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ACTAS DEL TERTIUM FORUM OPHTHALMOLOGICUM

A LOS COLABORADORES

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Arch. Soc. Amer. Oftal. Optom. (1981)-15-143

ACTAS TERTIUM FORUM OPHTHALMOLOGICUM

FACTORS OF DEPENDANCE REGARDING STABILITY OF RESULTS IN KM

JÖRG KRUMEICH
Bochum, Deutchland

The quality of our surgical refractive techniques is value and brought into question by the results of refractive correction as well as visual acuity and the stability of both.

That in mind I would like to propose several hypotheses based upon information taken from 50 myopic cases and 100 hypermetropic KM cases, all of which come from my personal files.

There are two conditions, which the surgeon currently has little control over:

THESE ARE:

1. Tension within the disc
2. Interface instability.

Both of these conditions lead to unpredictable refraction and unpredictable visual acuity. One should attempt to avoid both.

Tension within the lathe-treated disc may occur due to:

a) Difference in arc lengths
b) tight adaptation of borders, or
c) wings.

The disc is to be sutured to the same spot from where it had been removed before the change of the surface parallel curves were effected.

The wings sutured tightly to the corneal step may force the disc to take its former shape, there by prohibiting a tension-fee alignment of the new curves.

This prohibitive procedure creates spaces within the interface, which are readily filled with serous exsudate. Fluid also enters this interface due to capillary powers.

The wings basically meant for a better and tighter adaptation and for closure of the wound may themselves be a reason for tension within the disc and for instability of refraction.

The better the adaptation by the wings, the less the computerized curves can fulfill their purpose.

The surgeon should allow the difference of arc lengths of the new internal radii as compared to the original surface-parallel curves, ie. RI minus ED, to expand.

The drawing originally taken from Dr. Barraquer's manual and altered here for illustration makes this point clear.

Point A is to be aligned with point B, PA is longer than PB by the amount c.

Point P has to move down. This can only be achieved free of tension if there is space for it. The size of this space may range between 0,12 - 0,2 mm, depending on the steepness of the cutting radius.

ER is only 18/100 mm thick. This may seem to be a small quantity, but the mechanical sequellae may play a significant role.

If we assume there to be an elasticity module of 0,4 a tension of about 0,7 grms/mm^2, i.e. 27 grms is constantly exerted over the area of the disc.

If the sutures are loose, one may observe ectasia of the borders of the lenticule. If they are tight, the adaptation zone bulges.

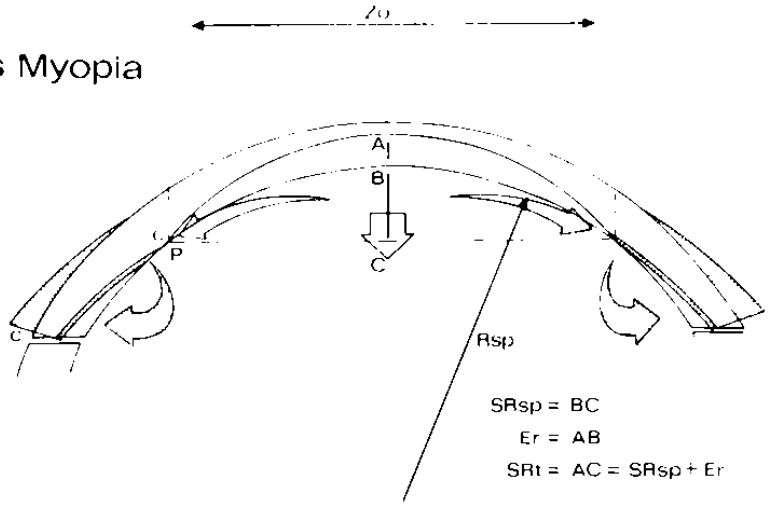
A similar situation occurs in hypermetropia due to the arc length differences which exist between RTA - RT and RB - ED.

In addition to this somewhat more mathematical and mechanical consideration, the above said is apparent if we look at an operated eye in coaxial light.

Shows a myopic case. One can see the circular bulging of the sutured area.

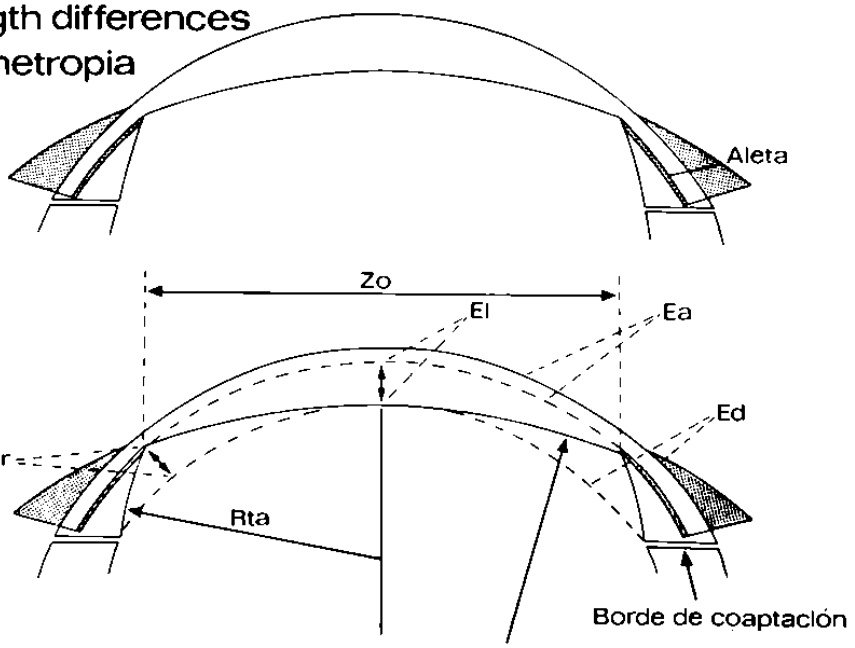
Shows no bulging of the alignment zone. In this case the wings were shortened by c/2.

Arc length differences Myopia



$$\frac{C'}{2} = \frac{\frac{ZO}{Fcc\ (RT+CH)} \cdot \text{arc sin } \frac{1}{2}(RT+CH) \cdot \pi \qquad \left(\frac{ZO}{\frac{1}{2}\ Fcc\ (RB-ED')}\right) \cdot \text{arc sin } \frac{1}{2}(RB-ED') \cdot \pi}{180 \qquad\qquad 2}$$

Arc length differences Hypermetropia



Similarly illustrates the same situation using a slit lamp photograph.

The interface itself may be the origin of instability of refraction and visual acuity.

The surgeon must combat both, irregular cuts and cells or detritus in the interface. Even the most meticulous operative techniques can not prevent cells of corneal or conjunctival epithelium from emerging within the separated corneal layers. As a result these cells grow and multiply exponentially producing metabolic products, which then appear as whitish infiltrations of the parenchyma.

Two possible explanations may account for the appearence of cells and detritus within the parenchyma:

1. The knife of the mikrokeratom forces under a presure of 0,7 kg/cm during the incision these cells of corneal origin into both sides of the parenchyma. This results in the growth of cells and the production of metabolic wastes in the interface.

2. Capillary suction around the entire incision draws detritus as well as cells of possible conjunctival origin into the interface within those areas where negative pressure occurs.

   In myopia they gather in the center.

   In hypermetropia they gather in the sulcus, which is around the optical zone.

The media, which is found within the newly formed spaces and which is ideal for the rapid growth of cells permits these cells to grow almost in a culture. This is true in myopia in regard to the optical zone. The better the surgery, i.e. the better the adaptation of the borders, the greater the amount of exsudate and of cells under the optical zone.

Looking with the broad slit of slit, lamp at the interface containing such cells, we see white patches which, when in the optical zone, impede acuity of vision considerably and cause blurring. If, during surgery, cells are brought into the interface, and if, before closing the wound, there exists the possibility that capillary action draws cells and detritus into the interface, current practice concerning wings and wound adaptation have to change.

The theory that cells are introduced into the interface surgery is supported by the fact that epithelial cells do not show any tendency to grow

into tissues anywhere in the body, unless they are traumatically brought there. They don't activily move but grow by continuity.

If both of these hypotheses are valid, it follows that the cells and detritus have to be removed from the parenchyma of the cut tissue and that the form of the adaptation edges has to be changed.

The surgeon could, however, prevent these problems by reducing the ED by 1/100 mm in myopia and 2/100 mm in hypermetropia.

This he could achieve by using a baseparallel cut.

The adaptation edges would then have to be cut down to 25/100th mm by a RB-parallel cut. The diameter of the disc has to be diminished by the amount of the arc length differences.

This would allow the borders to slide.

The interface would have to be throughly cleaned. The double-running suture would be usefull in all cases, even in myopia, in preventing capillarity. By employing these techniques, predictably satisfactory results could be achieved.

These two slides distinctly make clear the contrast between the slit lamp view of regular myopic case with unchanged borders and that of myopic case with cut down borders.

One can see a smoother curve on the cornea.

The wings obtrued more obviously in hypermetropic KM cases.

The wings in a similar case create a surface which looks quite normal.

In the last 50 of 150 cases I followed the above stated procedures. I am now able to report in respect of these 50 cases stabil refractions and water-clear corneas.

Arch. Soc. Amer. Oftal. Optom. (1981)-15-149

L'OPERATION COMBINEE DE CATARACTE ET DE GLAUCOME CHRONIQUE DE L'ADULTE DANS LES CAS PARTICULIERS

P. BREGEAT

Paris

1º La valuer de l'opération combinée dans les cas de glaucome chronique et de cataracte n'est plus à démontrer. Elle surclasse les interventions en deux temps:

—Soit opération de la cataracte puis opération du glaucome de l'ceil devenue aphaque;

—soit opération du glaucome puis opération de la cataracte par extraction intracapsulaire.

Avec l'opération combinée nu n'y a qu'une séance opératoire, qu'une anesthésie et les resultats son généralement meilleurs qu'avec le traitement chirurgical en deux plusieurs séances opératoires.

2º L'opération combinée la meilleure est la trabeculectomie associée à l'extraction intracapsulaire du cristallin.

(Projection du tableu des statistiques de la Clinique ophtalmologique du CHU Cochin-Paris V). La trabeculectomie est une opération filtrante, mais une "filtrante protégée" (Brégeat). II n'y a pas d'hypotonie post-opératoire, excessive avet les complications qu'ele entraîne comme les maculopathies; le décollement choroidïen

les hémorragies sous rétiniénnes ou vitrénnes sons moins fréquéntes qu'avec les autres opérations fistulisantes en chirurgie combinée. L'indication opératoire peut donc s'étendre aux cas des sujets âges artérioscléreux.

Cependant il faut attendre souvent 3 mois pour juger de l'équilibre tensionnel: Il est posible qu'après cette période, un traitement médical d'appoint soit utile pour conserver l'équilibre tensionnel D'autre part, l'évolution post-opératoire de la cataracte n'est pas exclue après une simple trabeculectomie quoiqu'elle soit moins fréquente qu'après les autres opérations fistulisantes. C'est pourquoi nous étendons l'indication de l'opération combinée aux cas dont le cristallin présente des opacités assez importantes malgré une acuité visuelle encore utile, parce que celle'ci risque de devenir assez rapidement inutilisable après la seule trabeculectomie.

3º Cas particuliers

Ce sont ceux qui nous intéressent actuellement.

a) **Cas facile**

Myopie élevée, cristallin transparent et hypertonie oculaire nécessitant une trabeculectomie. L'opération combinée, avec zonulolyse améliore la réfraction du même coup.

b) **Cas difficiles**

Cataracte à opérer sur un oeil ayant déjà subi une opération filtrante.

—après trabeculectomie, quand la filtration est correcte, avec nappe sous conjonctivale et que la tension oculaire est normalisée, on peut opérer la cataracte **par la voie supérieure** en dissèquant à nouveau lambeau conjonctival et trappe sclérale, en agrandissant l'incision et en complétant l'iridectomie périphérique par la sphinctérotomie avant d'enlever le cristallin. On peut également faire une incision cornéenne prélimbique au devant de la fistule. Mais l'expérience montre que 2 fois sur 3, surtout si la filtration est précaire, la fistule se tarif et l'hypertonie oculaire réapparaît. L'interposition d'une lamelle de gelfilm au devant et en arrière de la trappe sclérale expose à des effets hypotonisants excessifs avec leurs complications

—Aussi, dans tous les cas, après n'importe laquelle des opérations fistulisantes, quel que soit le degré de filtration, que la tension oculaire soit normale, un peu élevée ou équilibrée par un traitement médical, nous préferons pratiquer l'opération combinée par **la voie inféreure** à cause justement des bons résultats que donne généralement l'opération combinée; il ne faut pas ommetre l'iridectomie périphérique à 6h pour éviter les synéchies postopératoires facteur d'oedème cornéen et la sphinctérotomie à 12h pour faciliter l'extraction du cristallin. Les résultats obtenus dan nos premiers cas paraissent très satisfaisants.

Arch. Soc. Amer. Oftal. Optom. (1981)-15-153

METASTATIC TUMOR IMPLANTS IN THE ANTERIOR CHAMBER

GEORGES ASSIS MASRI, M. D.
Cali - Colombia

CASE REPORT:

V. B. years old, white male, came to the office complaining of pain, photophobia, lacrimation, blurred vision, and congested left eye for the last three days. He had lost 20 pounds in two months and noticed low back pain.

Examination revealed visual acuity of 20/25 in the right eye and 20/40 in the left eye with correction. Refraction: + OD: + 1.25; OS = + 0.75. The right eye was normal with incipient nuclear senil cataract. The left eye was tender to palpation and showed marked injection mainly circumcorneally. The pupillary reaction was impaired and the pupil was miotic and slightly distorted to the lateral side where a white, coliflowerlike, mass occupied part of the angle of the anterior chamber, mostly the upper lateral quadrant. This mass was avascular, well circumscribed and adherent to the adjacent iris. The aqueous was turbid, three plus Tyndall, and there were small keratic precipitates on the posterior surfacer of the cornea, composed of lymphocytes. The iris itself was edematous with loss of normal luster. There was absence of posterior or anterior synechiae at gonioscopic examination. Ophthalmoscopic examination was not done at this time. The intraocular preassure was 12 mm Hg. In the right eye and 17 mm Hg, in the left eye. The diagnosis was acute anterior uveitis, secondary to local necrosis of metastatic tumor in the anterior chamber. Few days, after treatment with local 2.5%. Hydrocortisone every 2 hours and Atropine 1% gtts. twice a day, the uveitis subsided and the ophthalmoscopic examina-

tion with indirect opthalmoscope was done. No tumor mass were seen up to the ora serrata. Transillumination was negative except at the side of the tumor mass. The vitreous showed moderate Tyndall of inflammatory cells and the optic disc and the posterior pole wers normal. The visual acuity improved to 20/25 cc, and the intraocular preassure dropped to 12 mm Hg.

GENERAL PHYSICAL EXAMINATION:

The only possitive findings were a nodule in the left lobe of the thyroid, hard, regular, non tender and mobile. There was small, firm nodule, in the left upper flank meassuring 1 x 1.5 cm fixed to the skin. Rectal examination disclosed an enlarged but benign prostate to palpation.

LABORATORY FINDINGS:

The hemogram as well as the peripheral smear were normal. Bone marrow was also unremarkable. Chest films revealed a wide mediastinum with a nodule in the right hilum and a prominent aortic arch. Bronchoscopy and bronchograms were normal (there was no deformity of the main bronchi).

Byopsies were performed of the thyroid and in the abdominal wall nodule. Pathology reported nodular thyroid and in the abdominal wall nodule many abnormal lymphocytes, with hyperchromatic nuclei, without any arrangement and with a sarcomaous stroma. The diagnosis was lympho-sarcoma, lymphocytic-type.

The patient was treated with Nitrogen Mustard, 0.2 mg x kg, divided in two dosis. The white bood count dropped to 3.000 but the drug was well tolerated.

DISCUSSION:

This is the case of a well demostrated lymphocytic lympho-sarcoma with metastasis to the left eye, abdominal wall and nodes. The probable mechanism of this anterior uvea metastasis is throught the long ciliary arteries though typically they appear more frequently in the posterior choroid by way of the short posterior ciliary arteries. The original tumor was probably located in the mediastinum.

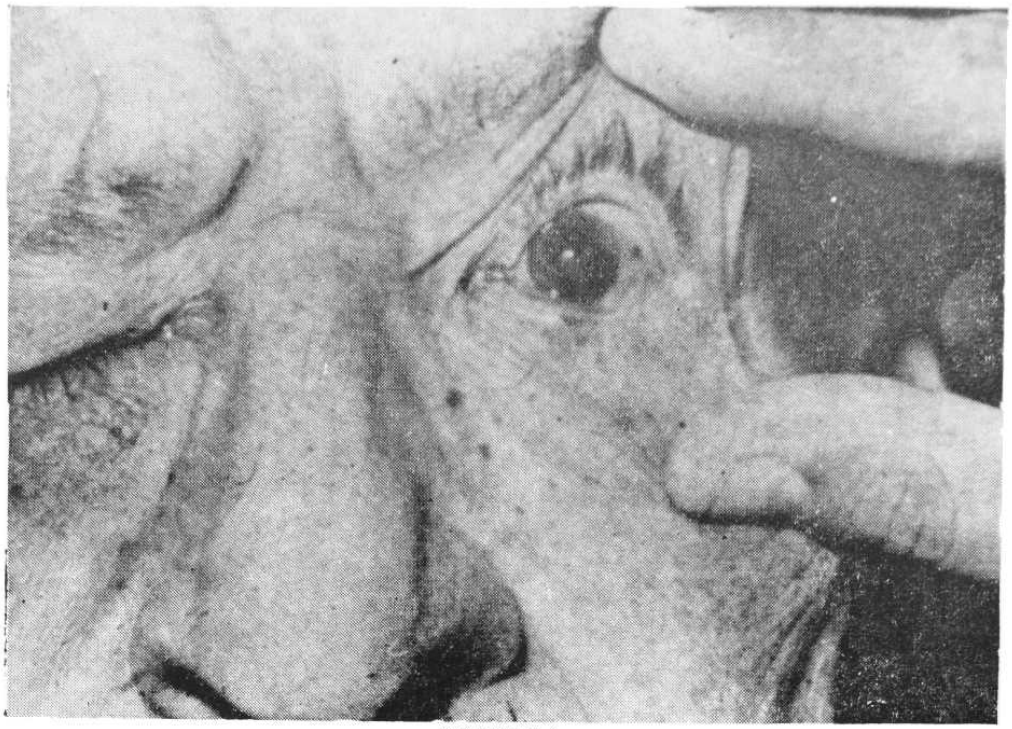


FIGURE 1
Photograph showing the tumor in the upper lateral quadrant.

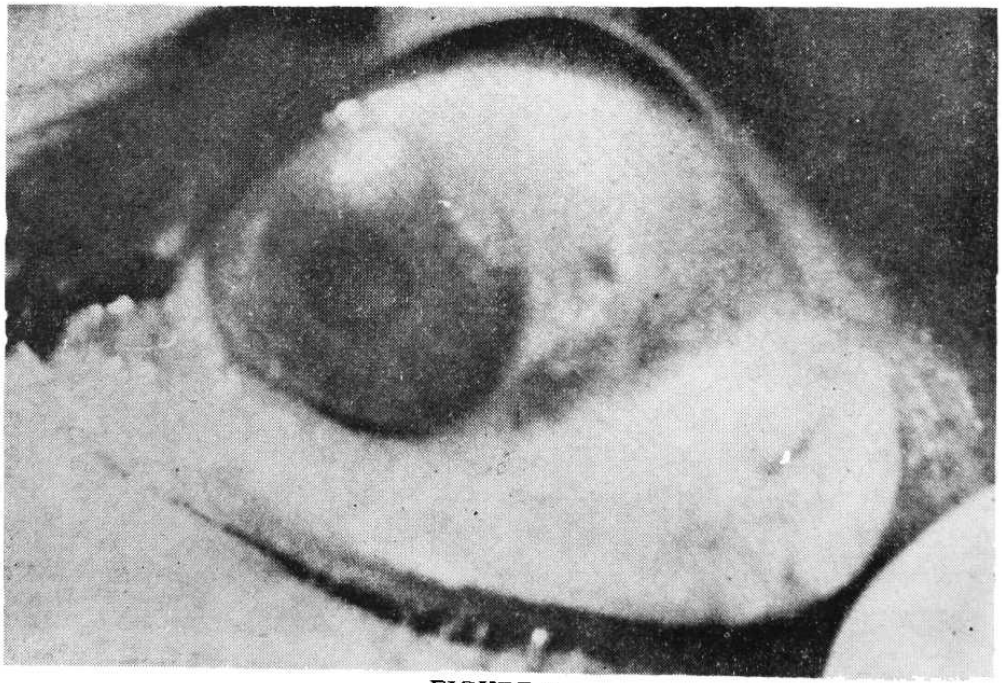


FIGURE 2
Close view of the tumor mass.

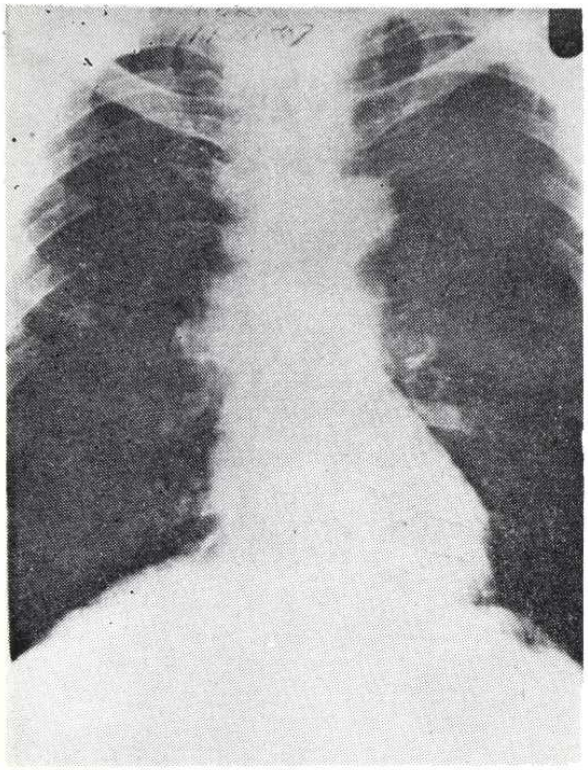


FIGURE 3

X-ray of the chest. Wide mediastinum and prominent right hilum.

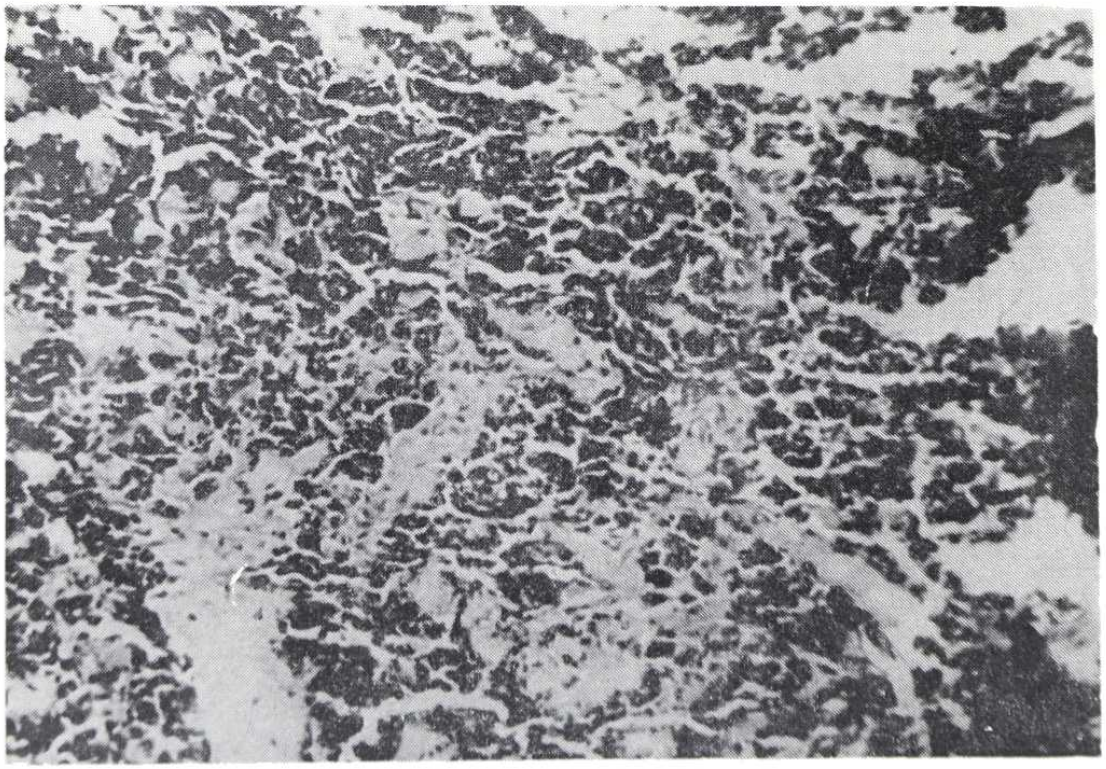


FIGURE 4

Microscopic view of the abdominal nodule. Numerous atypic lymphocytes without any arrangement and sarcomatous stroma.

We considered this an advanced case in view of the spread, age and condition of the patient. The eyes was not enucleated because the pain could be easily controled.

Nitrogen Mustard was the drug of election. In case of respiratory obstruction or hemopthysis, the mediastinum and the hilum of the right lung could be radiated.

SUMMARY:

This case showed that the eye is not infrequ~ntly the first site of malignant spread, and the eye symptoms occasionally precede the diagnosis of the primary tumor.

Arch. Soc. Amer. Oftal. Optom. (1981)-15-159

QUERATOCONO ELECTROCOAGULACION CIRCULAR Y RADIAL DE LA CORNEA PARA LA ADAPTACION DE LENTES DE CONTACTO

Dr. JORGE VASCO-POSADA
Colombia

Cuando un ojo con queratocono no puede ser adaptado con lentes de contacto, o la agudeza visual obtenida con él, no es satisfactoria y el adelgazamiento del estroma corneal llega a límites que contraindiquen su uso, la queratoplastia se hace necesaria.

En este momento una alternativa puede ser utilizada: la electrocoagulación circular y radial de la córnea.

En el queratocono avanzado, la ruptura espontánea central o paracentral de la membrana de Descemet, deja un leucoma cicatricial que aplana el radio de curvatura y engruesa el estroma. Es frecuente observar también, anillos y radios incompletos de cicatrización natural del proceso.

Una manera antigua de tratar el queratocono avanzado, era la cauterización térmica del vértice, lo cual dejaba un leucoma central y pérdida marcada de la visión.

Castroviejo hacia la electrofulguración del centro del cono, para facilitar la aplicación del trépano.

En 1964 presenté al XII Congreso Colombiano de Oftalmología el trabajo titulado "Autotrasplante y Autoplastias Reconstructivas de la Córnea", el cual publiqué en el mismo año [1].

En este trabajo se analizan los resultados obtenidos en queratoconos y queratoglobos avanzados con la electrofulguración en semicírculo y con

puntos separados, de la mitad inferior y periférica de la córnea. Esto con el fin de mejorar la topografía anterior, engrosar el estroma y permitir la adaptación de una lente de contacto.

En los últimos años el calor ha sido aplicado al estroma corneal en algunos queratoconos, para tratar de mejorar el espesor del tejido, es la termoqueratoplastia [2].

También, recientemente se ha utilizado el termocauterio, en el momento de practicar una queratoplastia, con el fin de disminuir el astigmatismo residual, aplicando caustia en cuadrantes astigmáticos opuestos del ojo receptor [3].

TECNICA QUIRURGICA

Bajo anestesia local y utilizando el Wet-Field Coagulator bipolar de Mentor, la pinza 221209 y una intensidad entre 30 y 35 de su escala, se practica una serie de puntos separados en forma de círculos y radios de acuerdo con la topografía corneal que se quiera corregir. Se trabaja en campo húmedo y en cada punto se deja pasar la corriente un segundo.

Primero se electrocoagula el epitelio y una vez retirado éste, se procede a coagular el estroma, con el fin de producir un pequeño leucoma y una zona de tensión y reforzamiento, que se aprecia por los pliegues radiales que se forman en la membrana de Descemet.

En los casos de queratoconos grado II, con proscidencia inferior y estroma central de espesor normal, se practica un semicírculo inferior en 180 grados de la circunferencia, a un milímetro del limbo, tres radios en el cuadrante de las 6 del reloj y dos radios en el de las 9 y en el de las 3. (Fig. 1). Cada punto se coloca a 0.8 mm, el uno del otro y los radios solo tienen dos puntos fuertes y uno muy débil hacia el centro.

En los queratoconos grados III y IV con proscidencia inferior o central, se practica un círculo completo de puntos a 1 mm del limbo y luego tres radios en cada cuadrante como indica la Fig. 2.

Si se presenta una zona astigmática mayor en los cuadrantes de las 8 y de las 2 del reloj, o en los contrarios, se practican dos semicírculos opuestos de puntos separados de 6 a 9 y de 12 a 3 del reloj, y se agregan tres radios en cada cuadrante. Fig. 3.

En algunos casos, o en el postoperatorio tardío de una queratoplastia puede presentarse una zona astigmática localizada en un solo sector de la córnea. En tal caso se practica solamente una línea de puntos circulares en dicho cuadrante y tres radios. Fig. 4.

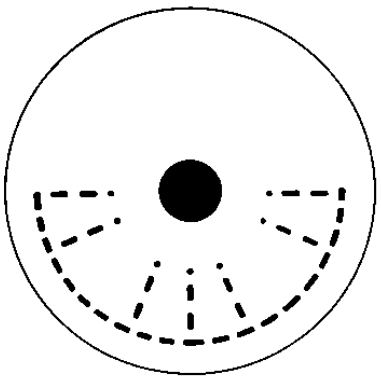


FIG. 1

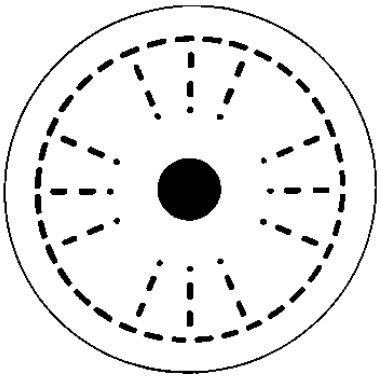


FIG. 2

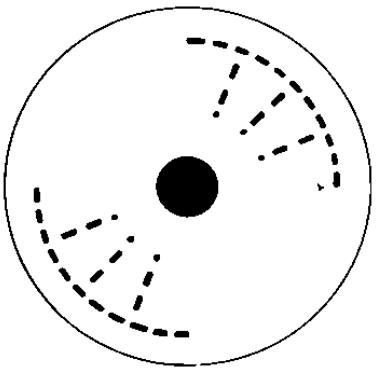


FIG. 3

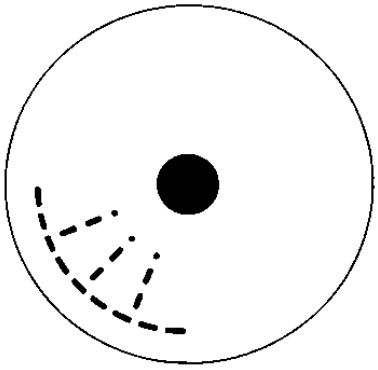


FIG. 4

QUERATOCONO. ELECTROCOAGULACION RADIAL

POSTOPERATORIO AMBULATORIO

Analgésicos orales o intramusculares, hielo local y reposo 24 horas. Al segundo día se inicia localmente un esteroide suave con antibiótico, esto

por ocho días. Al tercer día se retira la oclusión. Si se trata de un hidrops, se agrega la medicación hipotensora ocular.

A los 10 días se puede reiniciar el uso del lente de contacto, empezando con un período de cinco horas.

COMPLICACIONES

Ninguna de importancia.

RESULTADOS

El cuadro Nº 2, muestra los resultados obtenidos en un total de 281 ojos intervenidos con la técnica descrita y en un período de observación de 17 años.

Queratoconos grado II, se intervinieron 25 ojos, los cuales tenían un espesor corneal central cercano a lo normal, pero su topografía hacía imposible la adaptación del lente.

El resultado visual fue muy satisfactorio y la agudeza sin lentes, de un promedio de 20/100 mejoró a 20/25 con lentes de contacto.

QUERATOCONO. ELECTROCOAGULACION RADIAL

	Nº casos	*A. V. Preop. S. L.*	*A. V. Post. C. L.C.*
Grado II	25	20/100	20/25
Grado III	186	20/800	20/30
Grado IV	36	Dedos 1.5 m	20/50
Reoperac. Post. queratop.	16	20/800	20/30
Total	18	20/800	20/30
	281		

El grado III, es la mejor indicación para la técnica y 186 casos electrocoagulados mejoraron su agudeza de 20/800 sin lentes a 20/30 con lentes de contacto.

Los 36 ojos con grado IV, eran casos avanzados, córneas muy adelgazadas y algunos habían sufrido ya un hidrops o estaban en él. La visión preoperatoria de dedos a 1.5 m., mejoró a 20/50 en promedio, con un lente de contacto. Se apreció en ellos una franca mejoría en la disminución del astigmatismo irregular, se aplanó la superficie anterior y se engrosó el espesor del estroma.

En 18 casos que habían sido operados con queratoplastia penetrante, pero que el astigmatismo residual era tan grande que impedía la adaptación del lente, se logró mejorar la agudeza de un promedio de 20/800 sin lentes a 20/30 con lente de contacto.

Se reintervinieron 16 casos, unos porque la cirugía fue insuficiente y otros en los cuales continuó con el tiempo, el proceso del cono.

De los casos operados sólo 2 presentaron un hidrops tardío y 8 casos requirieron una queratoplastia, la cual se practicó sin inconvenientes.

COMENTARIOS

La estructura de la córnea se considera como la de una cúpula arquitectónica y para reforzarla se procede a practicar en su base, un anillo de puntos separados de electrocoagulación. De este anillo periférico salen hacia el centro de la bóveda radios o nervaduras incompletas con el fin de dejar clara la zona de visión central.

La técnica busca equilibrar las fuerzas de tensión, engrosar el estroma, evitar la ruptura del cono y facilitar la adaptación de una lente de contacto que recupere la función visual haciendo innecesaria la queratoplastia.

RESUMEN

Se describe una técnica quirúrgica que utiliza puntos de electrocoagulación circulares y radiales en ojos con queratoconos de diferente grado, que no pueden ser adaptados con lentes de contacto.

En 17 años se operaron 281 ojos. Se analizan los resultados visuales obtenidos.

Se presenta la técnica como una alternativa de la queratoplastia, en casos en los cuales aún el estroma corneal transparente, una vez modificada su topografía, permita la adaptación de una lente de contacto o en los cuales por distintas razones una queratoplastia no pueda realizarse.

SUMMARY

A new technique for keratoconus cases in which the contact lens fitting was impossible is described. Using the bipolar Wet-field coagulator a semicircle or complete circle of electrocoagulation poins was applied in the periphery of the corneal stroma. Also radius of coagulation points are applied in differente quadrants in accordance with the astigmatic error. This procedure has been performed in 281 eyes and the visual results are analized in the table Nº 2.

Emphasis is placed upon the ease with which this surgical procedure can be performed, the complete absence of postoperatory complications and the good visual results obtained.

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REFRACTIVE KERATOPLASTY USING PRE-LATHED PRESERVED CORNEAL MATERIAL

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ABSTRACT

Worldwide interest in refractive keratoplasty is evident from the steadily increasing number of cases being performed and from the rapidly growing number of applicants for instructional courses. Even greater utilization of this surgical technique will occur when pre-lathed and preserved corneal material is made available. Evaluation of methods of preservation in animal and clinical studies has shown no deleterious effects resulting from prolonged storage of the lenticules before surgical insertion. A randomized, prospective study of keratomileusis hyperopia homoplastica and epikeratophakia is currently underway to evaluate the relative efficacy and safety of these two forms of refractive keratoplasty.

Refractive keratoplasty, developed by Dr. Jose Barraquer, is an efficacious and precise extraocular method of correcting moderate to high degrees of myopia and hyperopia. Analysis of Dr. Barraquer's cases [1, 2] as well as of those cases performed by surgeons in the United States [3, 4] confirms that the Barraquer techniques of refractive keratoplasty yield excellent and predictable visual results.

International instructional courses were initiated in Bogota by Dr. Barraquer in july, 1977, and continue to be offered periodically. Because

of the overwhelming number of applicants, additional instructional sites have been designated in the United States.

Many surgeons in the United States performing the procedures are enthusiastic, but general acceptance by the ophthalmic community will very likely be delayed because of the limited number of instructional courses available, the problems associated with the delivery and ongoing modification of cryo equipment and other hardware, and the problems associated with persuading ophthalmologists to master techniques foreign to their surgical training.

At least some of these problems may be eliminated if preserved, precarved and custom lathed donor tissue can be made available. This will allow the anterior segment surgeon to order lenticules much like ordering a contact lens; refractive keratoplasty then can be accomplished by the ophthalmic surgeon using familiar corneal surgical techniques.

In the 1950's, Rycroft and Eascott [5, 6] demonstrated that using lamellar corneal sections stored at -75ºC yielded a high percentage of clear corneal grafts. King [7] showed similar results with corneal tissue preservation in glycerine and silica gel at room temperature. Barraquer [8] demonstrated that freezing destroys the keratocytes in the stroma of stored corneal tissue. These data were confirmed and expanded upon in recent studies by Rich, et al. [9]. Using vital staining and current techniques of cryolathing and preservation, it was shown that, although the donor stromal keratocytes are killed in the lathing process, repopulation of the non-living stromal matrix by keratocytes from the host cornea begins within ten days.

Based on the above experiments, we theorized that preservation and continued storage of the cryolathed lenticule would not affect its ultimate transparency. In animal studies using cats as the experimental model, we compared resulting graft clarity using freshly lathed and preserved lenticules [4]. Lenticules of similar dimensions were cryolathed using the computer program for keratophakia. The lenticules were divided into three groups: 1) lenticules implanted immediately after lathing; 2) lenticules stored in liquid nitrogen for at least one week before surgical implantation; and 3) lenticules stored in glycerine for at least one week before surgical implantation.

Three months after surgery, there was virtually no difference among the three groups in graft clarity, in increased corneal thickness, and in the anticipated increase in dioptric power of the cornea.

Fifteen keratophakia patients are being followed in the clinical trial of cryopreserved and fresh keratophakia lenticules [4]. Six received freshly carved lenticules and nine received liquid nitrogen preserved (cryopreserved) lenticules. A total of seven patients had keratophakia and intracapsular cataract extraction combined; eight patients had keratophakia alone (Table).

Selection was based on 20/40 or better vision in the fellow eye, contact lens failure or refusal to wear a contact lens, and desire for refractive keratoplasty instead of intraocular lens implantation. With one exception, all patients showed excellent corneal clarity postoperatively. There were no infections, no graft rejections, and no eyes lost. The one exception, patient Nº 2, had persistent edema of the cornea secondary to inadvertent entrance of the anterior chamber with the microkeratome.

Within six months, all patients had functional visual acuity, with three exceptions. Of the patients having keratophakia as a secondary procedure, one (Nº 2 as above) had accidental perforation of the anterior chamber (surgery related), one had cystoid macular edema (not thought to be related to the refractive keratoplasty procedure), and a third patient had a retinal detachment four months postoperatively (not thought to be related to the refractive keratoplasty procedure). The remaining 12 patients had visual acuities equal to or within one line of their preoperative best corrected visual acuities.

The excellent corneal clarity and visual acuity obtained in this study led to the conclusion that pre-lathed, liquid nitrogen preserved corneal tissue could be successfully used in refractive keratoplasty.

In the treatment of hyperopia, hypermetropic keratomileusis has to some extent replaced keratophakia. It appears that the recovery of final visual acuity is achieved more rapidly with hypermetropic keratomileusis, possibly because the healing process involves only one interface, in contrast to the two interfaces that must heal after the keratophakia procedure [10]. Dr. Barraquer's technique of keratomileusis involves the lathing of a lamellar section of the patient's own cornea. In the event of an accident rendering the patient's own corneal tissue unusable, a separate computer program for keratomileusis hyperopia homoplastica (KMHH) provides for lathing donor tissue as a substitute. We propose that KMHH be employed as a primary procedure, using the pre-lathed and preserved corneal tissue techniques that we developed in the keratophakia study [4].

In an attempt to further simplify the techniques of refractive keratoplasty in the correction of aphakia, Dr. Theodore P. Werblin suggested suturing a pre-lathed, donor corneal cap over the patient's de-epithelialized, but otherwise intact, cornea [11]. Dr Werblin, Dr. Herbert E. Kaufman and Dr. Miles H. Friedlander have development this idea into a practical surgical procedure called epikeratophakia and clinical trials are currently underway.

To evaluate the respective merits of keratomileusis hyperopia homoplastica and epikeratophakia, and to compare liquid nitrogen and glycerine preserved, pre-lathed corneal tissue, a long term, prospective study has been initiated at the LSU Eye Center in New Orleáns, Louisiana by drs. Kaufman, Werblin and Friedlander. This double blind, randomized study will follow at least 40 patients for two to five years. Selection criteria for this study will be aphakia in the operated eye and functional visual acuity in the fellow eye.

Patients will be randomized into four groups. Group I will have keratomileusis hyperopia homoplastica, using cryopreserved donor corneal tissue lathed according to the calculations of Dr. Barraquer. Group II will be similar to Group I, but will receive grafts stored in glycerine at room temperature. Groups III and IV will undergo epikeratophakia, using the programs and calculations of Dr. Werblin. Group II will receive grafts that have been stored in liquid nitrogen (cryopreserved); Group IV will reveive grafts stored in glycerine at room temperature.

Preoperative evaluation will include a complete ophthalmic examination, including the following specialized tests: ultrasound, keratometry, endothelial cell counts, Shirmer (severe dry eyes excluded), corneal sensitivity, pachometry, visual fields, and corneal photographs.

Techniques of suturing, suture material, and postoperative medications will be standardized. Periodic postoperative examinations with special emphasis on visual acuity, time of recovery of visual acuity, pachometry, and keratometry with mire quality will be conducted. Evaluation of visual results will be performed by an examiner other than a member of the surgical team who will have no knowledge of patient selection or surgical technique. In this way, we hope to eventually accumulate sufficient data to successfully evaluate the relative efficacy and safety of these two techniques.

TABLE: Comparison of Liquid Nitrogen Preserved and Fresh Lenticules in Patients (adapted from Friedlander, et. al.[4]).

	Followup to Date	Graft Clariti	Diopters to Correct [1]	Diopters Uncorrected [2]	Preop Visual Acuity	Postop Visual Acuity
LENTICULES USED INMEDIATELY AFTER LATHING						
1—Keratophakia	2 yr	0.5	+ 18.25	— 2.75	20/30	20/30 [5]
2—Keratophakia	2 yr	4 [3]	+ 18.25	+ 2.00	20/50	20/200 [5]
3—Keratcphaquia	2 yr	0.5	+ 13.25	+ 2.75	20/50	20/50
4—Intracapsular cataract extraction Keratophakia	21 mo	0	+ 17.00	+ 3.75	20/200	20/40
5—Intracapsular cataract extraction Keratophakia	18 mo	0	+ 18.00	+ 1.25	20/400	20/25
6—Intracapsular cataract extraction Keratophakia	18 mo	0	+ 14.00	+ 1.50	Count Fingers	20/25
LENTICULES STORED IN LIQUID NITROGEN						
7—Intracapsular cataract extraction Keratophakia	1 yr	0	+ 12.50	— 3 00	Hand Motions	20/30
8—Keratophakia	4 mo [4]	0	+ 13.00	+ 3.00	20/25	20/50 [4]
9—Keratophakia	9 mo	0	+ 12.00	+ 1.00	20/40	20/50
10—Intracapsular cataract extraction Keratophakia	9 mo	0	+ 15.00	+ 2.50	20/100	20/40
11—Keratophakia	6 mo	0	+ 11.00	+ 2.50	20/30	20/40 [6]
12—Intracapsular cataract extraction Keratophakia	6 mo	0	+ 16.00	+ 2.00	Hand Motions	20/40 [6]
13—Keratophakia	9 mo	0	+ 12.00	+ 0.50	20/40	20/50
14—Keratophakia	6 mo	0	+ 11.75	+ 1.75	20/25	20/30
15—Intracapsular cataract extraction Keratophakia	6 mo	0	+ 12.00	+ 1 50	Count Fingers	20/40 [6]

[1] Calculated dioptric correction for emmetropia; [2] Residual correction - spherical equivalent; [3] Anterior chamber inadvertently entered, residual corneal edema; [4] Retinal detachment ocurred 4 mos. postoperatively; [5] Developed cystoid macula edema documented by fluorescein angiography 4 months postoperatively; [6] Corrected to 20/25 or 20/30 with bandage plano T lens and over-refraction.

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TOXOPLASMOSIS OCULAR

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Estudio de su incidencia en pacientes de la Clínica Barraquer y del Instituto de Cirugía Ocular.

I. *INTRODUCCION*

La toxoplasmosis es una de las infecciones parasitarias más difundidas en el hombre y en animales de sangre caliente en general. La infección primaria en el hombre es generalmente subclínica, aunque puede dar lugar a diferentes cuadros que van desde una simple "gripa", hasta una encefalitis, muchas veces letal.

Janku, en 1923 (citado en Schlaegel, 1978), encontró el parásito en cortes de ojo de un niño con toxoplasmosis congénita.

Posteriores hallazgos histológicos del protozoo en el ojo, resaltan la importancia del *toxoplasma* como causa de lesión ocular en el humano.

El parásito llega al ojo por vía sanguínea, líquido cerebro-espinal o por la envoltura perineural del nervio óptico, originando una retinitis primaria que suele causar una coroiditis secundaria.

Aunque en Colombia se han publicado varios estudios epidemiológicos y clínicos sobre esta parasitosis, existen pocos trabajos, desde el punto de vista oftalmológico, donde se correlacionen los niveles serológicos de anticuerpos y las lesiones causadas por este organismo.

II. *MATERIAL Y METODOS*

Se revisaron 798 historias clínicas de pacientes de la Clínica Barraquer y del Instituto de Cirugía Ocular, de la ciudad de Bogotá. Estas historias se escogieron por tener incluido el resultado de alguna prueba de laboratorio realizada para detectar anticuerpos anti-*toxoplasma*. 18 historias correspondían a fechas anterior a 1976, cuando todavía no habíamos estandarizado la técnica de la inmuno fluorescencia indirecta (I.F.I.), por eso la determinación de anticuerpos se hizo por otros métodos (Sabin y Feldman y hemaglutinación indirecta). En las 780 historias restantes, la determinación de anticuerpos se efectuó por I.F.I.

La recolección de muestras se realizó a partir de sangre venosa, sin anticoagulante, de la cual se separó el suero mediante centrifugación. Antes de practicar la prueba, los sueros se mantuvieron en refrigeración a 4-6ºC. La reacción de inmuno fluorescencia indirecta, se realizó de acuerdo a la técnica de Camargo (1964), con una dilución de conjugado (nti-IgG marcada con fluoresceína de la casa Wellcome Reagents Limited) de 1/20 en una dilución de azul de Evans 1/10.000 en PBS.

III. *RESULTADOS*

En las 798 historias clínicas revisadas que mostraban un resultado de laboratorio relacionado con infección toxoplasmósica, se encontró una positividad del 82.9% o sea que, en 662 pacientes, se encontró título de anticuerpos anti-*toxoplasma*.

A. *EDAD*

En los pacientes estudiados, la edad varió desde meses hasta los 79 años. La edad tomada en cuenta se refiere al momento en la cual el paciente consulta y no la edad del episodio inicial. En la tabla I, se muestra la positividad de acuerdo a la edad, encontrando una mayor incidencia (18.4%) en los individuos cuyas edades fluctuaban entre los 10-19 años.

Teniendo en cuenta la distribución por edades, solamente en pacientes que muestran anticuerpos anti-*toxoplasma*, se encuentra un pico de incidencia (gráfica I), en pacientes con edades entre los 10-19 años, positividad que va disminuyendo hasta encontrar un mínimo en los 70-79 años.

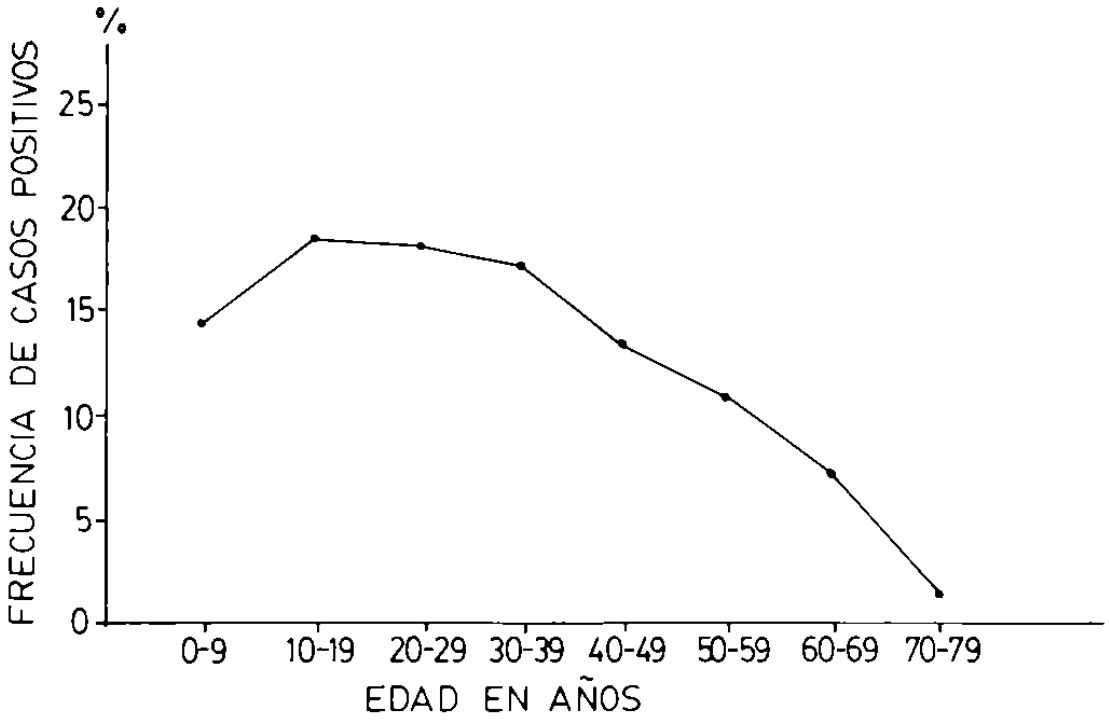
T A B L A I

RESULTADOS I.F.I. PARA TOXOPLASMOSIS

DEPENDIENDO DE LA EDAD

Edad en años		*0-9*	*10-19*	*20-29*	*30-39*	*40-49*	*50-59*	*60-69*	*70-79*	*Total*
Casos positivos	Nº de casos	97	122	120	114	56	70	45	8	662
I.F.I.	Frecuencia	14.6	18.4	18.1	17.2	13	10.6	6.8	1.2	100%
Casos negativos	Nº de casos	33	28	26	22	16	5	4	2	136
I.F.I.	Frecuencia	24.3	20.6	19.1	16.2	11.8	3.7	2.8	1.5	100%
TOTAL DE CASOS		130	150	146	136	102	75	49	10	798

GRAFICA 1. GRAFICA LINEAL DE LA FRECUENCIA DE CASOS POSITIVOS DEPENDIENDO DE LA EDAD.



En todos los grupos de edades, el título promedio fue 1/256.

B. *SEXO*

Se encontró una positividad del 56.9% en 453 pacientes del sexo femenino y del 43.1% en 345 pacientes del sexo masculino.

C. *CASOS DE POSIBLE ETIOLOGIA TOXOPLASMOSICA*

Estos casos se escogieron por llenar los siguientes requisitos:

1. Resultados de laboratorio que muestren pruebas positivas exclusivamente para *toxoplasma gondii.*
2. Retinocoroiditis como manifestación más frecuente de la toxoplasmosis ocular.

D. *RESULTADO DE LABORATORIO*

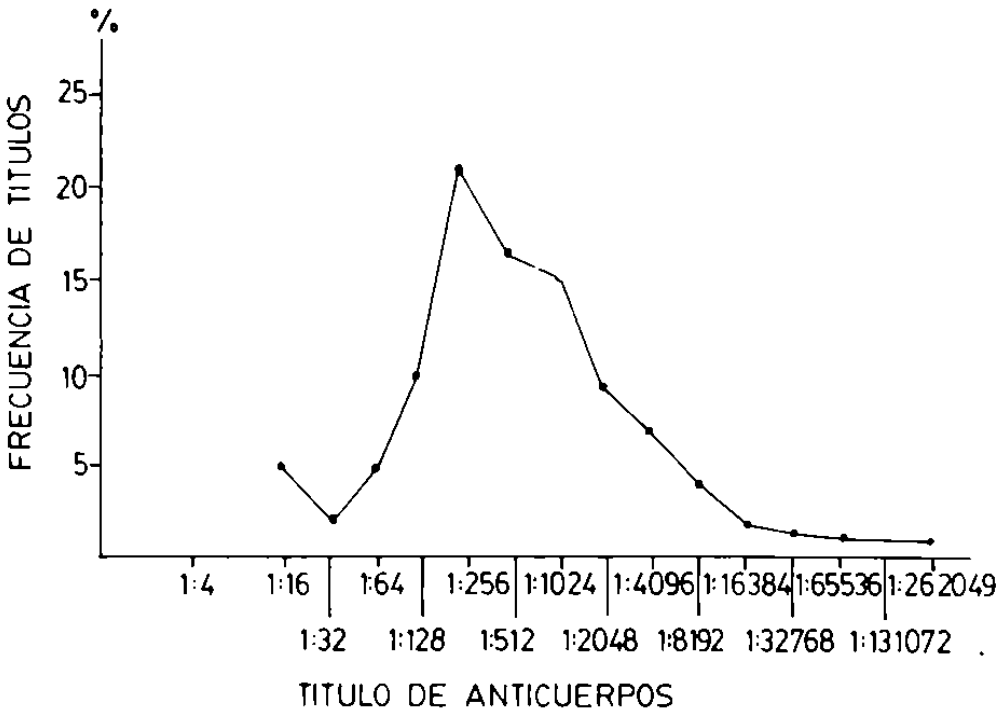
Se separaron aquellos casos que, por laboratorio, impliquen la posibilidad de otra etiología. Para esto se formaron dos grupos:

Grupo A:

Pacientes cuyos resultados de laboratorio sólo muestran título de anticuerpos anti-*toxoplasma*.

En estos, al igual que en el total de paciente, el título promedio fue 1/256 (véase gráfica 2).

GRAFICA 2. GRAFICA LINEAL DE TITULOS DE ANTICUERPOS EN PACIENTES POSITIVOS EXCLUSIVAMENTE PARA TOXOPLASMA (Grupo A)



Grupo B:

. Aquellos pacientes que presentan, además, prueba positiva para otras etiologías. Estos pacientes constituyen el 26.6% de los casos positivos. Este grupo fue descartado del estudio.

E. *RETINOCOROIDITIS*

Del 73.4% de los pacientes en los que se encontró prueba positiva exclusivamente para *toxoplasma* (grupo A), se determinaron las manifestaciones clínicas. Se encontró que el 59.5% mostraron una retinocoroiditis, en tanto que, el 19%, mostraron una uveitis anterior. Esto llama la atención si se compara con los resultados del grupo B, en los cuales la incidencia de la retinocoroiditis fue del 29.5% y la de la uveitis anterior del 39.2%.

De los 289 casos de retinocoroiditis del grupo A, se encontró:

Lesiones activas en el 33.6%.

Lesiones cicatriciales en el 53.4%.

Lesiones tanto activas como cicatriciales en el 11.4%.

No determinado en el 1.7%.

En la tabla II se relacionó el título de anticuerpos en pacientes con lesiones activas, cicatriciales, y activas y cicatriciales. Se encontró un máximo en las activas de 1/512 y 1/1024 (22.7%); en las cicatriciales en 1/256 (21.4%), y, en las activas y cicatriciales 1/1024 (27.2%).

F. *COMPLICACION BILATERAL*

El 69.2% de los pacientes mostraron unilateralidad de la lesión, en tanto que, el 30.8%, mostraron lesiones en ambos ojos.

Localización de las lesiones en el ojo. (Véase tabla III).

IV. *DISCUSION*

Dado que el aislamiento y la identificación del parásito en las lesiones retinocoroidales es muy difícil, el diagnóstico de la toxoplasmosis ocular se ha basado en los resultados obtenidos en encuestas serológicas, encon-

TABLA II

DISTRIBUCION DE CASOS DE RETINOCOROIDITIS EN PACIENTES DEL GRUPO A SEGUN ESTADO CLINICO DE LA LESION Y TITULO DE ANTICUERPOS TOXOPLASMOSICOS CORRESPONDIENTES.

Retinocoroiditis	TITULO DE ANTICUERPOS																TOTAL	
	<1:16	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8192	1:16364	1:32766	1:65536	1:131072	1:262144	N°	%
Activa	1	1	1	2	5	9	22	22	13	5	7	6	2	1			97	33.6
Cicatricial	7	7	1	7	12	33	22	21	12	12	11	4	2	2		1	154	53.3
Activa y Cicatricial			1	1	4	8	3	9	5	1	1						33	11.4
No determinado								1	2	1	1						5	1.7
Total	8	8	2	10	21	50	47	53	32	19	20	10	4	3		1	269	100.0

T A B L A III

SITIO DE LAS LESIONES

	Nº casos	*%*
Macular	66	38.3
Periférica	50	29.1
Paramacular	12	6.9
Peripapilar	12	6.9
Ecuatorial	10	5.8
No determinado	10	5.8
Haz papilo-macular	3	1.7
Macular y peripapilar	2	1.2
Macular y paramacular	2	1.2
Macular y periférica	1	0.6
Macular y papilomacular	1	0.6
Paramacular y peripapilar	1	0.6
Paramacular y periférica	1	0.6
Macular y ecuatorial	1	0.6
TOTAL	172	100.0

trándose una mayor incidencia de pruebas positivas en pacientes con retinocoroiditis o uveitis.

Frenkel (1949), encuentra en San Francisco una positividad del 82% por la técnica de Sabin y Feldman, en pacientes con retinocoroiditis, en tanto que, en individuos sanos, la positividad fue del 30% con la misma técnica.

Perkins (1961), en Londres, encuentra una positividad del 87% en pacientes con uveitis posterior y del 30% en controles.

Roch y Varela en México (citado en Roch, 1971), encuentran en pacientes con retinocoroiditis, una positividad del 80.7% por la prueba de Sabin y Feldman, en tanto que, en individuos sanos, la positividad fue del 47%.

En el presente estudio, encontramos que de 798 pacientes de la Clínica Barraquer y del Instituto de Cirugía Ocular en Bogotá, con problema ocular, especialmente retinocoroiditis, el 82.9% fueron positivos para *T. gondii*.

utilizando la prueba I.F.I. Esto resulta muy significativo si se compara con los resultados obtenidos por diferentes investigadores (Rodríguez Gómez, 1956; Restrepo *et. al.*, 1964; Jewell *et. al.*, 1973; Grogl, 1976 y otros), en encuestas serológicas realizadas en nuestro país que arrojan incidencias cercanas al 55%. Los resultados anteriores sugieren una alta incidencia de toxoplasmosis ocular en nuestro medio.

Se ha demostrado que la incidencia de la infección aumenta con la edad (Frenkel, 1971), y si como se ha sugerido, la retinocoroiditis toxoplasmósica resulta de una infección adquirida después del nacimiento, el número de casos puede aumentar con la edad. Encontramos que esto no es así, puesto que la incidencia es alta entre los 0.9 años, luego se eleva entre los 10-19 años y se mantiene elevada entre los 30-49 años, a partir de los cuales la incidencia empieza a disminuir. En los pacientes del grupo A, el título promedio fue de 1/256 (gráfica 1). En este mismo grupo se intentó relacionar título con forma activa o cicatricial, determinándose en la activa un título promedio de 1/768 (1/512-1/1024), en tanto que, en la cicatricial, fue de 1/256, lo que muestra que no existe una diferencia significativa de títulos entre las formas de toxoplasmosis ocular. Lo anterior indica que es más importante el hallazgo de una prueba positiva, que el mismo nivel de anticuerpos. Esto ha sido encontrado y aceptado por varios autores (Hogan, *et. al.*, 1964; Frenkel, 1971; Schlaegel, 1978).

Es necesario tener en cuenta que el *toxoplasma* puede coexistir con otros microorganismos y que la presencia de una prueba de laboratorio positiva no excluye la posibilidad de que el daño ocular sea debido por este parásito. Los resultados sugieren que es más común el compromiso unilateral (69.2%), así como la localización macular de las lesiones (38.3%).

RESUMEN

Se presenta una revisión de 798 historias clínicas de pacientes de la Clínica Barraquer y del Instituto de Cirugía Ocular, a los cuales se les ha practicado examen para toxoplasmosis.

El 82.9% de los pacientes mostraron prueba positiva para *T. gondii*. De este total se excluyeron los casos en que además de toxoplasmosis, había evidencia de infección por otros microorganismos.

En los casos restantes, se encontró que el 73.4% tenían retinocoroiditis, de los cuales, el 33.6% eran lesiones activas; el 53.4% eran lesiones cicatriciales, y el 11.4% tenían lesiones, tanto activas como cicatriciales.

En todo el grupo estudiado, el título promedio fue de 1/256, por medio de la técnica de inmuno fluorescencia indirecta (I.F.I.). En los pacientes con retinocoroiditis activa, el título promedio fue de 1/768 (1/512-1/1024). En las cicatriciales 1/256, y, en las activas y cicatriciales, 1/1024. El 69.2% de los pacientes mostraron unilateralidad y el 38.3% lesiones maculares.

SUMMARY

798 clinical records from Clínica Barraquer and Instituto de Cirugía Ocular patients were reviewed. These patients had a laboratory test for toxoplasmosis. 82.9% were positive.

The cases which had evidence of infection by other microorganims, were excluded. 73.4% of the remaining cases had retinochoroiditis. Of these, 33.6% had active lesions, 53.4% had scarred lesions and 11.4% had active and scarred lesions.

The average antibody titer was 1/256, determined by Indirect Inmunofluorescence technique. In the patients with active retinochoroiditis, the average titer was 1/768 (1/512-1/1024). In those with scarred lesions, the titer was 1/256 and 1/1024 in the cases with combined lesions. 69.2% of the patients, had unilateral involvement, and the most common site for the retinal lesions was the macula (38.3%).

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THE FATE OF CORNEAL STROMA CELLS IN KERATOPHAKIA

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SUMMARY

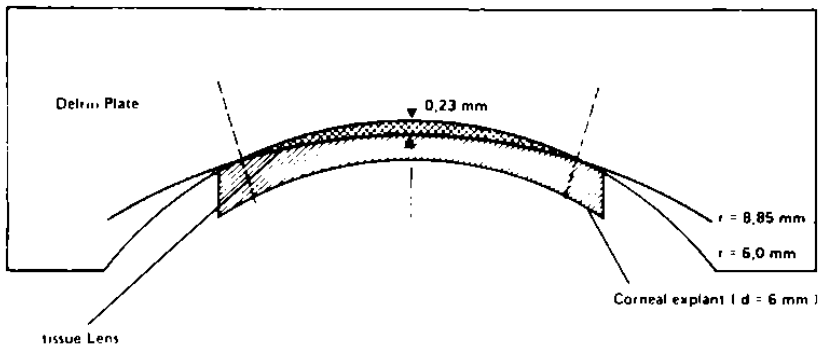
On 29 rabbits, we investigated with the aid of radiosulphate incorporation, histology and electron-microscopy the effect of the freezing process on corneal stroma cells during keratophakia with and without cryoprotection.

Histologic and electron-microscopic studies evidenced that the majority of keratocytes were destroyed 12 hours after keratophakia, and cleared by macrophages 24 hours after keratophakia. The findings pertaining to radiosulphate incorporation revealed that without cryoprotection 10% of the keratocytes survived, while after application of the cryoprotective agent KM 26 the keratocytic survival rate was 20%.

In 1973, Hernández tested various cryoprotective agents for keratophakia in the rabbit and found that the survival rate of keratocytes was most favorable when the solution KM 26 was used. It was now the purpose of our investigations to measure the effectiveness of the cryoprotective agent KM 26 with the aid of radiosulphate incorporation. The following experiments were carried out:

With the keratophakia device designed by Barraquer (1972), tissue lenses were obtained from corneal explants with a diameter of 6 mm., which had previously been freed from epithelium and endothelium. The thickness of the tissue lenses was 0.23 mm., the outer bend radius 6 mm., the

inner bend radius 8.85 mm. (Fig. 1); the refraction power of such tissue lenses used for interlamellar implantation into the cornea is approximately +12.0 diopters.



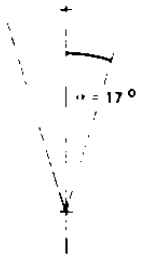


FIGURE 1

Technicall data on the tissue lens.

A total of 29 rabbits were used (Fig. 2); in 5 rabbits, the dry weight of 5 corneal explants and 5 tissue lenses was determined and the mean value calculated in each case. In 5 other rabbits, the dry weight of 5 explants and 5 tissue lenses was determined after they had been placed for one minute into the cryoprotective agent KM 26. The mean values of these two test series were almost identical.

In 3 further test series, the small tissue pieces were incubated while being shaken in calf serum for 2 hours at 37° with 0.5 mci/ml $Na_2{}^{35}SO_4$. Finally the S^{35} was washed in a saturated sodium sulphate solution, and the tissue pieces were dissolved in soluene. The radioactivity was determined using the tri-carb liquid-scintillation spectrometer of the Packard Co.

3 test series were done to determine the counting rate of the explants and tissue lenses without a cryoprotective agent, with KM 26 and finally

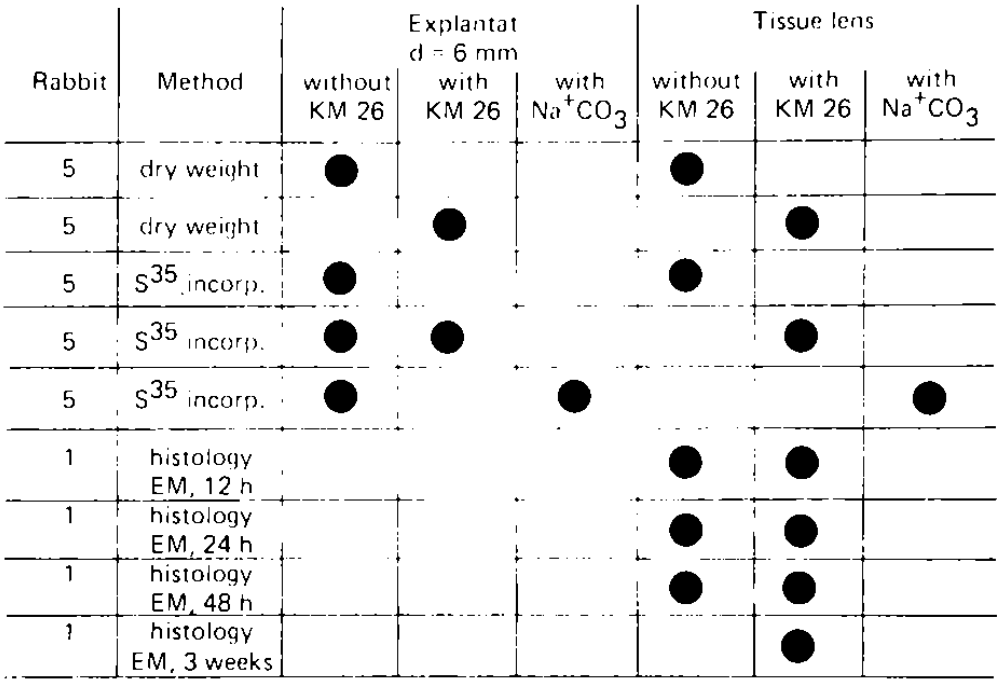


FIGURE 2

Experimental set up.

with a cryoprotective solution in which the kiton green had been replaced by a sodium bicarbonate buffer, while the pH value was maintained constant at 7.4.

In 4 rabbits, a histological and electron-microscopic examination was performed; 3 rabbits were bilaterally operated, the tissue lens having been produced with KM 26 in the right eye and without it in the left eye. The tissue lenses were examined once after 12 hours, once after 24 hours and once after 48 hours. In the 4th rabbit, the cornea of the left eye was used to produce the tissue lens, which was then implanted in the right eye. The histological investigation was done 3 weeks later. This procedure was chosen in order to avoid an immune reaction.

In the first test series, in which no cryoprotective agent had been used, the counting rate/min per mg dry weight was 10 times larger in the corneal explant than in the tissue lens (Fig. 3). The difference in the values was significant.

	Counts x min^{-1} x mg DW^{-1}* $\bar{x} \pm s$	relative radioactivity
corneal explant (d = 6 mm)	2086 ± 1022	100 %
tissue lens Thickness = 0.23 mm	209 ± 36	10 %

$p < 0.05$

* relating to the mean dry weight of 5 tissue samples

FIGURE 3

Influence of the freezing process of keratophakia on the corneal stroma cells without a cryoprotective agent (n = 5).

In the second test series, the counting rate of the corneal explant with the cryoprotective agent KM 26 was only 51% compared to that without cryoprotective agent. The difference was not significant. The counting rate of the tissue lens with the cryoprotective agent KM 26 amounted to only 20% compared to the counting rate of the corneal explants with cryoprotective agent. The difference was statistically significant (Fig. 4).

In the third test series, kiton green was replaced by sodium bicarbonate in the cryoprotective agent KM 26. The results of the measurements are almost identical with the results found with the cryoprotective agent KM 26. The dye kiton green thus appears to have no damaging effect on the keratocytes (Fig. 5).

While the tissue lens produced without cryoprotective agents evidenced only 10% radioactivity compared to normal corneal tissue, the relative radioactivity increased to 20% when the cryoprotective agent KM 26 was used. The capability of the cells to form basic substance is measured by sulphate incorporation. The reduced radioactivity of the cells after the freezing process, however, does not indicate whether damage to the cells is reversible or irreversible. It is possible that the cells still have some capability left to form basic substance shortly after the freezing process but later die. It is also conceivable that the activity of the keratocytes is only temporarily reduced after the freezing process and can regenerate again after a recovery period. For this reason, additional morphological investigations were performed.

THE HISTOLOGICAL INVESTIGATIONS EVIDENCED THE FOLLOWING:

12 hours after keratophakia the cells have lost their oblong shape and have taken on a ball-like shape. The corneal lamellae no longer show the characteristic parallel course. There are no differences recognizable between the tissue lenses produced with and without cryoprotective agents (Figs. 6, 7).

24 hours after keratophakia hardly any cells can be found in the tissue lens. The corneal lamellae do not run parallel to each other. The tissue lenses produced with and without a cryoprotective agent do not differ from each other (Figs. 8, 9).

48 hours after keratophakia the same clinical picture is seen as after 24 hours after keratophakia. Only isolated cells are visible in the tissue lens.

	Antifreeze KM 26	Counts x min^{-1} x mg DW^{-1} * $\bar{x} \pm s$	relative radioactivity	
corneal explant (d = 6 mm)	–	37712 ± 20166	100 %	$p < 0.1$
corneal explant (d = 6 mm)	1 min	19170 ± 5981	51 %	$p < 0.01$
tissue lens Thickness = 0.23 mm	1 min	7967 ± 7570	20 %	$p < 0.05$

* relating to the mean dry weight of 5 tissue samples

FIGURE 4

Influence of the cryoprotective agent KM 26 (DMSO 4% + glycerin 8.5% + kiton green V 0.25%, pH = 7.4), and the freezing process on the corneal stroma cells (n = 5).

	Antifreeze	Counts x min^{-1} x mg DW^{-1}* $\bar{x} \pm s$	relative radioactivity	
corneal explant (d = 6 mm)	–	64905 ± 33742	100 %	$p > 0.1$
corneal explant (d = 6 mm)	1 min	38050 ± 19054	59 %	$p < 0.05$
tissue lens Thickness = 0.23 mm	1 min	11856 ± 7476	18 %	$p < 0.1$

* relating to the mean dry weight of 5 tissue samples

FIGURE 5

Influence of the cryoprotective agent (DMSO 4% + glycerin 8.5% + Na + HCO3 --, pH = 7.4), and the freezing process on the corneal stroma cells (n = 5).

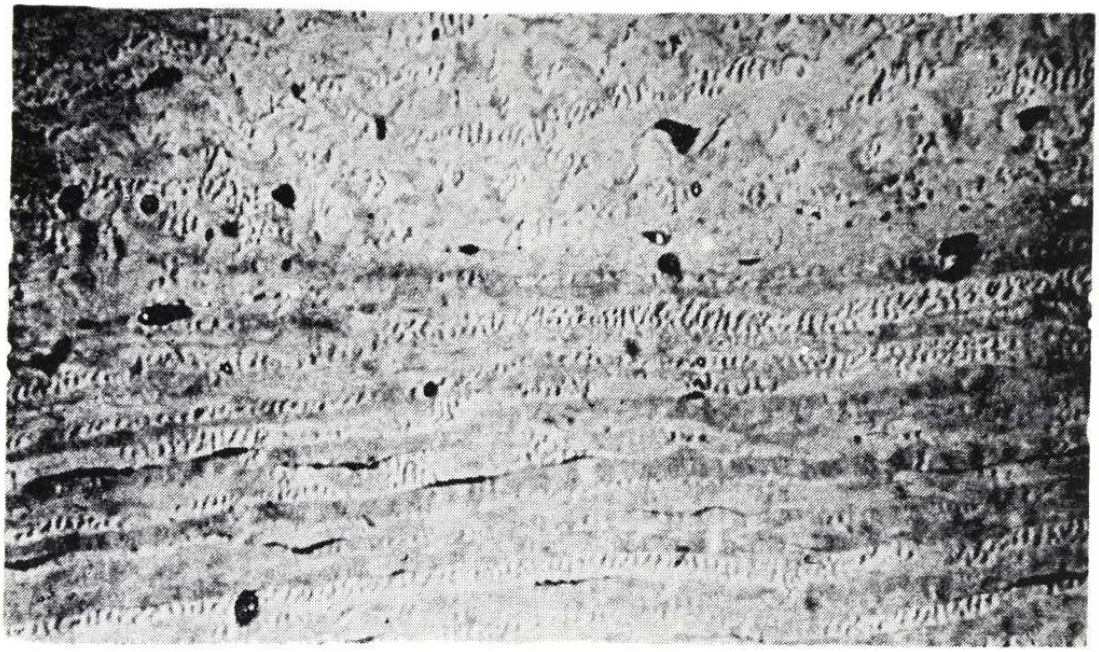


FIGURE 6

Semi-thin section 12 hours after keratophakia without cryoprotective agent, the lower part of the picture shows the recipient cornea, the upper part the donor cornea. The parallel course of the corneal lamellae is missing in the tissue lens, the keratocytes have taken on a ball-like shape.

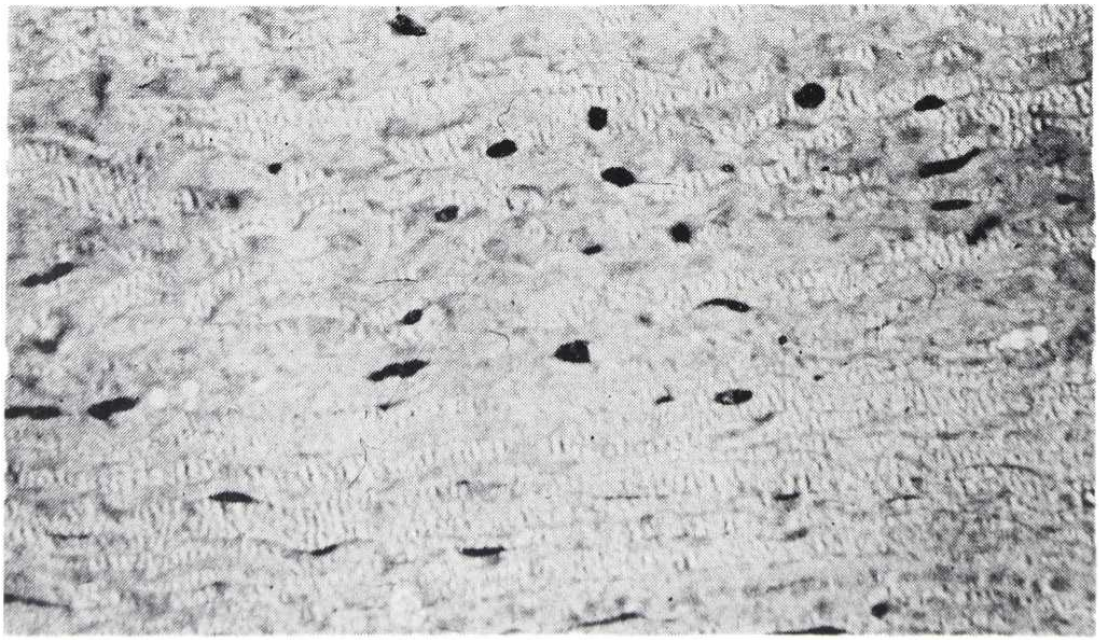


FIGURE 7

A semi-thin section 12 hours after keratophakia with cryoprotective agent KM 26. The lower part of the picture shows the recipient cornea, the upper part the donor cornea. The parallel course of the corneal lamellae is missing in the tissue lens, the keratocytes have taken on a ball-like shape.

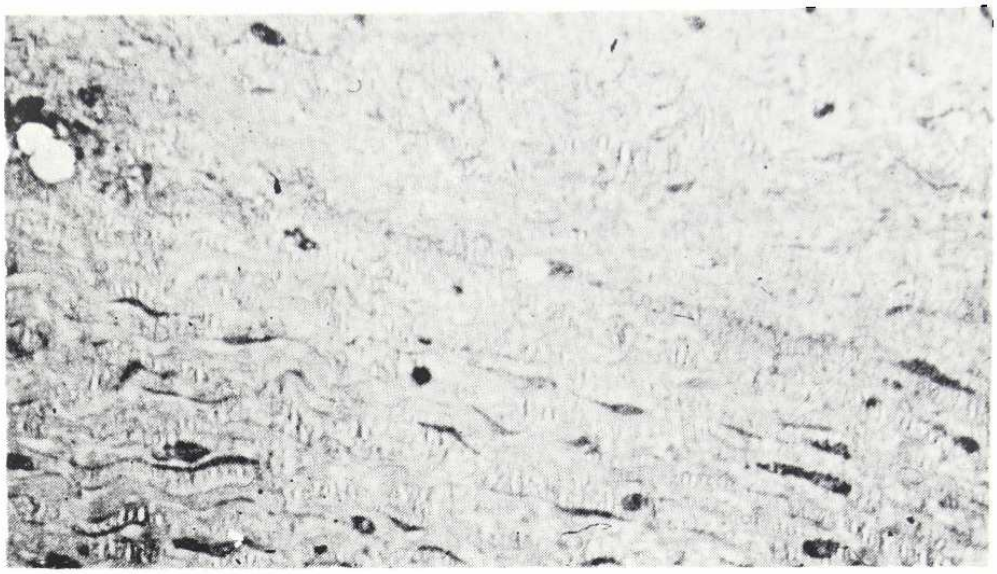


FIGURE 8

Semi-thin section 24 hours after keratophakia without cryoprotective agent. The lower part of the picture shows the recipient cornea, the upper part the donor cornea. The parallel course of the corneal lamellae is missing, cells are hardly recognizable any more in the tissue lens.

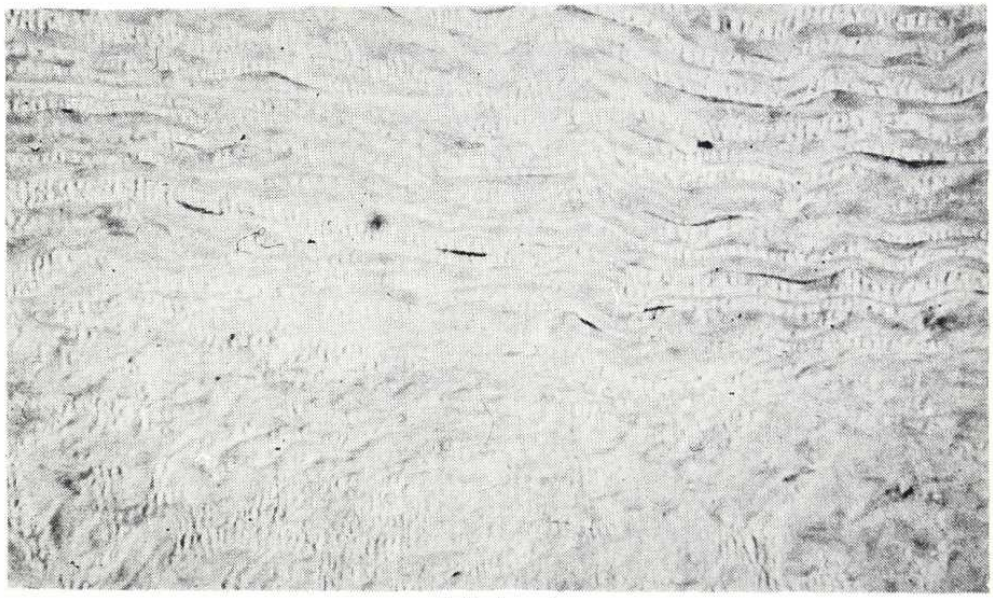


FIGURE 9

Semi-thin section 24 hours after keratophakia with cryoprotective agent KM 26. The lower part of the picture shows the recipient cornea, the upper part the donor cornea. The parallel course of corneal lamellae is missing, cells are hardly recognizable any more in the tissue lens.

In the electron-microscopic picture the tissue lens can be clearly distinguished from the normal corneal stroma (Fig. 10). The corneal lamellae no longer show the characteristic parallel course. The keratocytes in the tissue lens seem to be destroyed (Fig. 11). Under stronger magnification a clumping of the nuclear chromatin, a destruction of the cell membranes and a vacuolar change of the cytoplasm can be seen in the dying cell. There are no more cell organelles left (Fig. 12).

The keratocytes in the recipient cornea (Fig. 13), on the other hand, show an activation; there is a greater number of cell extensions. The endoplasmic reticulum is increased, and, in some places, a phagocytosis property of the cells can be observed in the form of a vesicle formation.

12 hours after keratophakia (Fig. 14), the recipient cornea shows macrophages situated within the lamellae and not between them like the keratocytes. With greater magnification, one recognized glycogen as an energy reserve in the cytoplasm in addition to numerous lysosomes.

48 hours after keratophakia, there are numerous macrophages, the cytoplasm of which is already full of phagocytized material. It is noteworthy that, in our material, macrophages 12 hours after keratophakia could only be found in the recipient cornea of the animal operated without a cryoprotective agent. 24 hours after keratophakia, macrophages were found in both eyes, but macrophages in the donor cornea were only found in the eye that was operated without a cryoprotective agent. Thus it appears that the greater stimulus for attracting macrophages was exerted in those corneas that were operated without a cryoprotective agent, an indication of the really protective effect of KM 26.

Three weeks after keratophakia, only individual cells are recognizable in the area of the tissue lenses. Apparently an increase in cells did not take place. At this time, electron microscopy no longer reveals any cells in the stage of cytoclasis. The cells now detectable in the tissue lens are fibroblasts with an increased and strongly broadened endoplasmic reticulum (Fig. 15).

On the basis of the investigations, it can be said in summary that the reduction of the relative activity in the tissue lenses measured by radiosulphate incorporation was caused by irreversible destruction of keratocytes.

If, during keratophakia, the tissue lens was produced without cryoprotection, the survival rate of the keratocytes is 10%. If, however, the cryoprotective agent KM 26 is applied, 20% of the keratocytes did survive.

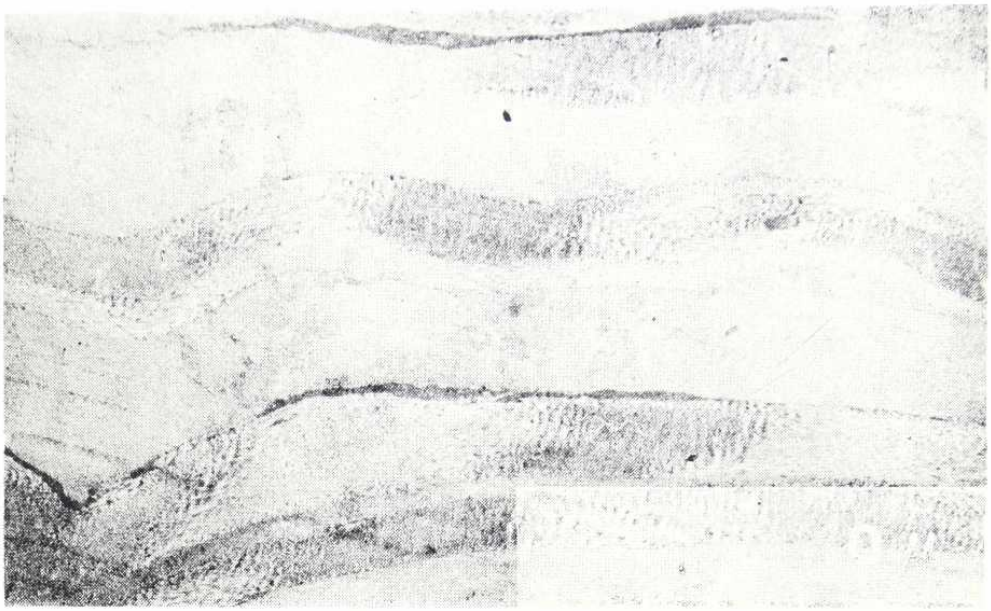


FIGURE 10

Recipient cornea: parallel arrangement of the corneal lamellae.

FIGURE 11

Tissue lens: the parallel arrangement of the corneal lamellae has been lost. Destruction of the keratocytes.

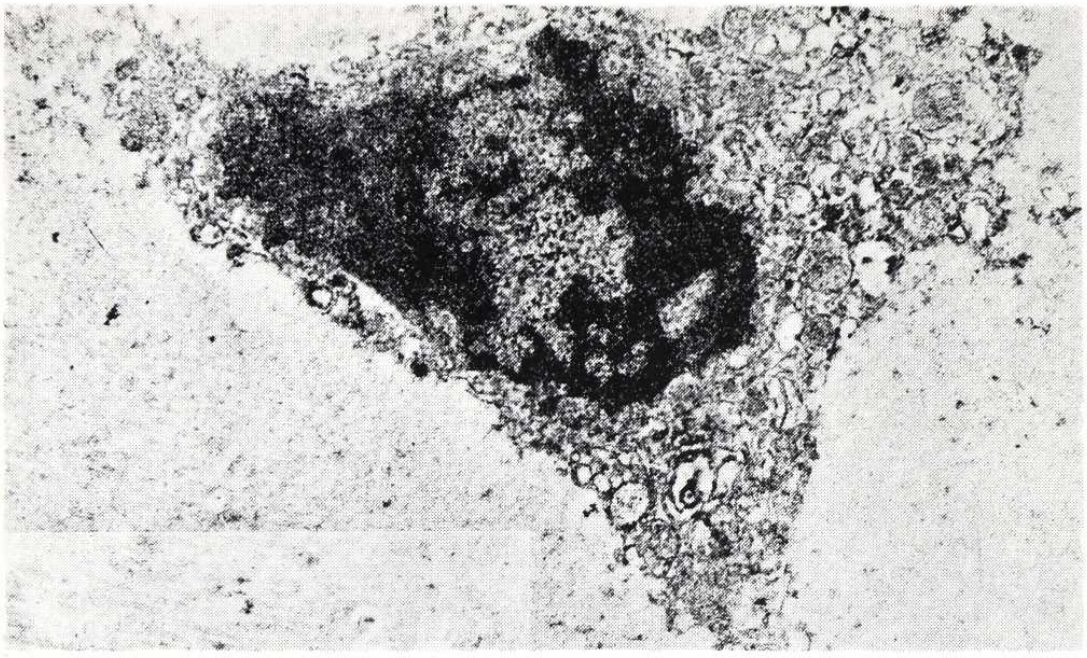


FIGURE 12

Dying keratocyte in the tissue lens 24 hours after keratophakia: Clumping of the chromoplasm. Destruction of the cell membrane. Vacuole change in the cytoplasm. Cell organelles are not recognizable.

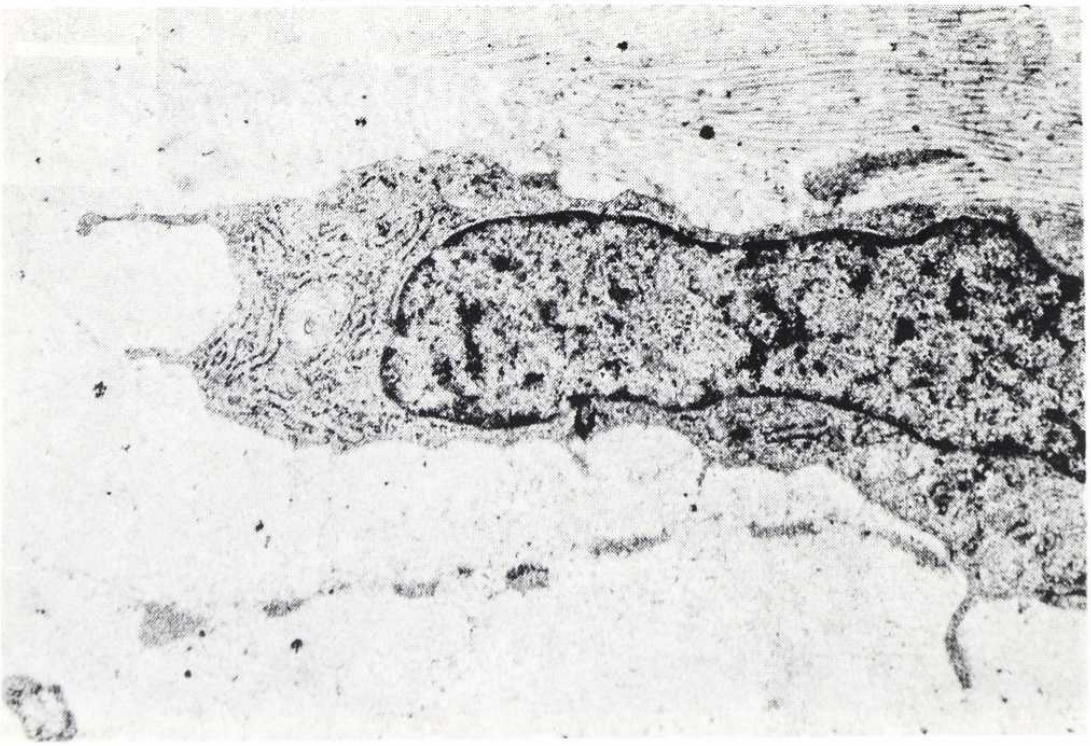


FIGURE 13

Activated keratocyte in the recipient cornea 24 hours after keratophakia. Increase of cell extensions and of the endoplasmic reticulum.

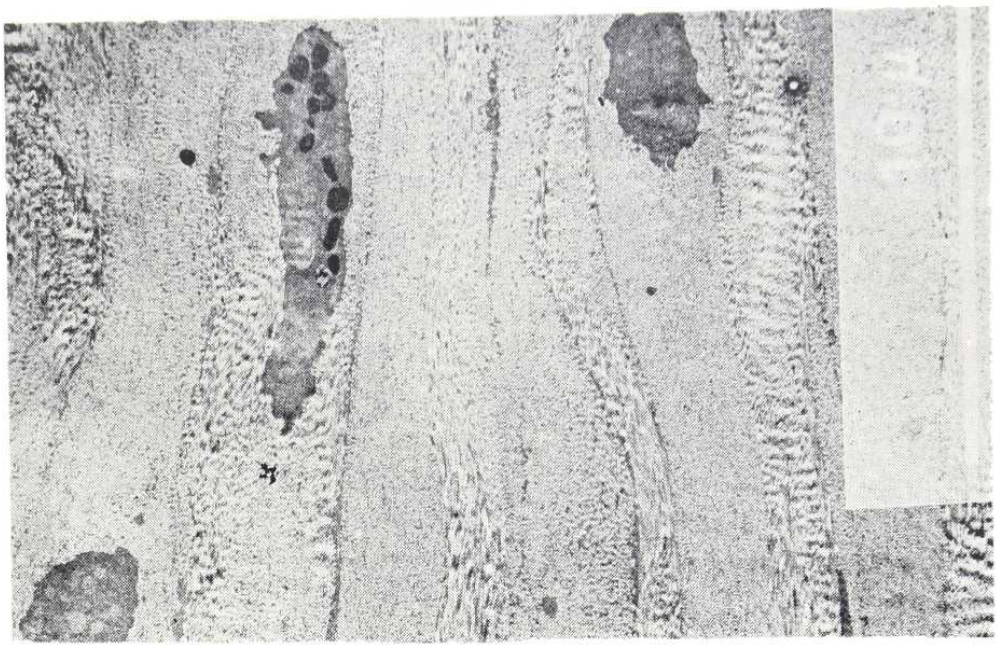


FIGURE 14

Macrophages in the recipient cornea 12 hours after keratophakia without cryoprotection.

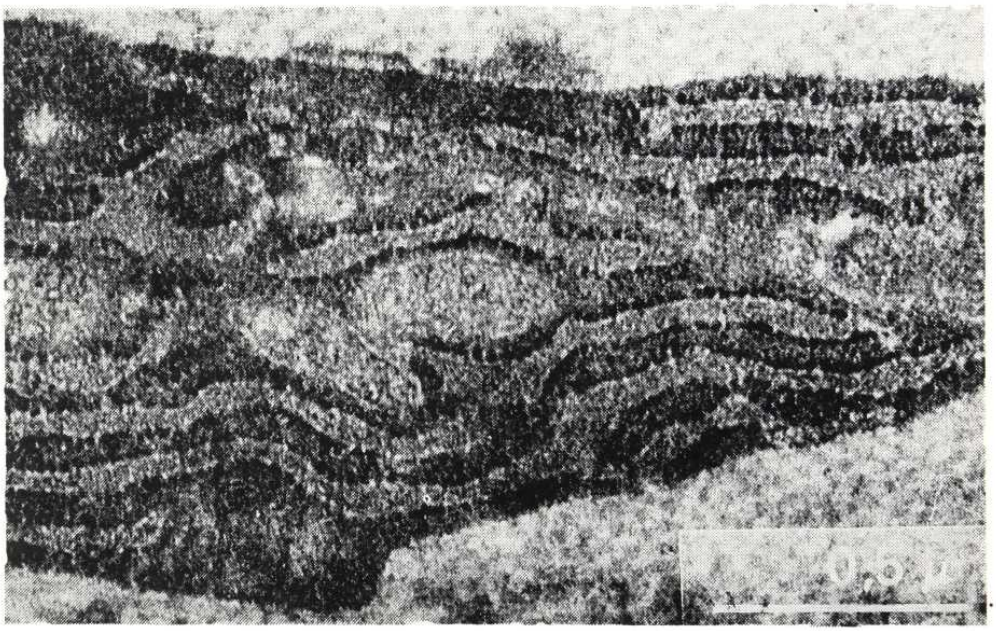


FIGURE 15

Fibroblast with increased and considerably broadened endoplasmic reticulum in the tissue lens 3 weeks after keratophakia.

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