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Trabeculo-Electropuncture in Cynomolgus Monkeys *(Macaca Irus)*

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Summary. Trabeculo-electropuncture (TEP) was performed in one eye of ten cynomolgus monkeys. In five control eyes, the TEP probe was inserted into Schlemm's canal and withdrawn without causing a spark discharge. In another five control eyes, a scleral window was produced and the canal was opened, but no probe was inserted.

The gross facility of outflow was determined prior to the operation and 2, 6, and 18 weeks postoperatively. TEP had no longlasting effect on the facility. Gonioscopy five months after the operation revealed marked changes in the anatomy of Schlemm's canal in treated eyes and in control eyes subjected to probe insertion. Control eyes without probe insertion appeared normal. Six months postoperatively three monkeys were killed and the eyes investigated. Light and electron microscopy indicated that insertion of the probe with or without spark discharge resulted in the formation of dense bridges between the inner and outer walls of Schlemm's canal. At the sites of TEP, the trabecular meshwork was replaced by dense scar tissue containing irregular, fine fibrillar material, elastic fibres and large amounts of curly collagen. There were no intertrabecular spaces in the scar tissue. In one eye, one lesion was covered by a thin endothelium resting on a basal membrane.

The results indicate that the failure of TEP in monkey eyes was due to the formation of dense scar tissue occluding the openings initially produced by the spark discharge.

Zusammenfassung. An 10 Cynomolgus-Affen wurde einseitig eine Trabeculo-Elektropunktur (TEP) durchgeführt. An 5 Kontrollaugen wurde die Elektrosonde ohne eine Funkentladung in den Schlemmschen Kanal eingeführt. An 5 anderen Kontrollaugen wurde ein Sklerafenster angelegt und der Schlemmsche Kanal geöffnet, es wurde jedoch keine Sonde eingeführt.

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Die Fazilität wurde vor der Operation und 2, 6 und 18 Wochen danach ermittelt. Die TEP hatte keinen längeren Einfluß auf die Fazilität. Eine Gonioskopie 5 Monate nach der Operation machte erhebliche Veränderungen in der Anatomie des Schlemmschen Kanals an den operierten Augen und an solchen Kontrollaugen deutlich, bei denen eine Sonde in den Schlemmschen Kanal eingeführt worden war. 6 Monate nach der Operation wurden 3 Affen getötet und die Augen für die Mikroskopie aufgearbeitet. Bei den Augen, bei denen eine Sonde in den Schlemmschen Kanal eingeführt worden war, waren dichte Gewebebrücken zwischen der Innen- und Außenwand des Schlemmschen Kanals zu beobachten. Im Bereich einer TEP war das Trabekelwerk durch dichtes Narbengewebe ersetzt, welches aus unregelmäßigem feinen fibrillären Material, elastischen Fasern und Curly-Collagen bestand. Im Narbengewebe waren keine Intertrabekulärräume sichtbar. An einem Auge war im Bereich einer Funkentladungsstelle das Gewebe mit Endothel und einer Basalmembran gedeckt.

Die Ergebnisse zeigen, daß der Mißerfolg der TEP an Affenaugen durch dichtes Narbengewebe bedingt ist, welches die durch die Funkentladung gebildeten Öffnungen verschließt.

Trabeculo-electropuncture (TEP) (Hager et al., 1970) is a procedure that is used to increase the facility of outflow in glaucomatous eyes. A probe consisting of two electric conductors insulated against each other is inserted into Schlemm's canal. The probe is in contact with a condenser discharge device. When a condenser discharge is released, a spark discharge takes place just below the tip of the probe, and a hole with a diameter of approximately 100 μm is torn in the inner wall of Schlemm's canal and in the trabecular meshwork. Several such apertures can easily be produced in the inner wall of the canal. In glaucoma patients, these apertures appear to stay for years in successfully operated eyes (Hoffmann and Harnisch, 1974). Since morphological examinations cannot be made in such eyes, we have investigated monkey eyes subjected to TEP. We report here the failure of the operation in monkey eyes and the structural changes caused by the procedure. The results may help to explain why TEP also sometimes fails in glaucomatous eyes.

Materials and Methods

Ten cynomolgus monkeys of both sexes and weighing 1.7–4.1 kg were operated on. Anesthesia was induced with sodium methohexital (Brietal Sodium®, Lilly) 50–100 mg, i.m. and maintained with pentobarbital sodium. Heparin, 1500-2500 IU, was injected i.v. to avoid coagulation of blood in the anterior chamber.

Operation

One eye in each of ten monkeys was subjected to TEP. The electroprobe was inserted from a scleral window established in the temporal upper quadrant, in nasal direction into Schlemm's canal over approximately 1/6 of the circumference. With gonioscopic control, three spark discharges were caused with 15 to 35 nF, which corresponded to an energy of 4–8 mWs. The scleral window was carefully sutured with 7 9-0 polyamide threads. Following the conjunctival suture, 2 mg dexamethasone were injected subconjunctivally, and a drop of 2 % pilocarpine was put onto the cornea. This operation was described in detail by Hager et al. (1972).

In 5 control eyes a scleral window was established, and the outer wall of Schlemm's canal was opened by a radial cut. In the other 5 control eyes, a scleral window was made and the TEP-probe was inserted into the canal but without causing a spark discharge.

Measurement of Facility and Follow-up Examination

Several days prior to operation, and 2, 6, and 18 weeks postoperatively, the gross facility of outflow was determined (Bill and Barany, 1966).

The first week after the operation, the monkeys were inspected every day: and during the first 2 weeks, they were examined twice with the aid of the slitlamp. After 5 months, they were viewed gonioscopically. A puncture of the anterior chamber was made to reduce the intraocular pressure and to fill all parts of Schlemm's canal with blood.

Preparation for Histology and Electron Microscopy

Six months postoperatively, 3 monkeys were killed, and, after rapid establishment of a large corneal window, the eyes were fixed in Karnovsky-solution for 3 days. Then parts of the iridocorneal angle tissue were removed under the microscope, refixed in 1 % osmiumtetroxide for 2-3 hours and embedded in micropal. Semi-thin sections at 60 levels and approximately 30 μm apart, as well as ultra-thin sections at 5 levels, were made. The semi-thin sections were stained according to Richardson (1960), and the ultra-thin sections were contrasted with lead-citrate and uranyl-acetate.

Results

After the spark discharge, a profuse hemorrhage into the anterior chamber occurred in all monkeys. In the control eyes where the probe was inserted into the canal, there was a light hemorrhage into the anterior chamber.

Gonioscopy 5 months after the operation revealed marked changes in most of the eyes subjected to TEP or probe insertion only. Table 1 presents the gonioscopic findings.

Gross Facility of Outflow

In experiments without probe insertion into Schlemm's canal in the control eyes, the facility of the experimental eyes tended to be lower than that in the controls. The difference was not statistically significant, however (Table 2). In the experiments with probe insertion into Schlemm's canal in the control eyes, the facility values of the experimental and control eyes were similar (Table 3).

Table 1. Gonioscopic findings 18 weeks postoperatively

	Exp. eye	Control eye with probe	Control eye without probe	
Synechia Iris-trabecular network	4	-	-	
Continuous wide blood filling	1	1	5	
irregular continuous blood fillings	5	4	-	
Interrupted blood column in the scleral window	3	-	-	
Interrupted blood column in 2 places	1	-	-	
Blood leakage into the anterior chamber	(1)	-	-	

Table 2. Mean values of facility ($\mu l \cdot min^{-1} \cdot mmHg^{-1}$) ± standard deviation (sd). Control eye without probe in Schlemm's canal

	Before surgery Fac I	2 weeks after surgery Fac II	6 weeks after surgery Fac III	18 weeks after surgery Fac IV
Exp.	0.25 ± 0.08	0.27 ± 0.13	0.18 ± 0.12	0.24 ± 0.17
Control (without probe)	0.26 ± 0.08	0.40 ± 0.30	0.26 ± 0.16	0.27 ± 0.15

Table 3. Mean values of facility ($\mu l \cdot min^{-1} \cdot mmHg^{-1}$) ± standard deviation (sd). Control eye with probe in Schlemm's canal

	Before surgery Fac I	2 weeks after surgery Fac II	6 weeks after surgery Fac III	18 weeks after surgery Fac IV
Exp.	0.33 ± 0.12	0.25 ± 0.10	0.18 ± 0.07	0.35 ± 0.15
Control (with probe in Schlemm's canal)	0.34 ± 0.10	0.22 ± 0.05	0.16 ± 0.07	0.34 ± 0.09

Histological Findings

In the control eye, where the probe had not been inserted, Schlemm's canal had a wide lumen separated into two parts by a small septum only in the region of a collector channel. Schlemm's canal of the experimental eyes was in large areas separated into 2-5 lumina (Fig. 1). The bridges between the inner and outer walls were wide and consisted of dense, collagenous tissue. In many places, the bridges and adjacent tissue in the region of the inner wall had densified to such an extent that trabeculae and intertrabecular spaces were no longer visible (Fig. 2). In one experimental eye, the corneal endothelium had grown over the trabecular meshwork at a width of approximately 300 µm. Behind the endothelium, the trabecular meshwork was densified so that trabeculae or intertrabecular spaces could no longer be seen (Fig. 3).

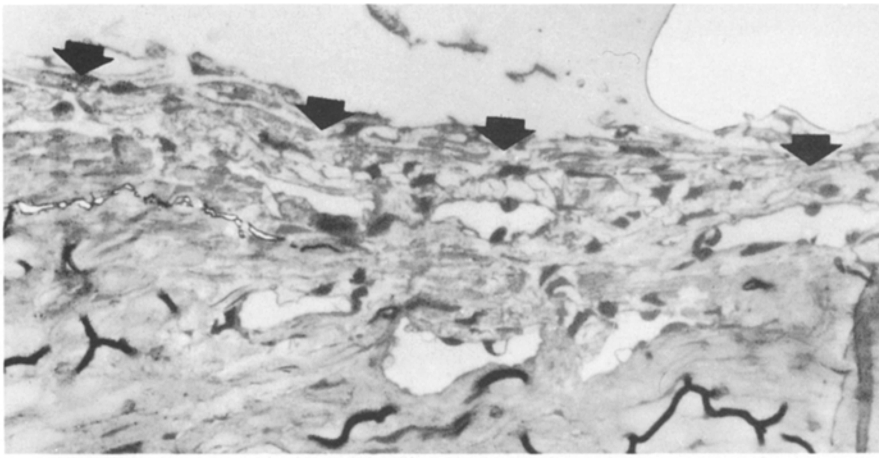
In the control eyes with inserted probe but no spark discharge, large areas of Schlemm's canal had up to 3 lumina. The tissue bridges between the canal lumina were wide and densified.

Electron Microscopic Findings

Figure 4 shows one of the regions with electropuncture. At the place of the trabecular meshwork, it consisted of dense tissue covered by endothelial cells. The dense tissue contained irregular, fine fibrillary material, many elastic fibres, and large amounts of curly collagen. There were no intertrabecular spaces in the tissue. The endothelium rested on a basal membrane only. A Descemet's membrane thus had not been formed.

At other regions subjected to a spark discharge, a tissue similar to that described above was seen at the place of the trabecular meshwork, but no endothelium covered the inner part of the tissue.

There were many pigmented cells in the trabecular meshwork of the experimental eyes (Fig. 5), and thickened basal membranes which included curly collagen were observed in many places. Some of the basal membranes were free in the tissue without any connection to the endothelial cells (Fig. 6).



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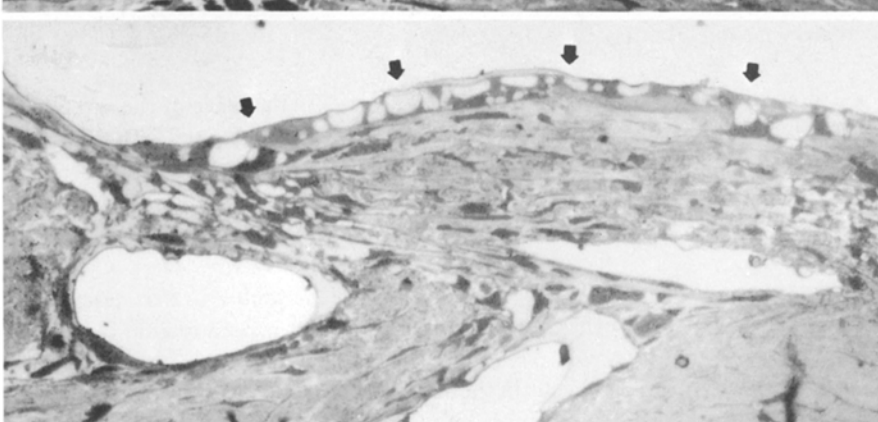

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Fig. 4. Electron-micrograph of the trabecular meshwork seen in Figure 3. The tissue consists of irregular, fine fibrillary material, elastic fibres (*e*) and curly collagen (*c*). The endothelium rests on a basal membrane. There is no Descemet's membrane

Fig. 1. TEP caused formation of dense bridges between inner and outer walls of Schlemm's canal. As a result, canal has several lumina. (*Arrows*)

Fig. 2. Dense bridges (*Arrow*) between inner and outer walls of canal in an experimental eye. Extremely densified tissue located in front of inner wall of Schlemm's canal does not show intertrabecular spaces

Fig. 3. Corneal endothelium (*Arrows*) in front of trabecular meshwork at place of spark discharge. Almost the entire inner wall of Schlemm's canal consists of dense scar tissue

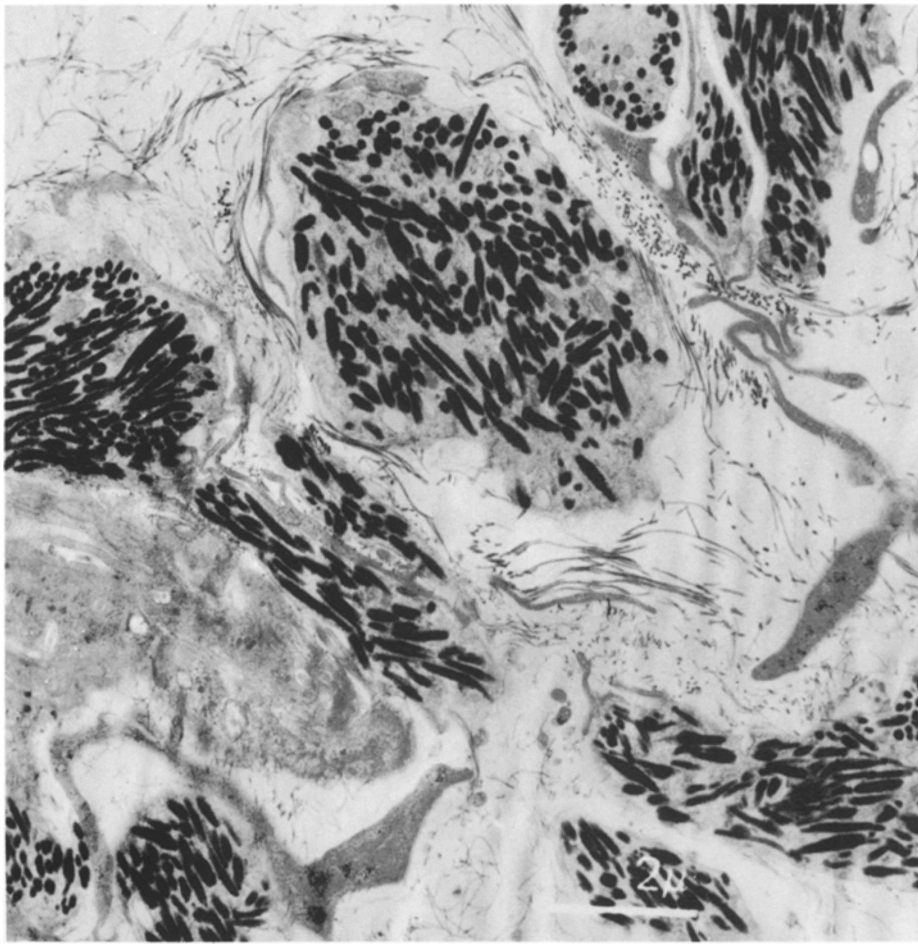

Fig. 5. Pigmented cells in trabecular meshwork of experimental eye

Discussion

In human eyes with glaucoma, successful TEP seems to result in a long-standing opening between the anterior chamber and Schlemm's canal which reduces the resistance to outflow of aqueous humor. The spark discharge usually does not cause a bleeding into the anterior chamber (Heppke et al., 1972). In healthy monkey eyes, immediate as well as long-standing effects of TEP are quite different. No permanent opening is created; there is no long-standing reduction in outflow resistance, and immediately after the spark discharge there is a profound bleeding into the anterior chamber.

In a study by Bárány et al. (1972), it was found that after trabeculectomy in monkeys, the open ends of Schlemm's canal became occluded with scar tissue and a flat endothelium. In most cases, facility of outflow did not change appreciably. It

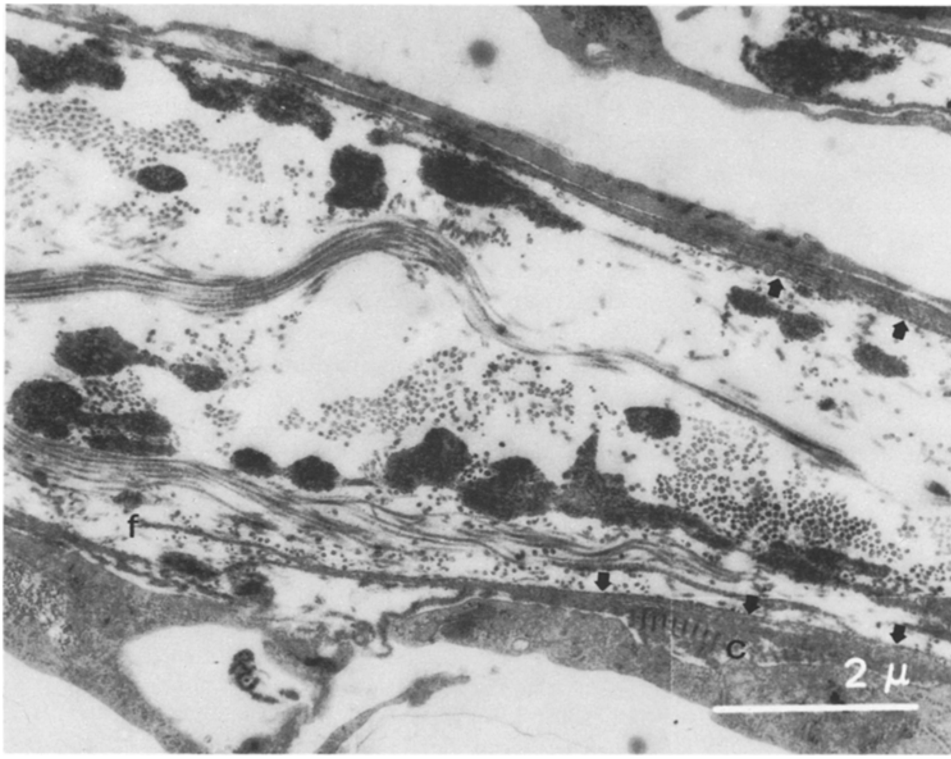

Fig. 6. Free (*f*) and densified (*Arrows*) basal membranes with curly collagen (*c*) in trabecular meshwork of experimental eye

was suggested that creation of several small openings in Schlemm's canal might be more effective than trabeculectomy.

The scar tissue that occluded the openings caused by TEP in the present experiments was similar to that occluding the ends of Schlemm's canal in the experiments of Bárány et al. The canal at the sites of TEP had also changed but could be demonstrated by means of gonioscopy in most cases. Sections of regions with TEP demonstrated very marked changes in the meshwork as well as within the canal. We observed an endothelium in the area of a spark discharge in one eye only. Assumedly this was not an operculum, for this would have had a Descemet's membrane (Rohen et al., 1967) with regular collagenous structure and a normal trabecular meshwork behind it.

Our results thus suggest that with TEP the lumen of Schlemm's canal usually is not totally obliterated with scar tissue as after trabeculectomy. In addition, the lesions may be less prone to become covered by endothelium. However, the dense scar tissue developing in the trabecular meshwork after TEP is such a barrier to aqueous drainage that the effect on the outflow resistance is just as poor as that after trabeculectomy.

There are several reasons why TEP may be more effective in glaucomatous eyes than in healthy monkey eyes. First, the tendency to wound healing in the trabecular meshwork of relatively old glaucoma patients may be less pronounced than that in young healthy monkeys. Second, the pressure conditions in glaucomatous eyes are such that most of the aqueous humor can be expected to drain through the openings created by TEP. A continuous, relatively rapid flow may help to keep the openings patent. In healthy monkeys, the resistance to outflow through the trabecular meshwork is low, and a considerable part of the aqueous humor may therefore be drained through the physiological routes even after TEP. Third, the hemorrhages seen in monkey eyes after TEP may contribute in the healing process. Our results, as well as those of Dannheim and van der Zypen (1970; 1972) and Bárány et al. (1972) and an unpublished study on the effects of cyclodialysis in monkeys by Bill, indicate that experimental glaucoma surgery in monkey eyes is much less successful than glaucoma operations in patients.

If a procedure can be found that results in a permanent opening between the anterior chamber and Schlemm's canal in monkeys and in an increase in outflow facility, it seems likely that it will be effective in glaucoma patients as well.

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