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## **Glass–metal keratoprosthesis: Light and electron microscopical evaluation of experimental surgery on rabbit eyes**

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**Key words:** corneal blindness, glass, keratoprosthesis, platinum, electron/light microscopy, rabbit, clinical

**Abstract.** A keratoprosthesis (KP) is the last and only surgical resort to regain some visual acuity in eyes with severely damaged corneae. Corneal blindness represents an important percentage of the blind in the economically poor countries. Commercially available KP's, e.g. those made of PMMA, which are difficult to sterilize and vulnerable to surface damage, are too expensive in these countries. To overcome these disadvantages, we developed a new KP, made of a glass core melted into a platinum cylinder with flange. They were implanted unilaterally in eyes of ten Hollander rabbits intralamellarily. They were fixated by two stainless steel traction threads passed around the whole eyeball. We investigated this type of KP in the rabbit cornea, its acceptance by stroma, epi- and endothelium, and its hydro-mechanical dynamics in situ. No signs of infection or extrusion were observed. No epithelial downgrowth, nor adverse tissue reaction could be detected. LM and SEM showed endothelialization of the newly formed stroma around the central column of the KP. We conclude that this type of KP (although optically still to be optimized) has been accepted by the rabbit cornea and a clinical trial on cornea-blind patients is justified.

### **Introduction**

World wide, at least 10 million people suffer from bilateral corneal blindness, most of them live in the so-called Third World. Corneal blindness is caused by a large variety in pathology (e.g. trachoma, vitamin A deficiency, traumata, ulcerations, rosacea keratitis, cicatricial pemphigoid, dry eye syndromes, chemical burns, Stevens-Johnson syndrome) in (sub-)tropical areas. This type of blindness is endemic in economically underprivileged areas. Due to a relatively high population growth, this figure may double the next 25 years.

Application of a corneal transplant can be precluded by social-economic factors, or is impossible if the opacified cornea is vascularized. In the latter situation, a keratoprosthesis (KP) is the last and only surgical resort to (re)gain some visual acuity.

In the past and present various designs and materials have been tested and are still being tested [1–6].

One type of KP which is applied at present, consists of a rivet ('mush-room') of PMMA (Clinical Quality) episclerally fixated with stainless steel wires [7]. There are, however, disadvantages connected to PMMA: If in the post-operative period tissue overgrowth occurs, repeated removal of this tissue may result in scratching of the prosthetic surface due to the relative vulnerability of PMMA. In addition, the wetting properties of PMMA are not optimal, thus possibly impairing access of lacrimal fluid (containing lysozyme which is lethal for some Gram positive bacteria) to the tissue-prosthesis interface. Furthermore, this KP cannot be autoclaved.

To overcome these disadvantages, and taking in account that commercially available KP's are too expensive for the less economically privileged world, a KP was developed made of either KF-9 glass and Inconel-600, or KF-9 glass and technically pure platinum [8].

The purpose of this study was to test these types of KP in the rabbit cornea, its acceptance by the stroma and the epi- and endothelium, and its hydro-mechanical dynamics in situ.

## **Materials and methods**

### *Description of the KP*

In cooperation with Mr. M. Kolenbrander of Philips Res. Lab. (Eindhoven), a prototype KP was developed consisting of a metal cylinder (length 3.5 mm, diam. 3 mm) with a flange (diam. 6 mm) and a glass core. The metal was either Inconel-600 or technically pure platinum (Fig. 1). Both Inconel and platinum have the same heat expansion coefficient as the KF-9 glass, which for technical reasons is favorable for production.

With Inconel, the glass core was shaped by heat-pressure moulding between a concave and a flat steel die. With platinum, the glass core was either heat-pressure moulded or melted into the cylinder. The curvature of the front was shaped non-mechanically while the rearside was flattened by polishing. Four holes of 0.1 mm diam. were drilled in the metal rim and loaded with 70  $\mu$ m stainless steel wires. The wires were fixed by a single throw knot under each hole.

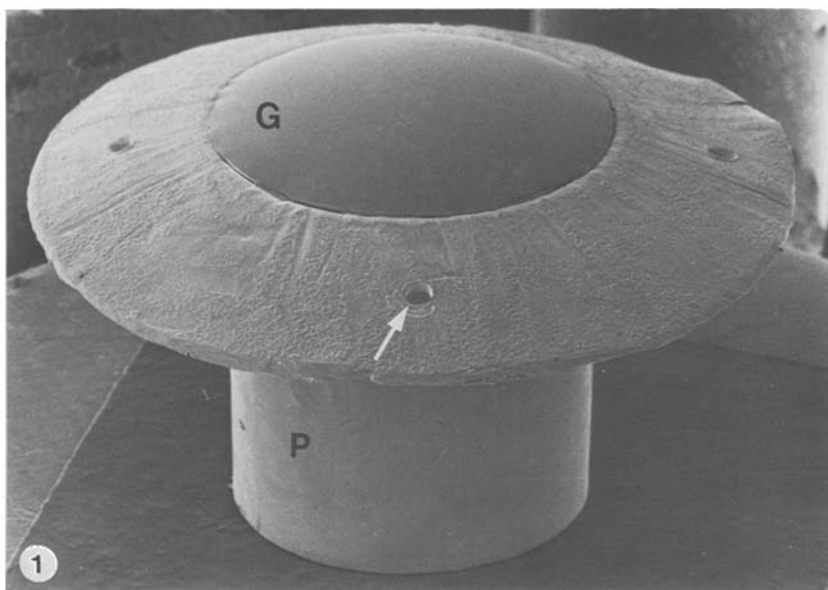


Fig. 1. SEM-image of *platinum-glass KP*; the edge has not been finished; P = platinum; G = glass; ↗ = hole for traction thread. Magn. 19 X.

### *Animals*

Chinchilla and Hollander rabbits were obtained from a local breeder, held individually in laboratory animal housings and fed *ad libitum*.

### *Material test*

To test the Inconel and the KF-9 glass for tissue inertness, discs of 2.5 mm diam. were placed into intralamellar pockets in the cornea, which were left unsutured.

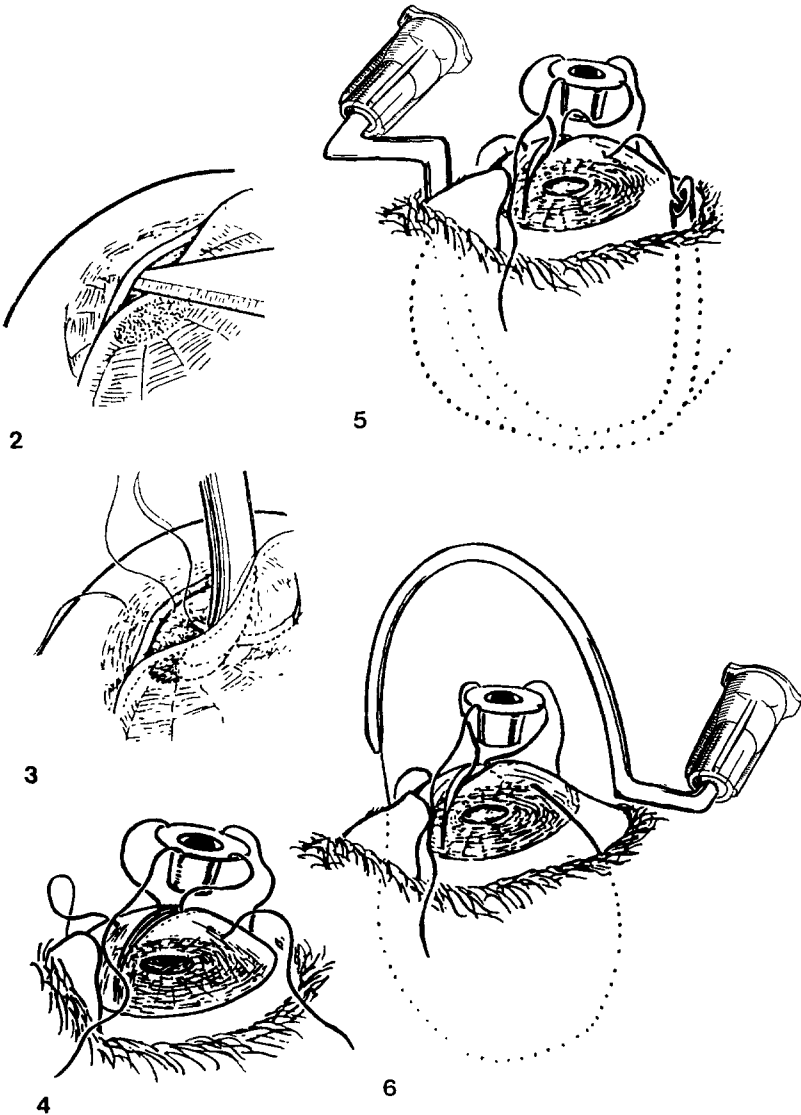
### *Surgery*

In eight rabbit eyes Inconel KP's were placed unilaterally, and in ten eyes platinum KP's.

Hollander rabbits were sedated with 1 ml Rompun™ s.c. in the neck, and ten minutes later anesthetized with 1 ml Ketavet™ bilateral i.m. to the highquarters. Due to greater average weight of Chinchilla rabbits, for these the amounts were 1.5 ml and 1.5 ml resp. Anesthesia was maintained as long as necessary by applying 0.5 ml, resp. 0.75 ml Ketavet every hour. Local

anesthesia, immobilization of the eye and pupil widening were achieved by an injection of 1 ml Xylocain (lidocain hydrochloride anhydrate 20 mg/ml, adrenaline 1:80,000) retrobulbar, and a few drops of oxybuprocain and phenylephrin onto the cornea.

The first step in the procedure was to make a 3H/9H incision of about half the thickness and over the middle of the cornea, long enough for lens extraction later on. Then intralamellar pockets at either side of the incision were made, together somewhat larger than the diameter of the hat of the KP



(Fig. 2). Two opposite stainless steel wires of the KP were carried outward through the edge of the pockets (Figs. 3 & 4).

Next step in the procedure was to 'careen' two stainless steel wires around the eyeball perpendicular to each other. This was done by passing a bent 15 G needle (of which the tip was blunted, and fixed on a firm grip) through a small conjunctival incision at 6 H around the eyeball. A stainless steel wire was slipped at 12 H into the needle as far as possible. The needle with wire was then pulled back to 6 H (Figs. 5 & 6). The same was done for the 3 H/9 H combination.

After this, anterior capsulotomy was carried out, the deeper half of the cornea was trephined with a 3 mm trephine, and the incision completed over the full thickness of the cornea. Then the contents of the lens capsule was gently rinsed out with heparinized saline, avoiding damage to the posterior capsule. The reason to make the rabbit eye aphakic, was to deepen the anterior chamber, since the KP is dimensionated for the aphakic human eye.

Subsequently, the deeper half of the incision was closed from both sides up to the trephination hole with 50  $\mu$ m stainless steel sutures (Fig. 7), whereby the sutures on either side of the trephination hole were kept loose. Then the KP was placed into position, with the brim positioned intralaminally (Fig. 8). The 3H/9H stainless steel wires were laid into the incision, and the sutures on either side of the trephination hole were also drawn tight. A macro-image of a KP is shown in Fig. 10.

Finally, the upper half of the incision was closed also using 50  $\mu$ m stainless steel sutures, and the 6H/6H and 9H/9H 'loose ends' of the stainless steel wires were twisted around each other at the level of the limbus to such an extent that the eye was forced into the shape of a tuffet (Fig. 9). The twisted ends were cut with scissors short and pushed back into the incisions in the

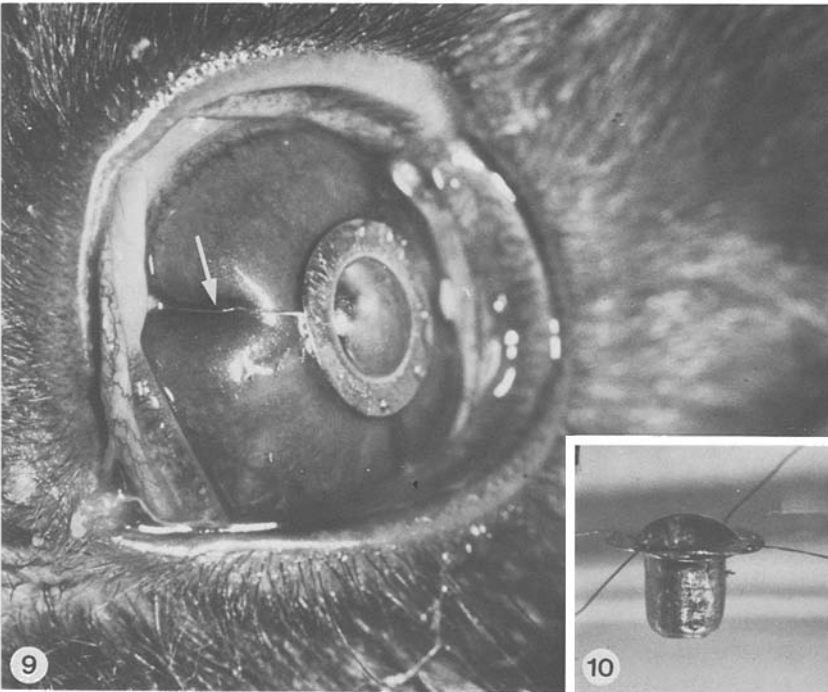
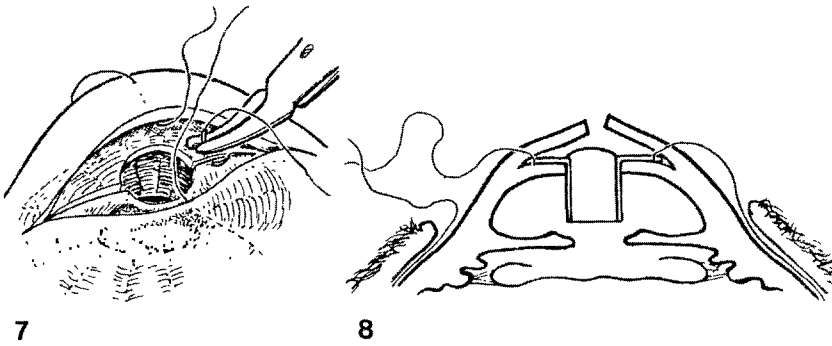
*Fig. 2.* After having made a half deep incision into the cornea, an intralaminar pocket for the hat of the KP is made.

*Fig. 3.* One traction thread is carried through an edge of the intralaminar pocket with a cornea suturing needle. A 27 G or 30 G needle can be used as well to 'tunnel' the thread through the edges. The other traction thread has already been carried through.

*Fig. 4.* Overview of KP with traction threads after having carried two threads through the edges of the intralaminar pocket.

*Fig. 5.* A bent and blunt 15 G needle has been passed around the eye ball through a conjunctival incision. One of the traction threads which was carried through an edge of the pocket previously, is slipped into the needle as far as possible. The grip on the needle is not shown.

*Fig. 6.* The bent needle has been pulled back, having carried the traction thread with it. When the traction thread has not been slipped deep enough into the needle, the thread may be lost somewhere under the eye ball, and the careening procedure must be carried out again.



*Fig. 7.* The deeper half of the cornea with Descemet's membrane is closed with stainless steel sutures. Previously, the deeper half of the cornea was trephined and the eye made aphakic.

*Fig. 8.* The hat of the KP has intralamellarily been placed into position. One 'pair' of traction threads with its loose ends (which are to be twisted around each other later on) are shown.

*Fig. 9.* Rabbit eye with implanted *platinum-glass KP* three days after surgery; note the tuff-like appearance of the eye; ↗ = one of the four traction threads.

*Fig. 10.* Macroscopical image of *platinum-glass KP* as used in the series.

conjunctiva, previously made for careening the wires. These small incisions were not sutured. In all cases the anterior chamber began to fill during suturing the upper half of the cornea, and in only three cases we considered it necessary to inject some air to deepen or enlarge the chamber angle.

Post-operative care consisted only of applying antibiotic ointment and taping to prevent desiccation and follow-up was carried out by slitlamp inspection until the animals were sacrificed.

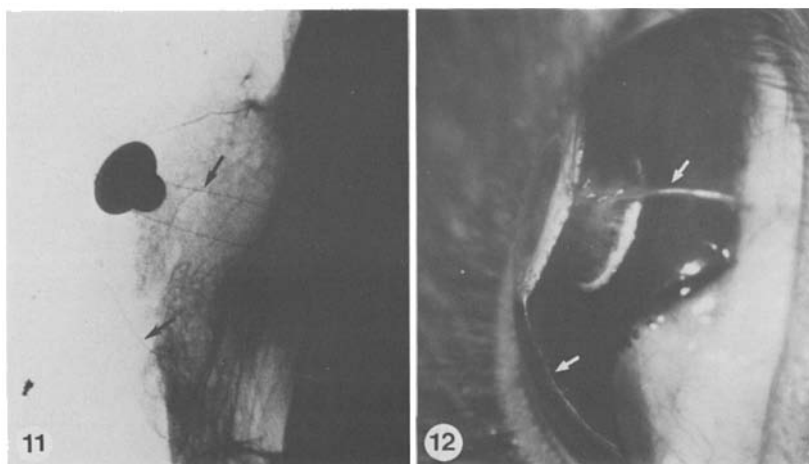
## Results

### *Material tests*

The tests with intralamellar Inconel and KF-9 glass proved that these materials are basically usable in cornea surgery. Although some vascularization occurs in the beginning, transparency was regained after five weeks, and is still present one and a half year later.

### *Post-operative trauma*

After the surgical procedure, the conjunctiva showed hyperaemia in all eyes for about two weeks. This must be attributed to the rather aggressive but



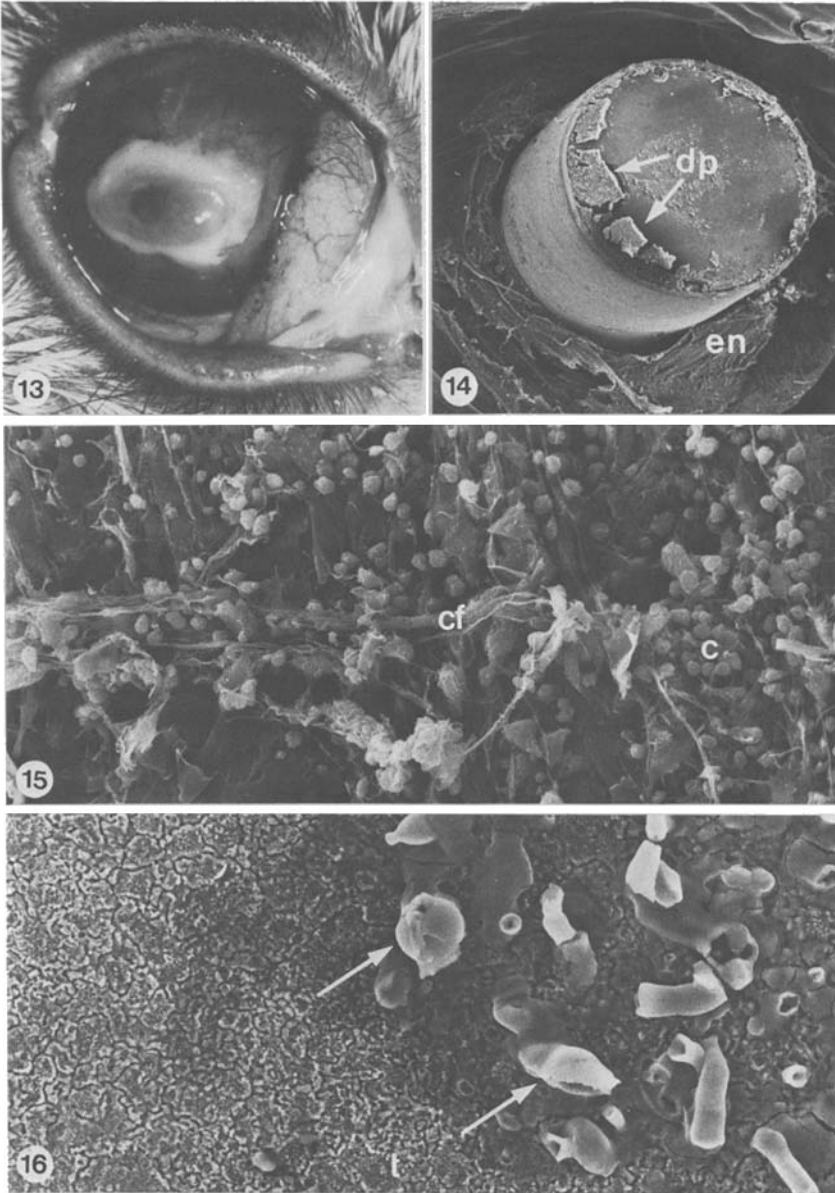
*Fig. 11.* X-ray image of rabbit eye with implanted *platinum-glass KP*; note the traction threads around the eye ball (↗ ↘).

*Fig. 12.* Rabbit eye with *platinum-glass KP* with healthy appearance, five weeks after surgery. The traction threads (↗ ↘) have become buried into the corneal tissue.



workable procedure to place the stainless steel wires around the eyeball by the careening method. On the X-ray image these wires can be clearly seen (Fig. 11).

In the beginning, all eyes appeared tuffet-like, as already shown in Fig. 9, due to the tightness of the wires. Within a few weeks the wires became



embedded in the cornea finally resting on Descemet's membrane, while the eyeball had regained its normal shape (Fig. 12).

Also common to all eyes, was massive vascularization of the cornea, although often limited to distinct quadrants. This phenomenon is most likely related to the blood supply of the muscles of the eye. Within a few weeks this vascularization became reduced and delta-like. Some opaqueness around the stainless steel wires and sutures was present a day after surgery, but disappeared within weeks.

### *The Inconel-glass KP*

Over a period of two to five weeks, the Inconel-glass KP seemed to behave well in the rabbit cornea. After that period, however, progressive melting away and ulceration started around the KP (Fig. 13) to such an extent, that it was necessary to sacrifice all rabbits in this series.

Under general anesthesia, the anterior chamber was rinsed with fixative, the cornea with KP removed and prepared for SEM. In all cases the internal condition of the eye was very poor, ranging from the presence of fibrous depositions and macrophages on the KP and endothelium (Figs. 14 & 15), to complete filling with purulent discharge. Endothelium was at best present only at the level of the limbus or could not be inspected due to the bad condition of the eye, and consequently may be assumed having disappeared completely. SEM examination of removed Inconel-glass KP's revealed the presence of spots of possibly oxides or salts on the metal (Fig. 16).

### *The Platinum-glass KP*

Only one of the ten eyes on which surgery was carried out, was lost due to melting away of corneal tissue following accumulation of aqueous humour

←

*Fig. 13.* Rabbit eye with *Inconel-glass KP* about six weeks after surgery showing ulceration and melting away of corneal tissue around the KP.

*Fig. 14.* SEM image of cornea with *Inconel-glass KP* viewed from the back-side. Most of the deposited material (dp) at the back-side of glass part of the KP was lost during critical point drying. The endothelium (en) around the KP is in rather bad condition. Magn. 14 X.

*Fig. 15.* Detail of endothelium around KP of Fig. 14 showing collagenous fibres (cf) and different types of cells (c), most likely granulocytes and macrophages. Magn. 710 X.

*Fig. 16.* SEM-image of the Inconel surface of a KP removed from an ulcerous rabbit eye. Spots of probably melted metal, or metal-oxides and/or salts (↗ ↘) are visible at the surface. Magn. 1250 X.

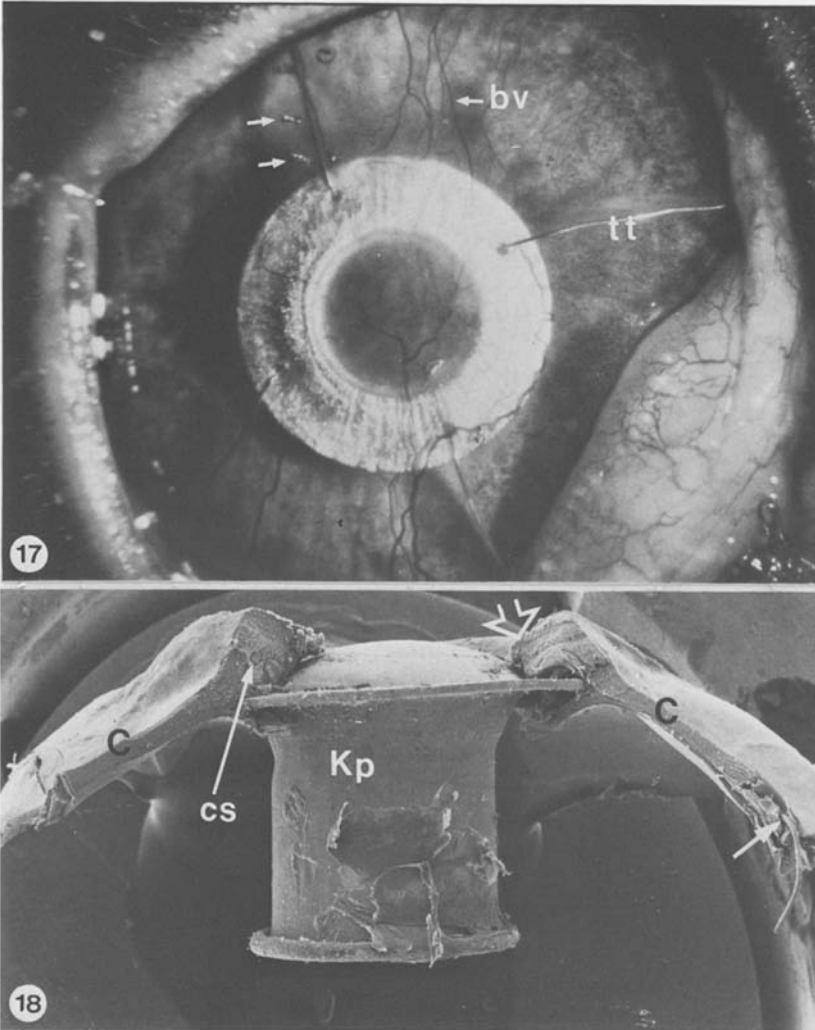
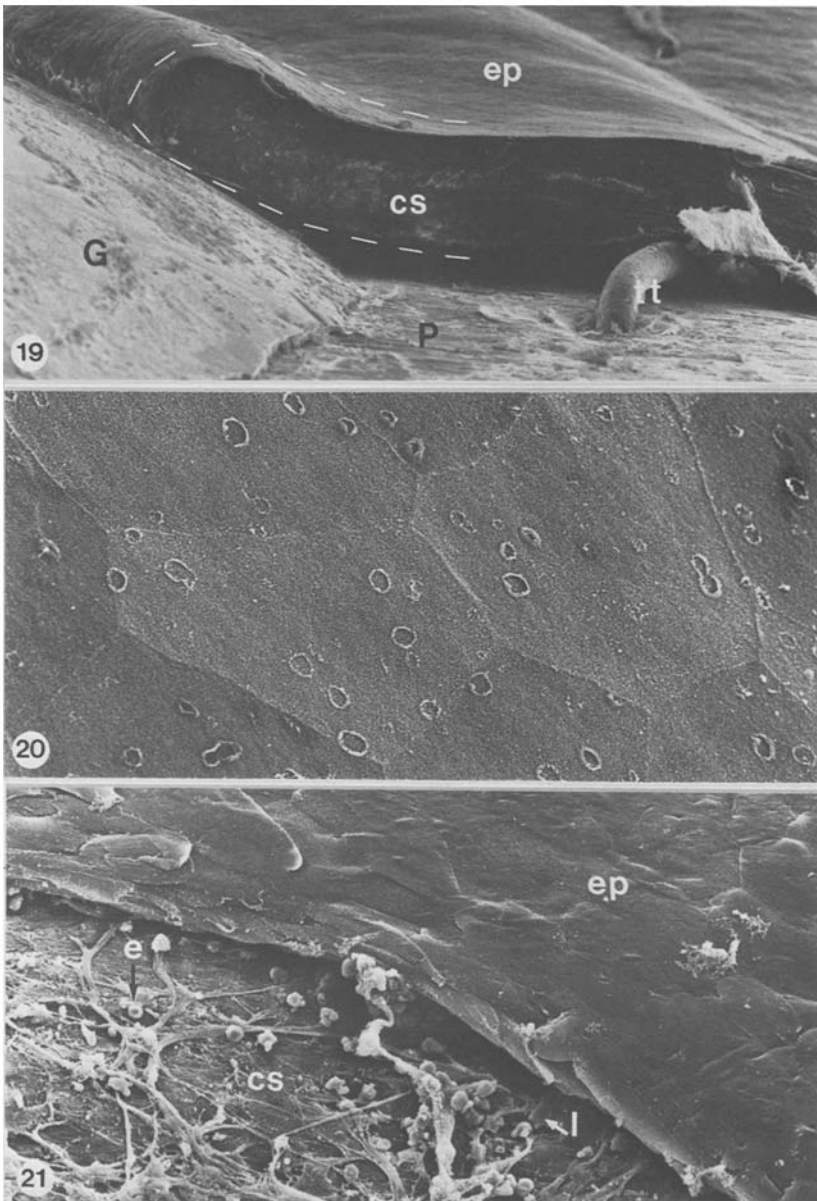


Fig. 17. Rabbit eye with *platinum-glass KP* seven month after surgery. The cornea is transparent; only a few blood vessels (bv) are present; tt = traction thread; (↗ ↘) indicate stainless steel sutures.

Fig. 18. SEM-image of a cross section of a rabbit cornea three month after implantation of a *platinum-glass KP*. Note one of the traction threads, buried into the cornea (↗); c = corneal tissue; cs = corneal stroma; ⇒ indicates corresponding area shown in Fig. 19. Magn. 18 X.

in front of the KP. Obviously, this was the result of a leak, caused by the absence of traction threads in this case.

Up till sacrificing, the other nine eyes stayed quiet. One of them is shown in Fig. 17. The cornea retained complete transparency although some vascularization remained.



*Fig. 19.* SEM image of part of rabbit cornea seven months after implantation of a *platinum-glass KP*; compare also with Fig. 22; cs = corneal stroma; P = platinum; G = glass; ep = epithelium; tt = traction thread; dashed line indicates area depicted in Fig. 24. Magn. 100 X.

*Fig. 20.* Corneal epithelium, seven months after implantation; detail of Fig. 19. Note relative large number of small holes (craters) in cell membrane. Magn. 1725 X.

*Fig. 21.* Detail of trephination rim bordering KP. Besides the presence of collagenous due to corneal stroma (cs) formation, erythrocytes (e) and lymphocytes (l) are present as result from cutting of small blood vessels during trephining; ep = epithelium. Magn. 640 X.

No signs of infections or extrusion were observed, while in all cases the anterior chamber had kept its depth. About four weeks postop., corneal overgrowth in front of the KP was trephined away. This overgrowth occurred repeatedly, taking four weeks to about two months to cover the whole KP. Repeated removal of corneal tissue in front of the KP did not affect the overall condition of the eye, and the glass surface of the KP stayed wet all the time.

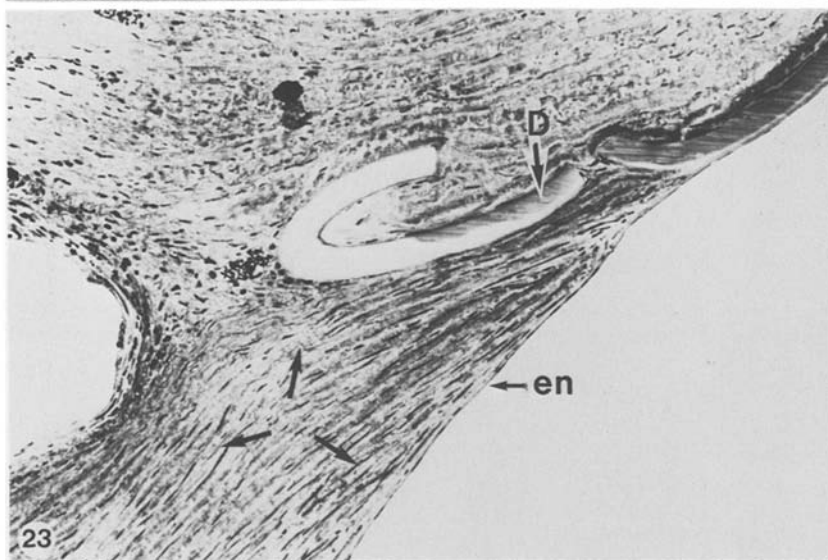
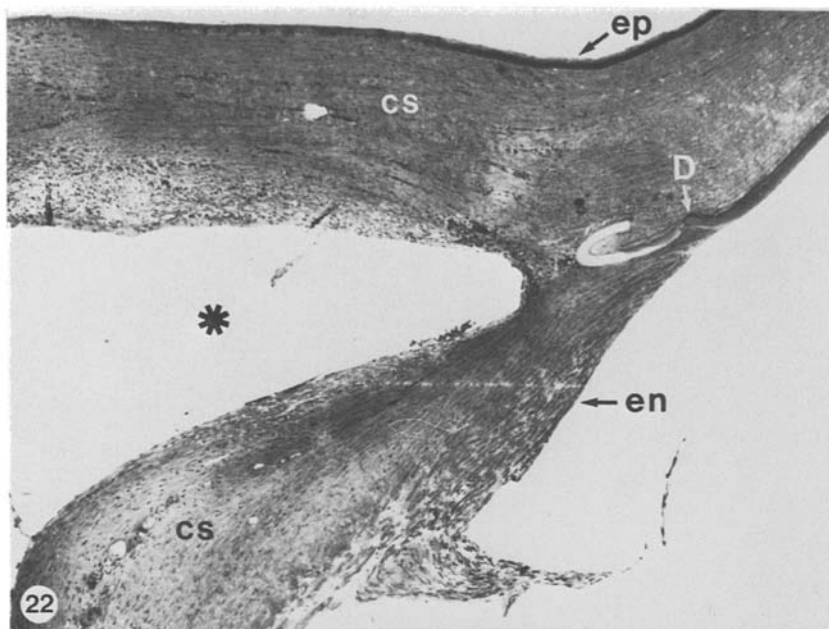
After 1.5, 3 and 7 months resp., the rabbits were killed under general anesthesia by an overdose of pentobarbital into an ear vein. The cornea with KP was quickly removed, prepared for (S)EM and LM in the usual way [5], and examined. In Fig. 18 a platinum-glass KP in the cornea is shown after 3 months residency; a part of the cornea was cut away to enable a better view on the intralamellar position of the hat of the KP. In Fig. 19 a detail of the 7 month KP (comparable to that of Fig. 18) is shown. Although having been trephined one month before, because of corneal overgrowth, the appearance seemed to be more stable. Up till now, we did not find a retroprosthetic membrane. The epithelium was normal for the major part of the corneal surface (Fig. 20), except for the trephination rim (Fig. 21). LM-pictures of a cross-section through the cornea adjacent to the KP (see Fig. 18) are shown in Figs. 22 & 23. The corneal stroma appeared quite normal, and only at the area adjacent to the KP some irregular organization of fibres is visible. The formation of newly formed corneal stroma in the 'fork-area' is clearly observable.

The rim of the trephination (see also Fig. 19, dashed line) is shown in a LM-section in Fig. 24. Corneal fibres close to the border with the glass of the KP, appeared less organized, while no epithelial downgrowth was seen.

With regard to the endothelial side of the cornea, it appeared that endothelialization had taken place over the newly formed corneal stroma around the trephination into which the cylinder of the KP is fitted (Figs. 22 & 23). At this site only a 100  $\mu\text{m}$  wide rim of endothelial cells bordering the cylinder of the KP showed abnormalities comparable with cases of local trauma (Fig. 25). Between 3 and 7 months, the 50  $\mu\text{m}$  stainless steel sutures through the Descemet's membrane were endothelialized completely.

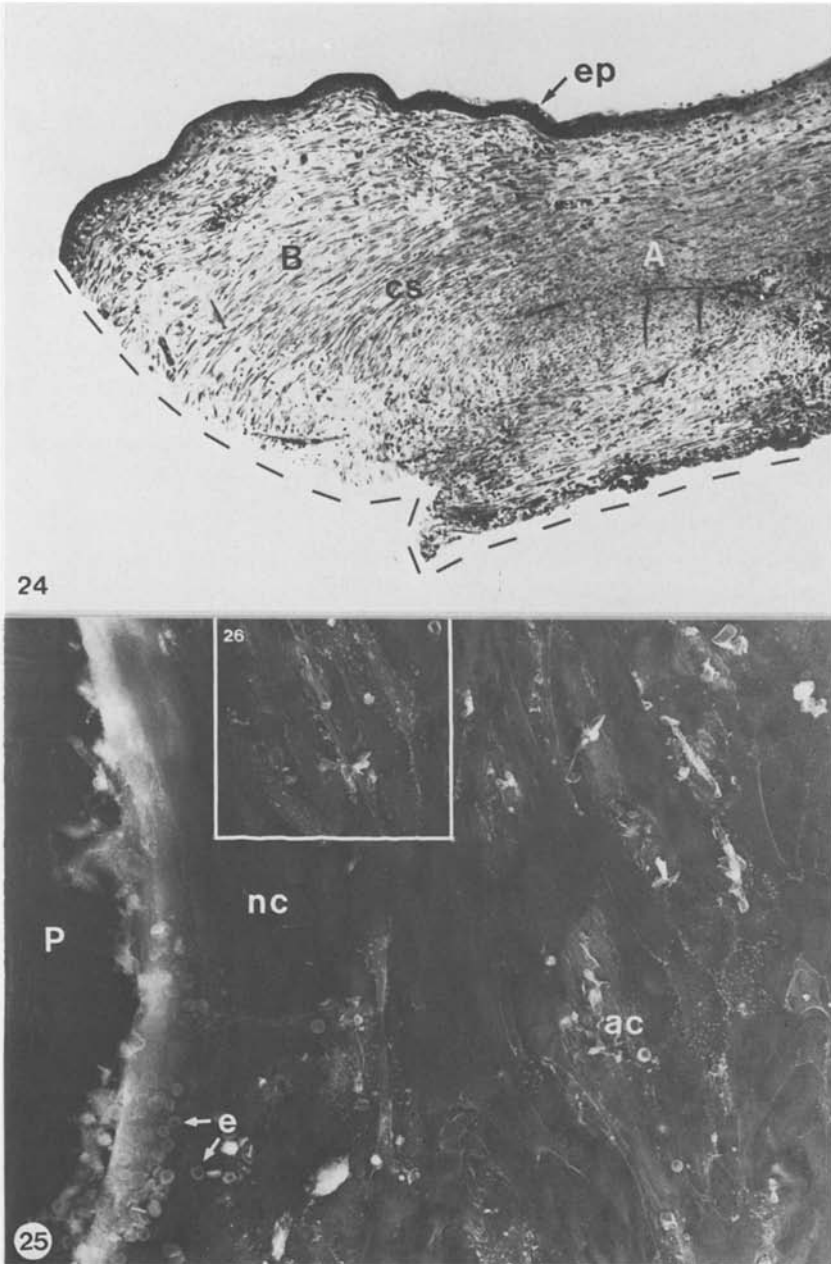
## Discussion

The fixation method of the KP, viz. by careening steel wires around the eyeball must not be considered as preferable for human application, since it cannot be excluded that the wires damage the optic nerve and blood vessels. The sclera of the rabbit eye however, is substantially thinner than in the



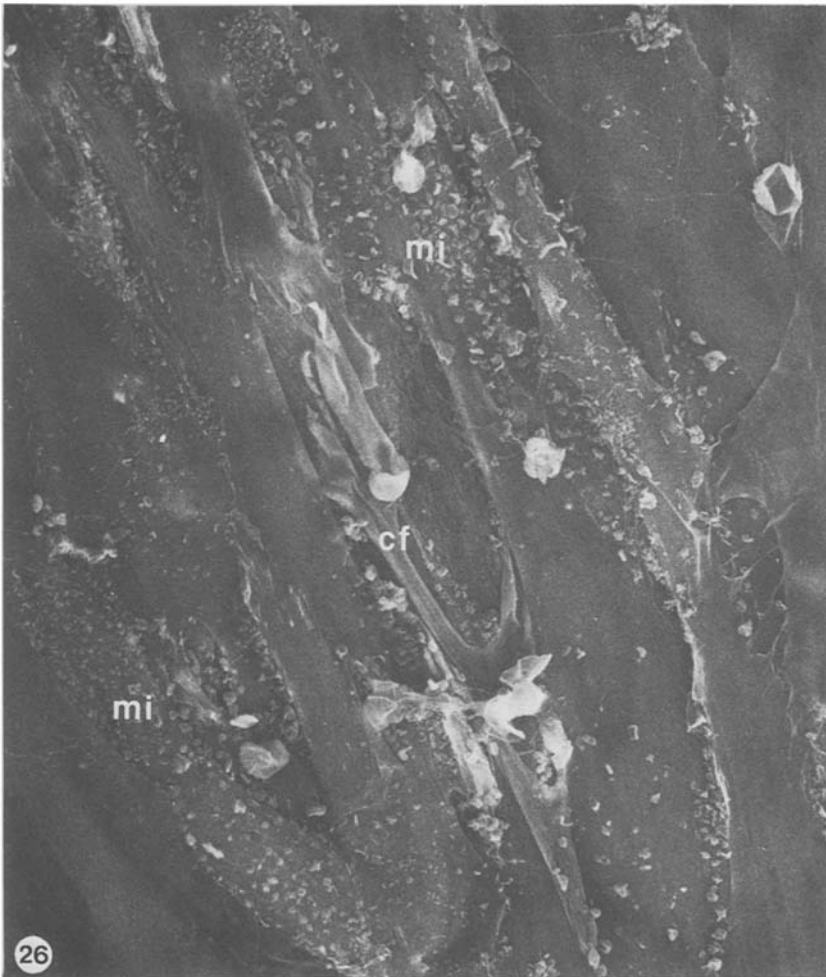
*Fig. 22.* LM image of cross-section of part of cornea after removal of the KP. Asterisk marks the place where the hat of the KP was located originally (compare with Figs. 18 & 19); ep = epithelium; en = endothelium; D = Descemet's membrane; cs = corneal stroma. Magn. 9 X.

*Fig. 23.* Detail of Fig. 22; ↗ ↘ indicate newly formed stroma; en = endothelial layer; D = Descemet's membrane (partly missing due to sectioning). Magn. 28 X.



*Fig. 24.* LM-image of trephination rim of corneal tissue laying on the KP as shown in Fig. 19. Ep = epithelium; cs = corneal stroma with regular (A) and somewhat irregular (B) organization of fibres; --- = border of corneal tissue originally adjacent to KP. Magn. 18.5 X.

*Fig. 25.* SEM image of endothelium bordering platinum cylinder (P) of the KP; nc = normal endothelial cells; ac = anomalous endothelial cells; e = erythrocytes. Magn. 510 X.



*Fig. 26.* Detail of anomalous endothelial cells of Fig. 25; note the numerous microvilli (mi) on top of the cells; cf = collagenous fibres. Magn. 2060 X.

human eye, and therefore cannot be used in a reliable way for fixation. Therefore, it was necessary to use this rather radical method, to obtain enough follow-up to draw conclusions concerning the retention of the prosthesis.

Only in the case without traction threads around the eye, we observed leakage of aqueous humour around the KP. This water-tight wound closure may be explained by assuming that the eye pressure pushes the posterior half of the cornea against the 'hat' of the KP in a valve-like manner. In this context, it was necessary to draw the stainless steel wires tight until the eye



appeared tuffet-like, to maintain tension in the wires after they are buried into the cornea and sclera after some weeks. This important principle of peripheral fixation avoids gaping of the interface between KP and cornea. Filling of the anterior chamber with air was more effective than with saline, in as far that leakage along sutures did hardly occur, and that opening of the chamber angle was easier. The air was resorbed within two days. To prevent possible accumulation of aqueous humour in front of the KP due to initial leakage, the cornea in front of the KP was not completely sutured.

Inconel-600 is an alloy of chromium, nickel and a trace of cobalt. This alloy was intralamellarly tested in the unmoulded, stainless steel form and then tolerated perfectly well. The problems we faced when we used it in a KP, however, may be due to loss of its stainless steel character during heat pressure moulding. Inspection of the KP with SEM revealed tiny spots which may consist of chromium/nickel oxides, molten metal particles or metal salts (Fig. 16).

Dohlman [9] pointed out that corneal ulceration is most likely the result of local release of proteolytic enzymes and collagenase by polymorphonuclear lymphocytes in a reaction to antigens. Metal oxides and salts may be considered as such.

A possibility to avoid these adverse tissue reactions is to deposit (artificial) hydroxyapatite onto the alleged metal surface. Because hydroxyapatite is a natural substance of teeth and bone, tissue reactions could possibly remain absent; implantation of small pieces of artificial HAP in intralamellar corneal pockets confirm this assumption. Alternatively, the cylinder and hat of the KP could be made of stainless steel (V<sub>2</sub>A AISI 316).

White and Gona [6] reported that a Proplast support, porous material prepared from Teflon fluorocarbon polymer and carbon fibres, for fixation of a PMMA cylinder in corneal tissue, was tolerated very well in rabbit cornea and that cellular infiltration into the material occurred. Whether or not Proplast may be combined with glass, is not known and has to be investigated.

In the mean time we therefore have confined our attention to the platinum-glass combination. The fact that platinum is a rather soft metal, and the hat of the KP consequently a little vulnerable, can be overcome by the addition of the metal rhodium (10% of the alloy).

One of the complications often encountered in prosthokeratoplasty is extrusion of the KP or loss of tissue around it [10–13]. We do not feel that tissue adherence to the KP is a 'conditio sine qua non'. Therefore, fixation of the KP should be done by means of wires attached to the stable and healthy sclera to avoid mobility of the KP in the soft and diseased cornea. In follow-up observations this far, we did not meet with extrusion of the KP

or 'melting away' of surrounding corneal tissue, provided traction threads were used.

A rather massive deposit was found at the rear of many *Inconel KP*'s (see Fig. 14). The fact that most of it was lost during the washing and critical point drying procedure for SEM, indicate that it was a more or less granular (powderlike) material, easily detached from the glass surface. The presence of granulocytes and macrophages at the KP bordering corneal endothelium indicates inflammation of the cornea at this place. Almost no healthy endothelium cells are found, but collagenous fibres covered by granulocytes and macrophages can be seen (Fig. 15).

Figures 18, 19 and 22/23 have a lot in common. The hat of the *platinum-glass KP* is placed approximately in the centre of the corneal stroma like a 'fork' (Fig. 18). In Fig. 22, a light microscopical image of the fork is shown (the KP was removed to enable sectioning). How the corneal stroma adheres to the hat and glass-optical part of the KP, seen from the epithelial side, is shown in Fig. 19. The difference in roughness between the trephined corneal epithelium and stroma bordering the glass and the corneal tissue bordering the metal hat is obvious. The latter tissue had a healing time of seven months (Fig. 20).

The effect of the trephination at the edge of the cornea is visible in Fig. 21. Epithelial cells around the rim, appear to be artificially aged, that is cells get detached and take a more or less rounded shape. Similar phenomena have been found at invaginations at the epithelium of keratoconus [14].

The presence of small pits in epithelial cells is quite normal [15], but the number and in particular the size of the pits found in the cells of Fig. 20 probably are a result of a surgical trauma to the epithelium.

Light microscopy did not reveal the presence of unusual cells in the stroma at the brim of the KP, nor the formation of a (water tight) closing layer along this border, at least up till seven months postop. The arrangement of stroma fibres is only slightly disorganized along that border.

At the *endothelial side* (Figs. 25 & 26), the bordering corneal tissue shows cells with numerous microvilli which is indicative for a high state of activity [16]. The corneal endothelium close to the metal cylinder of the KP shows disrapture and repair of cells, collagenous bundles and other products of cell activity (Figs. 25 & 26). At a few hundred microns from the transition area, normal endothelial cells are present.

Stainless steel traction threads as well as sutures did not evoke adverse tissue reactions. They become nicely incorporated into the tissue itself. At the endothelium, sutures are covered almost completely by Descemet's membrane although still somewhat irregular types of endothelial cells are present after approximately five months. After seven months covering is

complete [17, 18]. After 2.5 months a sort of embedding of the sutures has taken place, while the start of Descemet's membrane deposition is on its way. More research is currently being carried out to establish the initial laying down of Descemet's membrane in the process of total incorporation.

From the findings in this series of experiments, we conclude that the glass-platinum KP and the peripheral fixation is well tolerated in the rabbit-cornea and -eye. A clinical trial on cornea-blind is justified, provided that the optical quality is improved and made acceptable for this purpose. At Philips Res. Lab., attention is now paid to this.

Because of the low cost of manufacturing this type of KP may be suitable for so-called Third World countries.

### Acknowledgements

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A detailed description of the KP by Mr. P. van Andel may be seen at the office of "Verenigde Octrooibureau's" (The Hague; dossier no. 8501403, November 1986).

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