the system proposed by Porter and Crombie (6), i.e. to establish the degree of calcification in patients by comparing it to drawings depicting 5 stages of degree of calcification. The patient characteristics are shown in Table 1.

To monitor the changes in the serum concentrations of calcium, phosphate and ureum that were determined every one and a half months on the average, as well as the calcium and phosphate product, both Shewhart and Cumulative sum charts were used. The Shewhart method was based on the calculation of the mean value and the 95 and 99% confidence limits of 15 determinations for a particular parameter followed by plotting subsequent data on the chart.

As the Shewhart plot has the disadvantage that small changes in direction, i.e. deterioration or improvement of a particular parameter, are not readily seen, Cumulative sum (Cusum) charts were used as they do not have this disadvantage. In the Cusum plot the cumulative differences between the calculated mean of 15 previous determinations and subsequent data are plotted. Changes in the slope relative to the preceding slope are studied. In both the Shewhart and the Cusum plots the data were standardized for easy comparison. For statistical analysis the Mann-Whitney U-test and variance analysis were used.

Conjunctival biopsies were taken from patients with combined diffuse episcleral and conjunctival hyperaemia. The tissues were fixed in phosphate buffered neutral formalin (10%). The histologic sections were processed according to standard methods. The histochemical stains used were hematoxilin-eosin, to reveal the general cell structure, Giemsa stain and toluidine blue for the detailed study of polymorphonuclear leucocytes and mastocytes. The van Gieson stain was used to study elastotic degeneration of connective tissue. Calcium deposits were shown by the Von Kóssa stain. Complement activation was determined by direct immunofluorescence using anti-C1q and anti C3c.

Table I. The age distribution, the total number of hours dialysis and the degree of calcification, expressed in the Porter and Crombie (P&C) score, of 49 male and 26 female patients with chronic renal failure and on regular intermittent dialysis.

	males median (range)	females median (range)	Sig.
age	50 (26-73)	54 (35-71)	NS
Hrs dial	3931 (72-14196)	2769 (273-9360)	NS
P&C	1.5 (0.5-4)	2 (0.5-3)	NS

Hrs dial = total number of hours dialysis
P&C = degree of calcification
NS = not significant

Results.

Incidence of hyperaemia.

Fourteen patients were observed with periods of exacerbations and remissions of various forms of hyperaemia: either a diffuse combined episcleral and conjunctival hyperaemia (5.3%), a diffuse "conjunctivitis" (6.7%) or a "pingueculitis" (6.7%). A patient could at one time present with one type of inflammatory reaction and at another time with a different one. In Table II the incidence of all types of inflammatory reactions are shown and compared to the data of other clinicians. Although the reported incidences fluctuated markedly, it is of interest to find that the ratio of "red eyes" to "white eyes" is surprisingly low.

Table II. The percentage of patients with inflammatory reactions reported by the stated authors in patients with chronic renal failure.

Author	Year	RE/T	Pct
Berlyne	1968	3/13	23.1
Caldeira et al.	1970	5/16	31.3
Ehlers et al.	1971	1/78	1.3
Harris et al.	1971	2/18	11.1
Porter & Crombie	1973	4/38	10.5
Klaassen & v.Bijsterveld	1993	14/75	18.7
Total		29/238	12.2

RE = the number of patients with inflammatory reactions
T = total number of patients
Pct = percentage

Diffuse combined hyperaemia of the episclera and conjunctiva (CECH).

This type of hyperaemia is characterized clinically by a congestion of the episcleral vessels and the conjunctiva over it. Its incidence is low and recurrence uncommon. We have observed this form of diffuse hyperaemia in 4 patients; in only one patient there was a recurrence. This inflammatory reaction developed if the serum calcium concentration increased rapidly: initially presenting as a diffuse conjunctival hyperaemia that increased in intensity, later followed by a diffuse episcleral inflammatory reaction (Figure j, see addendum). The serum calcium and phosphate concentrations as well as their product and the serum ureum concentration of this group of patients are

Table III. The serum concentrations of calcium (mmol/l), phosphate (mmol/l) and ureum (mmol/l) as well as the serum calcium and phosphate product are shown in patients, at the moment of an inflammatory reaction, and in 50 healthy persons.

	calcium		phosphate		Ca*P		ureum	
	av.	sd(n-1)	av.	sd(n-1)	av.	sd(n-1)	av.	sd(n-1)
CECH	3.43	0.70	2.63	1.67	8.26	3.28	28.76	5.74
DCH	2.32	0.25	2.61	0.80	5.94	1.42	27.10	1.83
FHE	2.63	0.23	2.09	0.24	5.44	0.40	37.20	1.23
Co	2.41	0.10	1.30	0.17	3.13	0.46	4.71	0.86

av. = average value,
sd = standard deviation.
CECH = combined episcleral and conjunctival hyperaemia
DCH = diffuse conjunctival hyperaemia
FHE = focal hyperaemia associated with elastosis
Co = healthy persons

shown in Table III.

It is at once apparent that the serum calcium concentration in the CECH patients is markedly and statistically significantly increased in comparison to the serum calcium values in patients with other forms of hyperaemia. In Figure 1 the Shewhart plot of the serum calcium concentrations is shown for two patients of this group. A sudden and significant rise of the serum calcium concentration preceeds the hyperaemia. This pattern is nearly identical for all CECH patients and for the single recurrence in one patient.

During the period of acute episcleral and conjunctival hyperaemia, and also shortly afterwards, a shedding of lime salts could be observed clinically

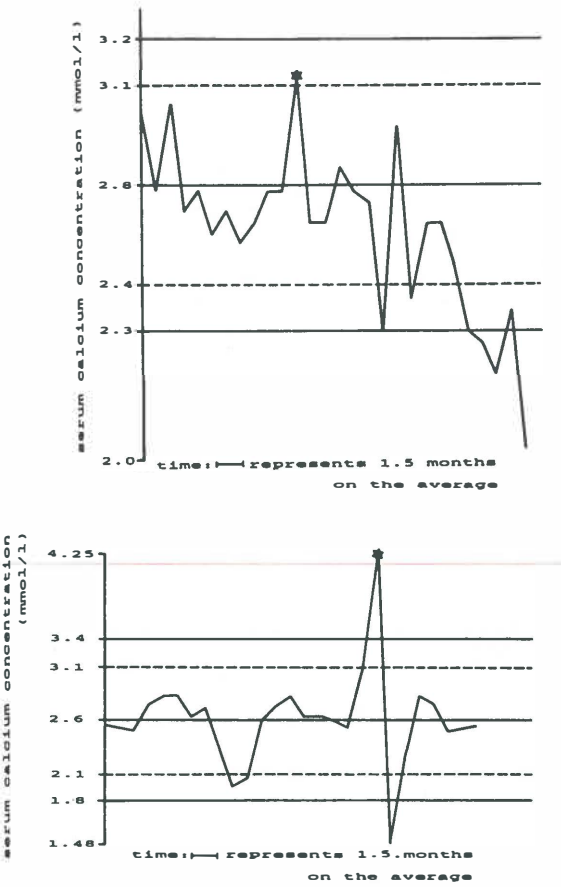

Figure 1. Shewhart plot of the serum calcium concentration in two patients with combined episcleral and conjunctival hyperaemia. There is a sudden and marked increase in the serum calcium concentration, that coïncided with an acute inflammatory reaction of the episclera and conjunctiva (*). (—) represents the 95% confidence limits. (—) represents the 99% confidence limits.

the limboconjunctival area in the particular patients. The serum values of phosphate and ureum in these patients showed no consistent tendency to increasing or decreasing levels during the observation period.

A conjunctival biopsy taken in the acute phase of the inflammatory

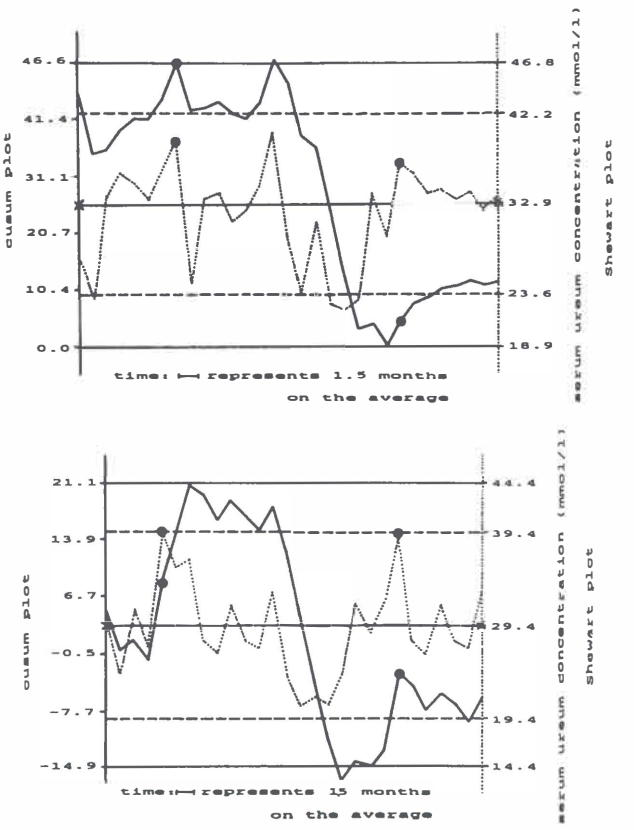

Figure 2. The Shewhart plot (dotted line) and the Cusum plot (solid line) of the serum ureum concentrations in two patients with pingueculitis. In the Shewhart plot it can be seen that an exacerbation of the pingueculitis can be expected if the serum ureum concentration is at a peak (●). From the Cusum plot it is clear that the exacerbation develops generally when the serum ureum concentrations are on the rise, indicating "deterioration".

reaction in a CECH patient showed a number of degranulating mastocytes adjacent to marked subepithelial calcium deposits (Figures k and l, see addendum). Elastotic degenerated collagen fibers, distended episcleral and conjunctival vessels with polymorphonuclear leucocytes, eosinophils and plasma cells were also observed. No crystal phagocytosis was observed nor was

complement activation detected by direct immunofluorescence.

Focal conjunctival hyperaemia (FHE) associated with elastosis ("pingueculitis").

In 5 patients with pingueculitis the serum calcium and phosphate concentrations did not seem to play an important role in the development of the inflammatory reaction (Table III). Also from the Cusum plots it was apparent that a relation with the serum calcium and phosphate concentrations was unlikely. The serum ureum concentration, on the other hand, was statistically significantly higher than in the other groups at the height of the inflammatory reactions.

As can be seen on the Shewhart plot of two FHE patients (Figure 2), the maximum serum ureum concentration coïncided with the peak of the inflammatory reaction. In this figure also the Cusum plot of these patients with episodes of pingueculitis is shown. Almost invariably the exacerbations developed after an extended period of increasing serum ureum concentrations.

Diffuse conjunctival hyperaemia or pink eyes (DCH).

Some of the inflammatory reactions present as diffuse smooth pinkish hyperaemia mostly of the bulbar conjunctiva that did not extend to other tissues. This type of hyperaemia tended to be chronic and eventually could last for months. Discharge, as in the other forms of hyperaemia, was not present. In fact if anything, the conjunctiva has a somewhat dry aspect. In 5 patients we observed this type of inflammation. The recurrence rate was about once a year on the average if also mild forms are included. The serum calcium and phosphate concentrations were not particularly high at the peak of the inflammatory reaction. The only consistent observation was the relatively low serum ureum concentration.

Discussion.

In only 18.7% of all our patients an inflammatory reaction of the conjunctiva, occasionally also of the episclera, was observed at one time or antother. Other investigators also found a relatively low incidence of these inflammatory reactions. The average incidence from all reports (2-6) can be

put at 12.2%. Histopathologically the inflammatory reactions were characterised by the absence of crystal-phagocytosis whereas in patients with a diffuse combined conjunctival and episcleral hyperaemia a large number of degranulating mastocytes could be observed.

We found in patients with a combined conjunctival and episcleral hyperaemia the inflammatory reaction to be associated with a sudden and marked rise in the serum calcium concentration that proved to be pathognomonic for this type of inflammatory reaction. In a few patients with "white eyes" nearly identical levels of serum calcium levels were observed. Hyperaemia, however, did not develop presumably because of a slow instead of a rapid rise in the serum calcium concentration.

An association between inflammatory reactions and the nervous system has been known for a long time (9). Recently, evidence has been brought forward that a functional relationship exists between primary afferent neurones and cells mediating inflammation (10,11). Peptides with a neurotransmitter function may be involved in the stimulation of mast cell responses and on other mediator cells involved in inflammation, such as neutrophils, macrophages and some T-lymphocytes (12). This concept of inflammation is generally referred to as "axonreflex" (13).

After some form of tissue injury, that, by the way, can directly mediate mastcelldegranulation, impulses then travel centrally to relay sensory information and, at the same time, antidromically into terminal arborisations of the sensory nerve that are located at a distance from the original injury. This results in the release of neuropeptides, such as substance P, that are able to generate an inflammatory reaction through mastcelldegranulation at some distance from the original injury.

We believe, therefore, that the diffuse combined episcleral and conjunctival hyperaemia in 4 of our patients is the result of neurogenic inflammation. The shedding of limesalts in the subepithelial tissues of the conjunctiva, that resulted directly from a sudden and marked elevation of serum calcium, could have mediated directly or through the axonreflex mastcelldegranulation. Focal hyperaemia surrounding elastosis, that was present in five dialysis patients and that also can develop in healthy persons, was preceded by a steady elevation of the serum ureum concentration. The pathophysiology of this inflammatory reaction is, both in uremic and healthy persons, unknown. The mechanism of the development of the diffuse conjunctival inflammatory reactions, or "pink

eyes", is also unknown.

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GENERAL SUMMARY

In patients with chronic renal failure, conventional dialysis eliminates body fluids and electrolytes resulting in a loss of body weight and a decreased blood urea osmolality. It is conceivable that the loss of body fluids causes a decrease in tearflow. We, therefore, measured several tear function parameters during one haemodialysis session.

Some twenty percent of the dialysis patients formally could be classified as having keratoconjunctivitis sicca to begin with. Moreover, in the majority of patients a significant decrease in tearflow was observed after one haemodialysis session that was on the average thirty-one percent. Porter and Crombie observed poor tearflow between the several dialysis sessions and theorized that inflammatory reactions of the outer eye could be associated with poor tearfunction. In our patients, however, such an association could not be demonstrated.

It is also likely that fluctuations of the intraocular pressure during haemodialysis are dependent on the dynamics of dehydration and hypo-osmolality. Regulatory forces, however, stabilize the intraocular pressure to such a degree that statistically significant fluctuations were not observed.

As in many diseases affecting the metabolism of the body as a whole, degenerative changes can be expected and chronic renal failure is no exception. In the ocular tissues conjunctival and limbal degeneration is known as pinguecula and the white limbus girdle of Vogt type II respectively. Although these changes can also be observed in healthy individuals as age advances, and particularly in the presence of actinic exposure, they are more common and also of greater extension in patients on regular dialysis.

In all dialysis patients a mild or moderate degree of limbal and/or conjunctival calcification is present, even in the early stages of renal disease. These calcium deposits consist of hydroxyapatite. Only when a medicamentally uncontrollable hyperparathyroidism with sustained high serum calcium levels sets in, a true corneal component is added: initially visible as the white limbus girdle of Vogt type I, later recognizable as band-shaped keratopathy.

The calcium salts are deposited preferentially at the areas with degeneration. Small wonder that many investigators did classify calcification of the outer eye in dialysis patients as, at least partly, degenerative in nature. However, on histopathologic examination the calcium precipitates are located superficially rather than deeper within the degenerative areas, a prerequisite for the definition of dystrophic calcification. Moreover, in dystrophic calcification one would expect an association between the magnitude of these degenerative

areas and the degree of calcification, and such an association was not found.

As metastatic calcification is defined as the precipitation of calcium deposits in an abnormal chemical environment, the soft tissue calcification in chronic renal failure falls per definition into this category. Quite contrary to the expectation in this respect is the finding that the degree of limboconjunctival calcification is independent on the serum calcium and phosphate concentrations at any given time. Although in a strict sense calcification in renal failure confirms to the definition of metastatic calcification other factors are most certainly operative.

The concept of calciphylaxis refers to the development of local calcification after minor local trauma in the absence of markedly elevated calcium and phosphate levels. It is a condition of induced hypersensitivity in which tissues respond to an appropriate challenger with local calcification. Renal failure can act as such an indirect sensitizing calcifier, as it gives rise to increasing levels of parathyroid hormone. Minor tissue injury of the eye, being referred to as a "local challenger", was represented by devitalisation of the interpalpebral limboconjunctival and corneal epithelium being the result of the markedly decreased tearflow after each dialysis session. As the degree of calcification is dependent on the duration of renal failure and also on the total number of dialysis sessions the limboconjunctival calcification can best be understood by this process of local calciphylaxis.

In patients with renal transplants the degree of limboconjunctival calcification decreases. This supports the theory of calciphylaxis, as the effects of the indirect sensitizer, i.e. renal failure, are disappearing. If the transplantation proves to be successful the levels of serum PTH will return to normal. When the haemodialysis sessions are discontinued, there are no recurrent decreases in the tearflow anymore and, therefore, no precipitous tissue devitalisation, i.e. the challenger disappears. The deposited calcium can then be slowly resorbed.

It is known that, apart from a variety of diseases characterized by long-term high serum calcium levels leading to band-shaped keratopathy, such a condition can also be encountered in degenerations of the eye. We observed a dialysis patient with a combination of markedly elevated serum calcium levels and ocular degeneration as a result of serious proliferative diabetic retinopathy, vitreous hemorrhages and extensive treatment with panretinal coagulation. Our patient developed centrally located small greyish white disc-shaped lesions that

was later followed by the development of a white limbus girdle of Vogt type I. The peripherally located white limbus girdle developed into a band-shaped keratopathy that rapidly spread towards the centre and finally resulted in a dense complete band.

In approximately twelve percent of the patients on regular dialysis an inflammatory reaction of the outer eye develops. The type that seems to be most specific for patients with chronic renal failure is the diffuse combined conjunctival and episcleral inflammatory reaction associated invariably with a sudden rise in serum calcium concentration be it as a consequence of a sudden disregulation of the metabolism or as a result of therapeutic measures. It appears, however, that under the term "red eyes" of renal failure a number of inflammatory conditions is included and their differentiation appears to be of importance with regard to the theories of the pathophysiology of the inflammatory reactions in renal failure.

Some of the inflammatory reactions are indistinguishable from "pingueculitis" and are also encountered in otherwise healthy individuals. As we shall see later, Berlyne tested his daring hypothesis of crystal-induced inflammation on the histopathology of biopsies from patients with pingueculitis. The "pink eye" of renal failure, a mostly bulbar conjunctivitis, has some peculiar characteristics not encountered in other forms of conjunctivitis. True, the soft, evenly distributed redness reminds one of H. aegyptius (Koch-Weeks) conjunctivitis, but the mucopurulent exsudate is lacking. In fact any type of exsudate is lacking and if anything the conjunctiva has a dry aspect.

Berlyne considered the conjunctival sac as a specialized joint in which the moving surfaces are the lids and the eyes, the conjunctiva being analogous to the synovial membrane. Hydroxyapatite has a powerfull phlogistic potential and, just as monosodium urate crystals in gout, will lead to membranolysis of leucocytes after phagocytosis with the release of large quantities of enzymes that lead to inflammatory reactions. The presence of polymorphonuclear leucocytes, mononuclear cells, as well as hydroxyapatite crystals small enough to be phagocytosed in conjunctival biopsies of patients with "red eyes" seems to favour the concept of crystal-induced inflammation in the red eye of renal failure.

Clinically one can observe occasionally the actual shedding of hydroxyapatite crystals. In our conjunctival biopsies that were taken at the height of the inflammatory reaction these crystals were nor found within the

polymorphonuclear leucocytes. Also, leucocyte accumulation did not seem to be present at the site of calcium precipitates, which indicates that chemotaxis due to hydroxyapatite precipitation is not an important aspect of the inflammatory reaction nor was there any evidence of complement activation. On the basis of these findings the concept of crystal phagocytosis had to be rejected.

In histopathological slides of patients with diffuse combined hyperaemia, the one thing that struck the mind was the abundance of lamina propria mastcells in the conjunctival connective tissue, many of them degranulating. As in none of these patients there was any evidence of allergy, the release of vasoactive amines had to be mediated by a different mechanism. On the evidence of histopathological examination the diffuse combined conjunctival and episcleral hyperaemia seems to be a neurogenic-driven inflammation in which an inflammatory reaction because of the wide area covered by the terminal arborisations of the nerve has a diffuse aspect.

NEDERLANDSE SAMENVATTING

I. Historische achtergrond.

Tot ver in de 20e eeuw was aan patienten met ernstige nierfunctiestoornissen geen lang leven beschoren. In de afgelopen 70 jaar echter zijn de behandelingsmethoden voor deze patienten sterk verbeterd: vooral de ontwikkeling van de kunstnier was van zeer grote betekenis. Vermeld dient hier te worden het baanbrekende werk dat gedurende de tweede wereldoorlog door W.J. Kolff in Kampen werd verricht.

Het zou echter nog enige tientallen jaren duren voordat deze therapie, die nu tot de routine behoort, voor elke patient toegankelijk werd. In 1993 bedroeg het aantal dialyse-patienten in Nederland 3400 (1 op de 5.000 inwoners). Hiervan werden 2400 behandeld met haemodialyse en 1000 met peritoneaal dialyse (gegevens zijn ontleend aan de Stichting "Renine"). De behandeling met de kunstnier betekent voor patienten die vroeger voortijdig overleden een langer en ook beter leven.

Ondanks deze vooruitgang is men echter nog steeds niet in staat om alle complicaties van nierlijden te verhelpen. Dit proefschrift beschrijft de oogafwijkingen die kunnen ontstaan ten gevolge van de veranderde vochthuishouding en de gestoorde calcium en fosfaatstofwisseling bij dialyse-patienten. Het geeft een antwoord op twee belangrijke vragen: welke factoren zijn van belang voor het ontstaan van kalkneerslagen in het oog bij dialysepatienten? En, ten tweede, wat is de oorzaak van ontstekingsreacties van het uitwendige oog bij deze patienten?

II. Nierfunctiestoornissen en de gevolgen voor de stofwisseling.

De nieren spelen een belangrijke rol in de uitscheiding van in het bloed opgeloste stoffen door middel van de afvoer met de urine. Chronisch nierlijden wordt gekenmerkt door retentie van vocht en elektrolyten waardoor hypertensie en atherosclerose ontstaan. Hyperkaliaemie en acidose zijn vaak voorkomende verschijnselen evenals hyperuricaemie en uremie. Een verhoogd ureum gehalte in het bloed veroorzaakt waarschijnlijk op directe wijze celbeschadiging en wordt wel in verband gebracht met pericarditis en thrombocytopathie. Ten gevolge van een gestoorde erythropoietine productie bestaat gewoonlijk een anaemie.

Een gestoorde nierfunctie leidt al heel vroeg tijdens de nierziekte tot een gestoorde calcium-fosfaat stofwisseling. Er bestaat een onvermogen tot het uitscheiden van fosfaat en ten gevolge van de metabole acidose is er een verhoogde botresorptie waarbij calcium vrijgemaakt wordt. Vrijgekomen calcium ionen vormen complexen met fosfaat ionen waardoor de serum concentratie van vrije calcium ionen zal dalen. Het gevolg hiervan is dat de bijschildklieren gestimuleerd worden tot een verhoogde productie van bijschildklierhormoon (parathormoon, PTH) in een poging het serum calcium te normaliseren. Bij de meeste patienten met chronisch nierlijden zal er dus een verhoogd serum calcium en fosfaat product bestaan.

Wanneer met dialyse behandeling begonnen wordt is de urine productie, en daarmee de uitscheiding van fosfaat, gewoonlijk minimaal. Omdat het echter, zelfs met de moderne dialyse technieken, moeilijk is de serum fosfaat concentraties volledig binnen normale grenzen te brengen, ontkomt men vaak niet aan het voorschrijven van fosfaat-bindende medicamenten. Toch zal bij de meeste dialyse patienten, ondanks deze medicamenten en eventuele aanvullende dieetmaatregelen, een verhoogd serum calcium en fosfaat product blijven bestaan.

De twee dialyse technieken die het meest gebruikt worden zijn haemodialyse en peritoneaal dialyse. Bij **haemodialyse** wordt bloed gepompt langs dialyse membranen waarbij een tegenstroom op gang gebracht wordt met behulp van een zogenaamde dialyse-buffer of dialysaat. Haemodialyse patienten hebben meestal 2 tot 3 behandelingen per week nodig die elk zo'n 3 tot 4 uur duren. Bij **peritoneaal dialyse** (CAPD = continuous ambulantory peritoneal dialysis) wordt gebruik gemaakt van het buikvlies als dialysemembraan. Het dialysaat wordt in de buikholte gebracht door middel van een semipermanente catheter. Met behulp van deze methode kunnen CAPD-patienten 24 uur per dag dialyseren.

III. Oogafwijkingen ten gevolge van chronische nierinsufficiëntie en dialyse.

III.1 Veranderingen in de vochthuishouding tijdens dialyse.

Het doel van dialyse is tweeledig: enerzijds wordt er vocht onttrokken aan het lichaam en anderzijds vindt er diffusie plaats waardoor er meer en minder osmotisch actieve stoffen geëlineerd kunnen worden; dit laatste leidt tot een daling van de serum ureum osmolaliteit. Het vochtverlies dat optreedt tijdens elke dialyse is aanzienlijk en kan wel 2 liter bedragen. Het is daarom niet onaannemelijk dat hierdoor de traanvocht productie afneemt. Hoofdstuk 1 beschrijft verschillende belangrijke klinische traanfunctie toetsen en hoofdstuk 2 toont aan dat de traanvochtproductie inderdaad gemiddeld met 30% afneemt tijdens elke dialyse.

Zowel onder clinici als onder laboratorium onderzoekers bestaat reeds geruime tijd een controverse over de vraag welk effect dialyse nu precies heeft op de oogdruk. Inderdaad zouden factoren zoals vochtverlies en een daling van de osmolaliteit kunnen leiden tot een verandering in de kamerwater productie en daarmee tot een verandering van de oogdruk. Wij hebben dit probleem opnieuw bestudeerd (hoofdstuk 2). De waargenomen oogdrukschommelingen tijdens dialyse waren echter statistisch niet significant. Kennelijk beschikt het gezonde oog over adequate adaptatiemechanismen om pathologische dalingen of stijgingen van de oogdruk te voorkomen.

Hoofdstuk 3 gaat in op de hypothese van Porter en Crombie die veronderstelden dat door de daling van de traanvocht productie ontstekingsreacties van het uitwendige oog bij dialyse patienten konden voorkomen. Ons onderzoek, dat zich richtte op de acute traanvochtproductie daling tijdens dialyse, kon deze hypothese niet bevestigen.

III.2 Een gestoorde calcium-fosfaat stofwisseling en het ontstaan van weefsel-verkalkingen.

Ondanks optimale dialyse behandeling en eventuele aanvullende medicatie bestaat bij de meeste dialyse patienten een verhoogd calcium en fosfaat product in het bloed. Het gevolg hiervan kan zijn het neerslaan van kalkzouten

in de vorm van hydroxyapatiet in verschillende weefsels, zoals in de ogen, de bloedvaten, de gewrichten en de huid. De verkalkingen in de conjunctiva en in de cornea komen het meeste voor.

Deze weefselverkalkingen werden vanoudsher gerangschikt onder de term metastatische calcificatie, een term die door Virchow geintroduceerd was op basis van de gelijkenis van dit proces met dat van cel-metastasering bij een maligniteit. Bij metastatische calcificatie ontstaan kalkneerslagen op afstand nadat calcium-zouten vanuit de botten door het bloed versleept zijn. De term werd ook wel gedefinieerd als het optreden van kalkneerslag in voorheen gezond weefsel vanuit een omringend "ziek" milieu. Het omgekeerde komt voor bij dystrophische calcificatie waarbij kalkneerslag optreedt in ziek weefsel dat omringd wordt door een "gezond" milieu.

Hoofdstuk 4 gaat nader in op de vraag omtrent de aard van de kalkneerslagen in het oog. Opvallend was altijd geweest dat, met uitzondering van één studie, de graad van kalkneerslag niet direct geassocieerd bleek te zijn met de hoogte van de calcium en fosfaat spiegels in het bloed en de hoogte van het calcium en fosfaat product. Ook wij konden deze relatie niet aantonen.

Klinisch en histopathologisch lijken de conjunctivale en corneale afwijkingen bij dialyse patienten op degeneraties zoals die ook wel voorkomen bij gezonde personen en die wij kennen onder de respectievelijke namen van pinguecula en de limbus gordel van Vogt type II. Hoofdstuk 4 analyseert de relatie tussen de grootte van het gedegenereerde conjunctiva-gebied en de graad van kalkneerslag. Wij konden niet aantonen dat er een relatie tussen deze beide parameters bestond. Ook bleek uit histopathologisch onderzoek dat de kalkneerslagen niet **in** het gedegenereerde bindweefsel liggen maar er door overdekt worden.

Dat celbeschadiging toch een rol kan spelen bij het ontstaan van kalkneerslag was door eerdere onderzoekers zowel in dierexperimenteel als in humane klinische studies aangetoond. Hoofdstuk 5 is de uitwerking van de hypothese dat de forse traanvocht productie daling na elke haemodialyse (hoofdstuk 2) kan leiden tot aanwijsbare epitheelbeschadiging van het uitwendige oog. Deze epitheelbeschadiging, die dus elke week zo'n 2 tot 3 keer voorkomt, bleek inderdaad mede verantwoordelijk te zijn voor de ernst en omvang van het limboconjunctivale kalkneerslag.

Bij de meeste dialyse patienten kan de overactie van de bijschildklieren (secundaire hyperparathyreoidie) onder andere met behulp van medicamenten

min of meer onder controle gehouden worden. Bij enkele patienten echter kan zich een situatie voordoen waarbij de secundaire hyperparathyreoidie niet meer reageert op conservatieve therapie: de hyperparathyreoidie is autonoom geworden. Deze situatie wordt door sommige clinici ook wel tertiaire hyperparathyreoidie genoemd.

In Hoofdstuk 6 onderzochten wij de lokalisatie van de kalkneerslagen in het oog bij patienten met een secundaire en tertiaire hyperparathyreoidie. Wij vonden een bandvormige keratopathie bij patienten met een tertiaire - en niet bij een secundaire - hyperparathyreoidie die klinisch niet te onderscheiden was van de keratopathie bij primaire hyperparathyreoidie. Bij één patient ontwikkelde zich een bandvormige keratopathie op een atypische en zeer snelle wijze. Hoofdstuk 7 beschrijft de ziektegeschiedenis van deze patient.

III.3 Kalkneerslagen in het oog en het ontstaan van ontstekingsreacties.

Bij een groot deel van de dialyse patienten ontstaan in de loop van de ziekte van tijd tot tijd ontstekingsreacties van het uitwendige oog. Wij waren in de omstandigheid een grote groep dialyse patienten gedurende meer dan 6 jaar te kunnen bestuderen en er ontstond bij ons de indruk dat zich waarschijnlijk verschillende vormen van uitwendige hyperaemie kunnen ontwikkelen. Hoofdstuk 8 beschrijft deze verschillende verschijningsvormen waarbij vooral de differentiatie tussen focale en diffuse ontstekingsreacties van belang leek voor de etiologie.

Traditioneel werden de ontstekingsreacties in het oog in verband gebracht met de aanwezigheid van kalkneerslagen in de conjunctiva en limbus. Men veronderstelde dat de conjunctivale hyperaemie een irritatieve reactie was op de vaak kleine kalkkristallen. Berlyne zag als eerste de analogie tussen de conjunctivale ontstekingsreactie bij dialyse patienten en de ontstekingsreactie bij jicht waarbij in het laatste geval uraatkristallen toxisch zijn voor leucocyten; de leucocyten gaan te gronde onder vrijkoming van ontstekingsmediatoren. Hij formuleerde de hypothese dat ook hydroxyapatiet kristallen, eenmaal neergeslagen in het oog, in staat waren om polymorphonucleaire leucocyten aan te trekken en op deze wijze een conjunctivale ontstekingsreactie in gang konden zetten. Hoofdstuk 9 geeft een literatuuroverzicht betreffende de mogelijkheden van hydroxyapatiet kristallen om ontstekingsreacties te veroorzaken.

De hypothese van Berlyne was uiteraard zeer interessant en in hoofdstuk 10 hebben wij deze getoetst. In geen van de ontstekingsvormen zoals wij die bij dialyse patienten hadden waargenomen konden wij echter kristalfagocytose en celdestructie aantonen in de omgeving van de hydroxyapatiet kristallen.

Ondanks de aanwezigheid van kalkkristallen in de ogen van bijna elke dialyse patient is het opvallend dat de meeste patienten geen ontstekingsreactie tonen. Bovendien is het opmerkelijk dat de hyperaemie zich soms uitstrekt tot buiten het gebied van de kalkneerslagen. Bij histopathologisch onderzoek vonden wij een groot aantal mestcellen zowel in de directe nabijheid als op enige afstand van de kristallen en dit leidde ons ertoe te veronderstellen dat neurogene componenten een rol zouden kunnen spelen bij het ontstaan van ontstekingsreacties van het uitwendige oog bij dialyse patienten. Hoofdstuk 11 is de uitwerking van deze hypothese.

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In 1986 as a first year resident in ophthalmology I was assigned to monitor ocular complications in patients with renal disease on chronic haemodialysis treatment. These patients were treated in the Foundation for Home Dialysis (STD), then located on the premises of the Royal Dutch Eye Clinic in Utrecht, and at that time under the medical supervision of Dr R.J.C. de Bos Kuil. So, early in my residency I had the opportunity to closely work in the field of ocular complications in systemic disease. Dr O.P. van Bijsterveld was asked to supervise my work. Ever since, ocular complications in renal disease has had my full interest and I developed a most cordial relationship with this group of patients and particularly with Dr de Bos Kuil, consultant for Internal medicine, whose wisdom and sympathetic counseling was a source of inspiration, not only for me but for all residents in ophthalmology.

Impressed with the loss of body fluids as a result of haemodialysis I wondered wat effect this would have on the intraocular pressure. Therefore, I tried to resolve the conflicting views on this topic and this study was completed successfully. Studies on the effect of haemodialysis on the tear function, on the other hand, were then only in part completed, since during many years I missed noticing the significance of the relation between tear function disturbances and ocular calcification. Only after stumbling on the unifying theory of Selye on soft tissue calcification and only after having developed sufficiënt analytical skills, I experienced the thrill of seeing the unexpected. I am particularly greatful to Dr Paul Kempenaers from Belgium, who was at that time a fellow at the Royal Dutch Eye Clinic. He meticulously performed all postdialysis tear function tests.

In the transitional period of leaving the beloved Royal Dutch Eye Clinic for the F.C. Dondersinstitute of Ophthalmology at the University Hospital Utrecht in the Uithof, AZU, the studies on ocular complications in renal failure were temporarily abandoned because of the hubbub of moving to a different hospital and later for the pressing requests to contribute most of my time to clinical practice. At that time, in August 1989, also the STD moved to a new building in Utrecht.

In 1991 the studies were renewed. I am very grateful to Dr J. Vos, presently the supervising nephrologist at the STD, for his support, advice and encouragement. He was always ready to explain the complicated and difficult matters on the metabolic consequences of chronic renal failure and dialysis

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I sincerely thank the patients of the STD who were always prepared to undergo biomicroscopical examinations, sometimes under difficult circumstances; for example during a dialysis session while being hypotensive so that an upright position was very difficult to achieve. Despite their suffering from serious renal disease these patients were always willing to supply - again and again - serum samples and to undergo - additional - tear function tests. The fact that succesfull treatment of the several eye complications associated with renal disease was quite uncertain to achieve despite our common efforts, makes the coöperation of these patients even more valuable.

I was assigned to take over the section on external eye diseases which allowed me to work closely with Dr van Bijsterveld, who during all the period supervised my work on ocular complications in renal disease. I am very grateful to Celia de Ruiter who taught me how to perform a perfect tear function examination and who introduced me in the daily practice of dealing with patients with external eye disease.

Because of knowing my affinity for the subject of ocular complications in renal disease, Dr van Bijsterveld suggested to take this topic as a model to study the basic concepts in external diseases. Dr Twan Lim did an extensive litterature search on the concept of crystal-induced inflammation for which I am very grateful. In rapid succession several studies were done on the various types of the "red eye of renal failure", the role of crystal induction in inflammatory reactions associated with renal failure, studies on conjunctival degeneration and calcification, limbal and corneal degeneration and calcification, challengers in limbal calcification and last but not least the nature of the red eye in renal failure.

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CURRICULUM VITAE

The author of this thesis was born on July 10th 1960 in Leeuwarden, the Netherlands. After completing primary and secondary (Stedelijk Gymnasium, Leeuwarden) education, she received the baccalaureat in June 1978. Medical education was attended at the Rijksuniversiteit Groningen (1978-1983). After 2 years of general internship in the University Hospital Groningen and Ziekenhuis de Weezenlanden in Zwolle from 1984 to 1985, she received the medical degree in 1985.

In October 1985 she enrolled in a residency programme at the Royal Dutch Eye Clinic in Utrecht in the Netherlands (head of the Department of Ophthalmology: till 1987 Prof. R.W.J.M. Hoppenbrouwers, since 1987 Prof. Dr J.S. Stilma) and she received the certificate of Eye specialist in October 1989.

From October 1989 till December 1993 she worked as a staff member in the F.C. Donders Institute of Ophthalmology, University Hospital Utrecht. The author of this thesis is a member of the scientific board of the European Society of Dacriology, and she is a member of the Editorial Board of "Dacriology News". Since 1993 she is working in private practice in the "Oogcentrum Houten", Houten.