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THE FATE OF CORNEAL STROMA CELLS IN KERATOPHAKIA

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SUMMARY

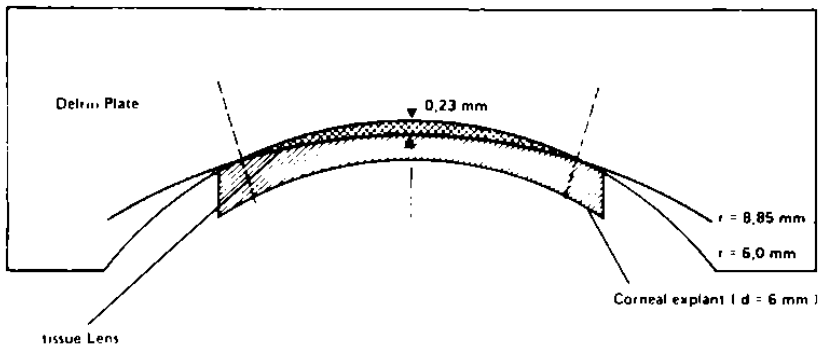
On 29 rabbits, we investigated with the aid of radiosulphate incorporation, histology and electron-microscopy the effect of the freezing process on corneal stroma cells during keratophakia with and without cryoprotection.

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.



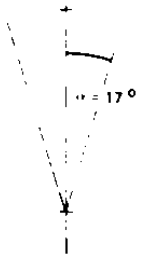


FIGURE 1

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

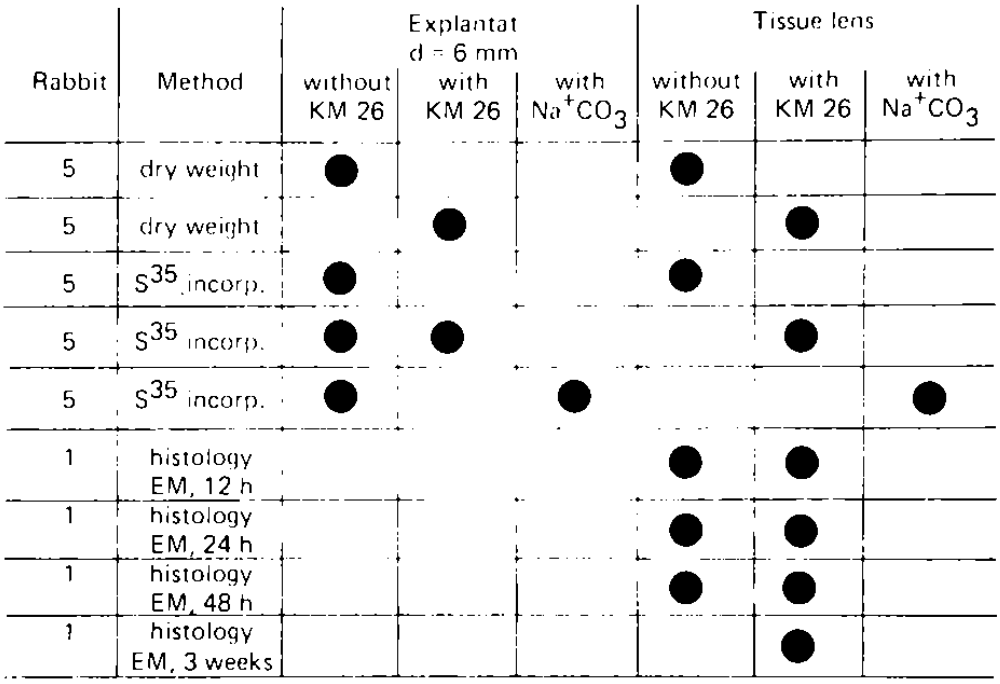


FIGURE 2

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).

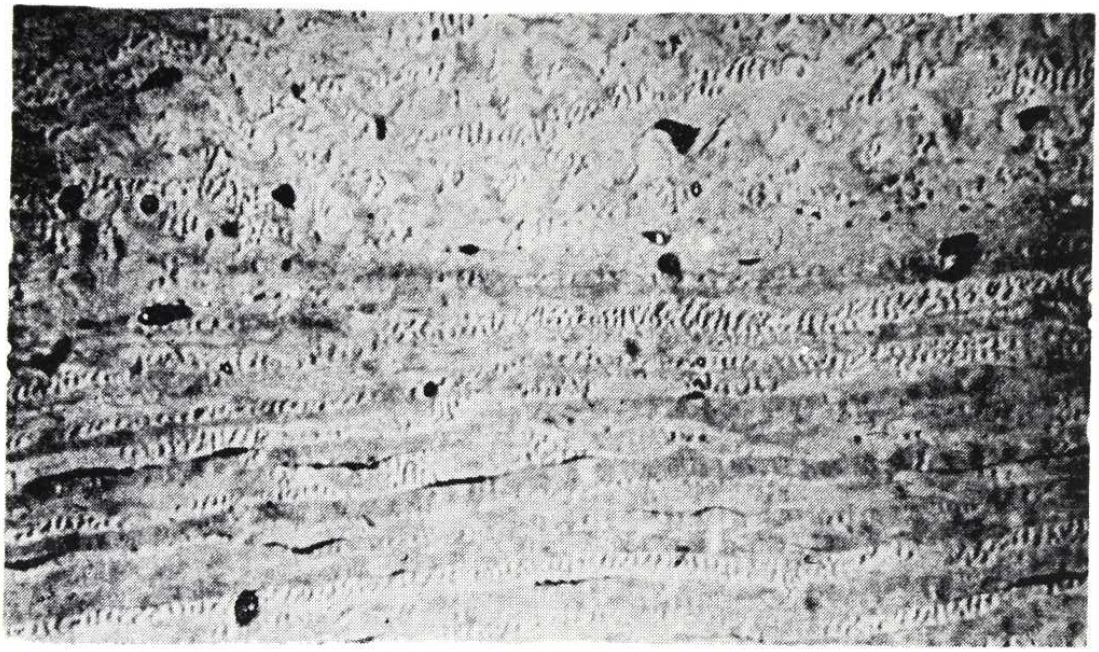


FIGURE 6

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.

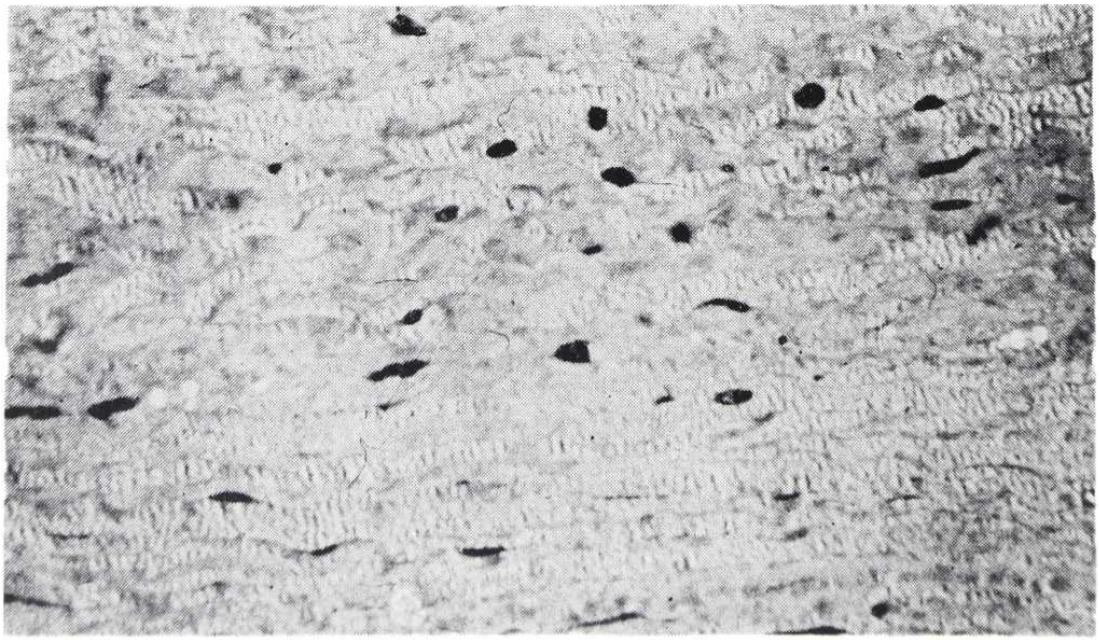


FIGURE 7

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.

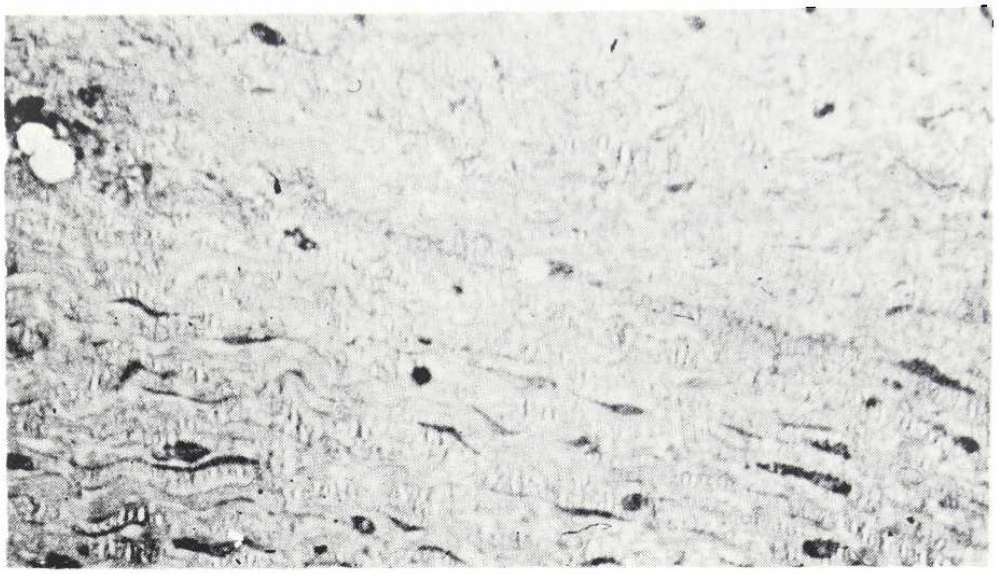


FIGURE 8

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.

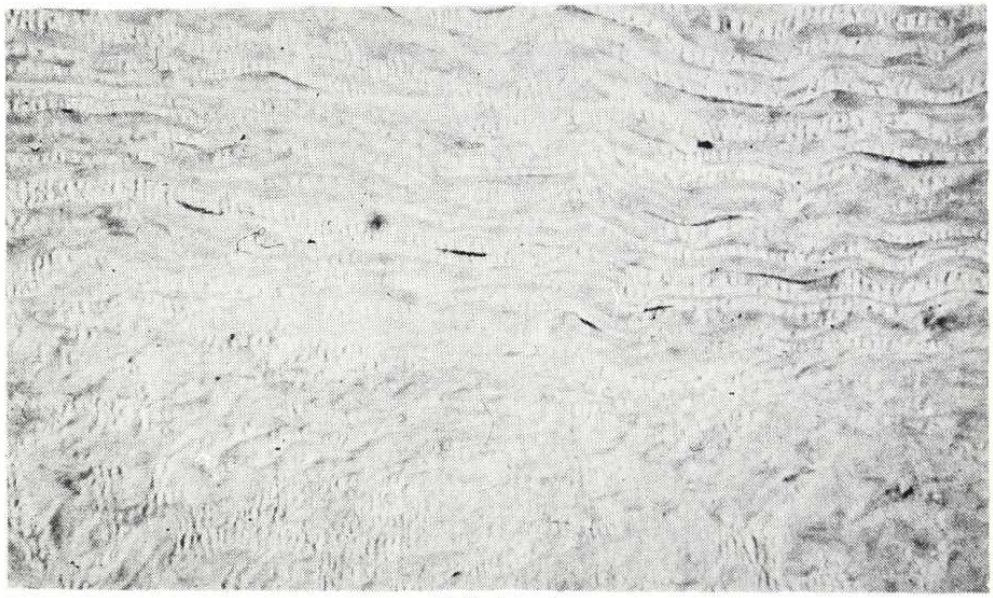


FIGURE 9

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.

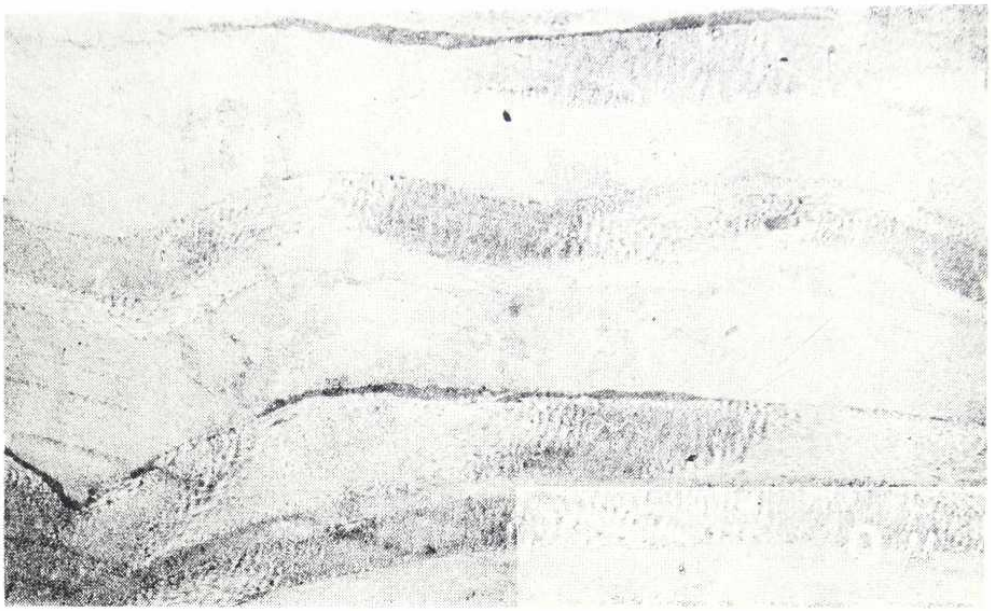


FIGURE 10

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.

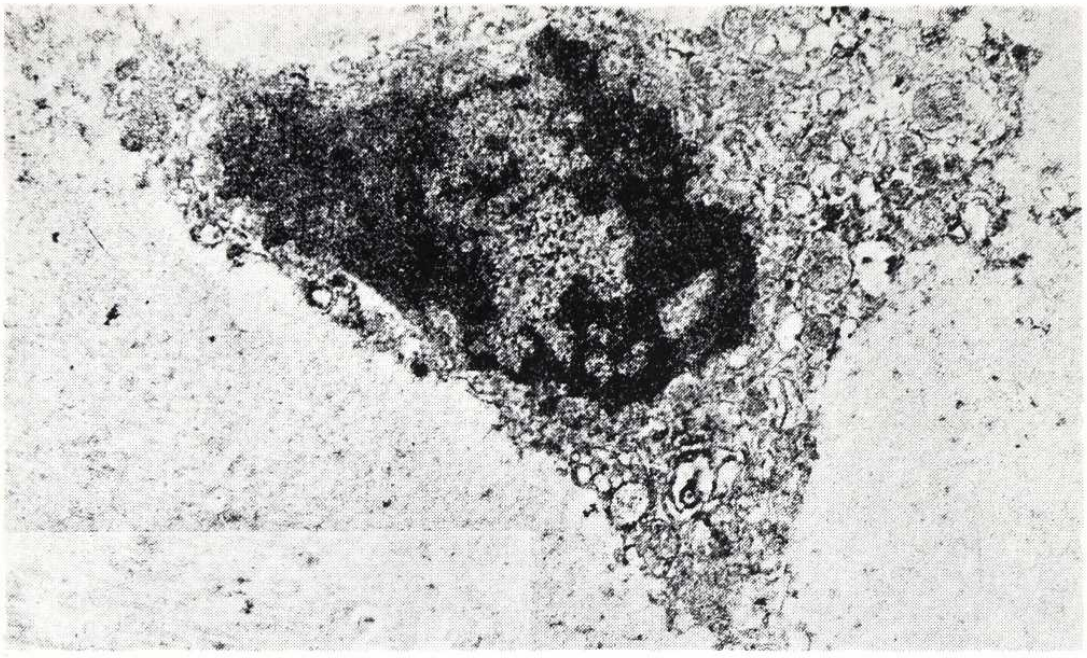


FIGURE 12

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.

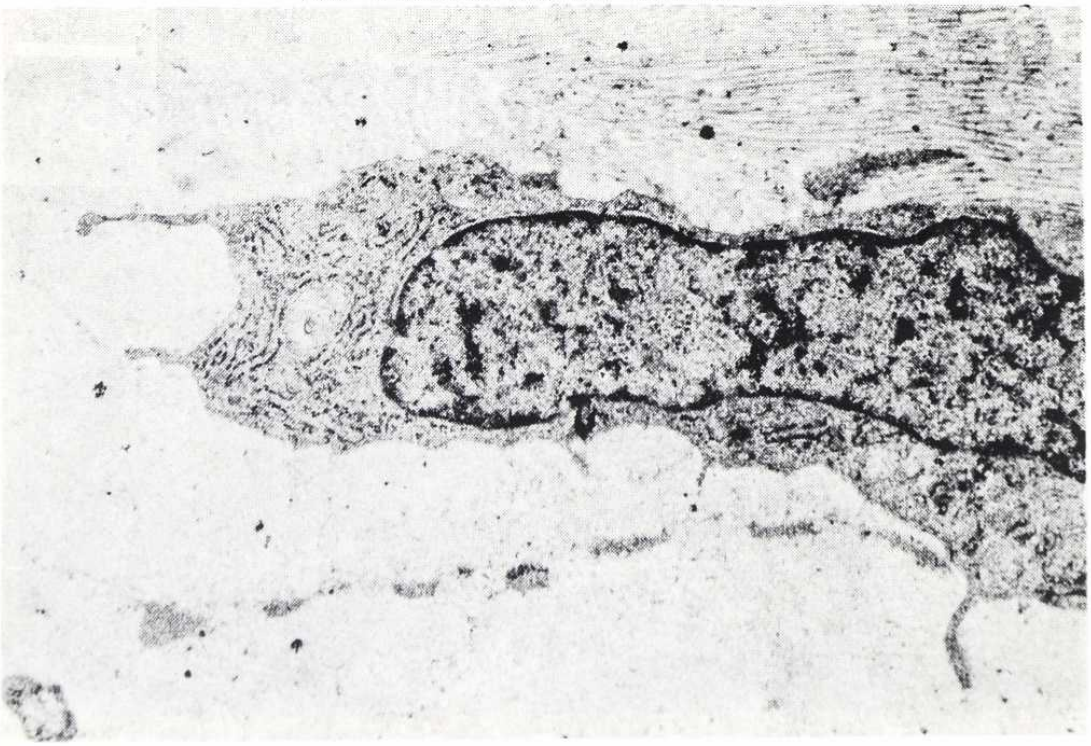


FIGURE 13

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

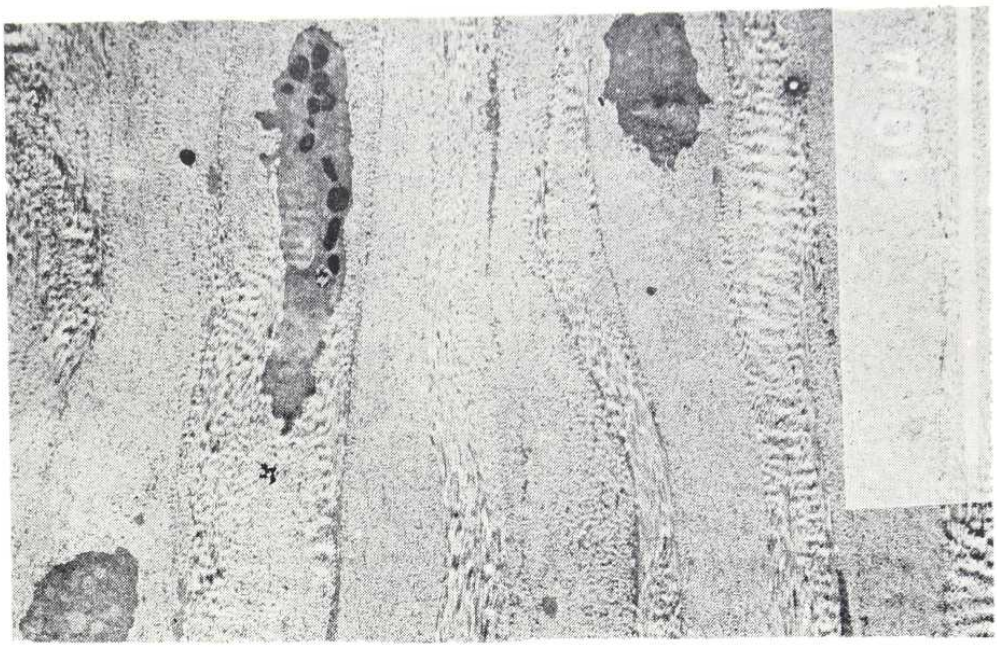


FIGURE 14

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

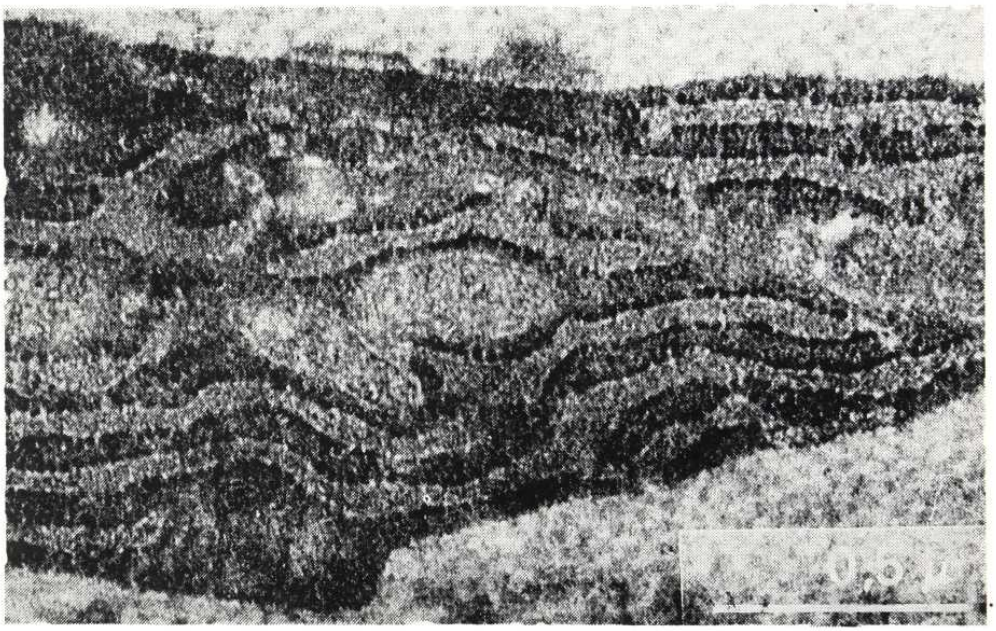


FIGURE 15

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

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