EXPERIMENTAL INTRAOCULAR TOLERANCE TO LIQUID PERFLUOROOCTANE AND PERFLUOROPOLYETHER

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Abstract: Three kinds of perfluorinated liquids (a perfluorooctane and two perfluoropolyethers) were evaluated as vitreous replacements. Tolerance to these liquids in rabbit eyes was investigated for periods of up to 2 months. Clinically, droplet formation of the liquids occurred within a few days of intravitreal injection. Histologic examination revealed no pathologic retinal changes 8 hours after surgery. At 6 days after surgery, hypertrophy of the Müller cells with bumplike protrusions into the interphotoreceptor space could be observed. At 1 month after surgery, light and electron microscopic examination showed larger droplike protrusions of Müller cells related to localized foldings of the outer retinal layers and rarefication of photoreceptor nuclei and loss of outer segments. Frequently disarranged, granule-loaded macrophages appeared in these areas. At 2 months after surgery, vesicles with low electron density appeared in some areas at the border between receptors and retinal pigment epithelium. In other areas pigment epithelial cells showed distinct hypertrophy (with drusen) toward the droplike Müller cell protrusion, together with narrowing of the interreceptor space. These findings were almost totally confined to the lower part of the retina that had been in permanent contact with the liquids. No histologic differences were noted between perfluorooctane- and perfluoropolyether-injected eyes. The results suggest that the liquids are not candidates for long-term vitreous replacement, but may be suitable for short-term intraoperative use.
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Liquid perfluorocarbons are useful tools during vitreous surgery for complicated retinal detachment; their high specific gravity facilitates reattachment of the retina considerably.[1–5]

Of the various perfluorocarbons, perfluorotributylamine, perfluorodecalin, and perfluorooctane have been used clinically. When injected as a short-term vitreous replacement, these liquids are tolerated equally well by the eye.[2] In addition, several perfluoropolyethers have been examined experimentally.[6–9] Compared to other fluorochemicals, perfluorinated ethers are available in different molecular weights and viscosities. This offers a potential advantage, as further experiments show that compounds with a higher viscosity demonstrate less droplet division. The long-term intraocular tolerance of liquid fluorochemicals is usually poor, because of the dispersion of the liquids, foam cell reaction, and photoreceptor toxicity, which has been observed at 1 month after injection.[10] Use of perfluoropolyether has also resulted in preretinal membrane formation and tractional retinal detachment.[6]

The purpose of this experimental study was to compare short- and long-term intraocular tolerance to perfluorooctane with tolerance to perfluoropolyether with low

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and high levels of viscosity. We used the new perfluoropolyether Hostinert (Hoechst AG, Frankfurt, Germany), a commercial product with a stated high purity of 99.9%.

Materials and Methods

Perfluorooctane that demonstrated a purity of 99.9% by gas chromatography was obtained for use in this study. The purity and uniformity levels of the commercial perfluorinated polyethers Hostinert 130 and Hostinert 245 (Hoechst AG, Frankfurt, Germany) were 99.99% and 99.96%, respectively, as determined by nuclear magnetic resonance spectroscopy and by gas chromatography. Details of the chemical and physical properties of the three fluorochemicals are listed in Table 1. The liquids were not subjected to further purification by alumina treatment. Sterilization was performed through 0.22 μm Millipore filters directly before intraocular injection.

A total of 30 eyes of pigmented rabbits weighing 3.5 kg to 4.0 kg were used in this study (Table 2). The rabbits were anesthetized with intramuscular ketamine (35 mg/kg of body weight) and xylazine (5 mg/kg of body weight). A 20-gauge infusion cannula attached to a Ringer's solution was inserted through the inferotemporal pars plana, 1 mm behind the corneoscleral limbus. The vitrectomy probe was then passed through another sclerotomy in the superonasal area, 1 mm posterior to the limbus. Coaxial illumination was provided by an operating microscope (Zeiss OPMI 6, Oberkochen, Germany). Under constant infusion, as much of the vitreous was removed as possible, but no attempt was made to completely remove the cortex. At the completion of vitrectomy, the infusion line was closed and 0.5 ml to 1.0 ml of Ringer's solution was aspirated. The vitrectomy probe was then withdrawn and 0.8 ml of the different perfluorocarbons were injected into the vitreous cavity. At the end of the procedure, the sclerotomies were closed with preplaced 7-0 Vicryl sutures (Ethicon, Hamburg, Germany). Subconjunctival injections of 10 mg gentamicin sulfate were given.

Table 1. Physicochemical Properties of Perfluorocarbons Studied

	Perfluoro-octane	Hostinert 130	Hostinert 245
Molecular weight	438	570	1068
Density, g/cm^3 (+20° C)	1.76	1.77	1.85
Viscosity, mPa-s (+20° C)	0.8	2.13	16.9
Boiling point (C)	+100°	+130°	+245°
Surface tension, mN/m (+25° C)	14	13.6	16.8
Vapor pressure, mbar (+25° C)	29	10	1
Refractive index	1.27	1.27	1.28

Table 2. Number of Rabbit Eyes Studied and Time of Enucleation After Surgery

	8 hrs.	6 days	1 mo.	2 mos.	Total
Perfluoro-octane	2	2	2	3	9
Hostinert 130	3	2	4*	3	12
Hostinert 245		2	2	4*	8
Ringer's solution		1			1
					30

* In 1 eye removal of the perfluorocarbon was performed 2 weeks after injection.

Initially, the eyes were examined daily after surgery, and then examined weekly, using slit-lamp biomicroscopy and indirect ophthalmoscopy. A Draeger applanation tonometer (Moeller, Wedel, Germany) was used to check intraocular pressure (IOP) on the first day after surgery and then sporadically every second to third week. The clarity of the lens and the degree of cells and opacities in the vitreous cavity were estimated, and the level of the perfluorocarbon liquid and the size and amount of droplets into which the liquid had divided were recorded.

Two eyes injected with Hostinert 130 and Hostinert 245 underwent a second surgery two weeks after injection to remove the perfluorocarbon. An infusion cannula connected to Ringers's solution was inserted through the pars plana and a blunt needle was introduced into the vitreous cavity through an additional sclerotomy. Due to the droplet division, the fluid could not be completely aspirated. Numerous small droplets remained at the surface of the retina, where they were probably captured by a thin cortical layer of vitreous. Such removal of the fluid was therefore abandoned in other cases. The eyes from which Hostinert 130 and Hostinert 245 were removed were enucleated 2 weeks and 6 weeks after surgery, respectively.

The rabbits were anesthetized with intramuscular injections of ketamine and xylazine at 8 hours, 6 days, 1 month, and 2 months after injection of the fluorochemicals. After intravenous injection of heparin (500 I.U./kg of body weight) a transcardiac perfusion was performed with 600 ml of 37°C Dulbecco solution, followed by 500 ml of 37°C 4% glutaraldehyde-phosphate buffer. Immediately after enucleation, a razor blade was used to open the eyeball through a small horizontal cut 2 mm posterior to the corneoscleral limbus. In order to avoid deformation, the globe was placed in 4% glutaraldehyde-phosphate buffer, and the section was completed within the fixative with pointed scissors. After the anterior segment was removed, the eyes were studied with a dissecting microscope to evaluate changes of the remaining vitreous and retina. In selected cases, photographs were

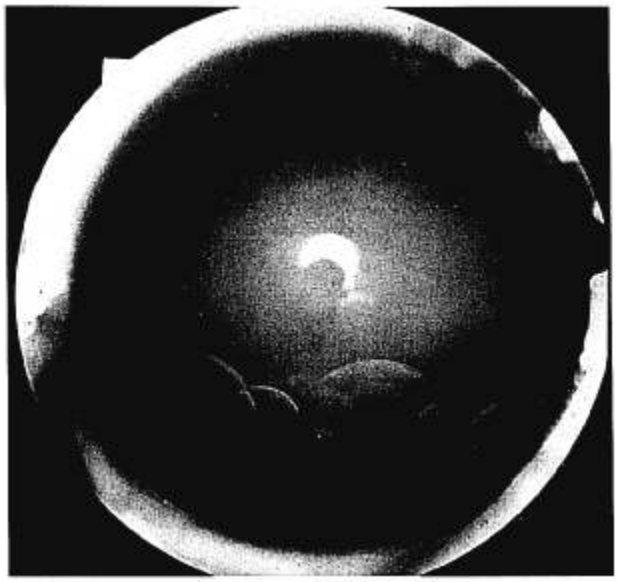

Fig. 1. Rabbit eye 6 days after vitrectomy and intraocular injection of perfluorooctane shows total division of the liquid into numerous droplets of different size.

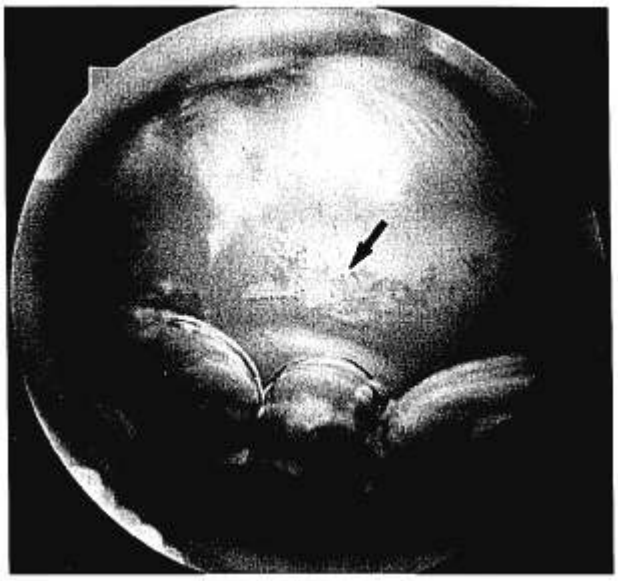

Fig. 3. Rabbit eye 3 weeks after vitrectomy and intraocular injection of perfluorooctane shows numerous flakelike white precipitates (arrow) in the vitreous cavity above the liquid, which is divided into several drops of different size.

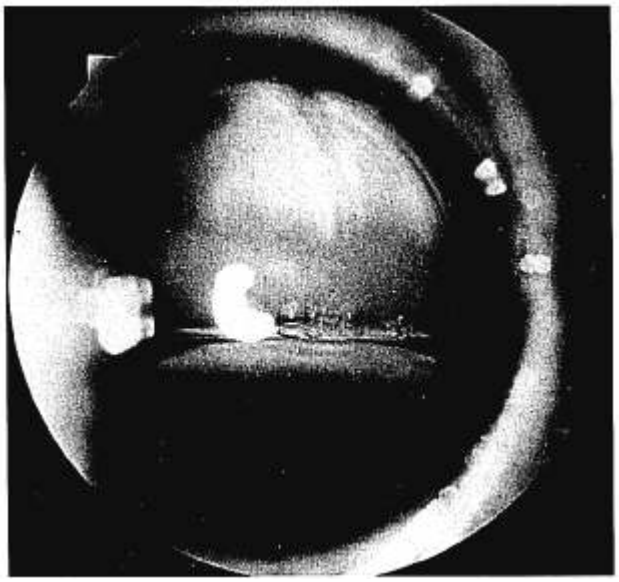

Fig. 2. Rabbit eye 6 days after intraocular injection of Hostinert 130 shows some droplet division above the level of liquid.

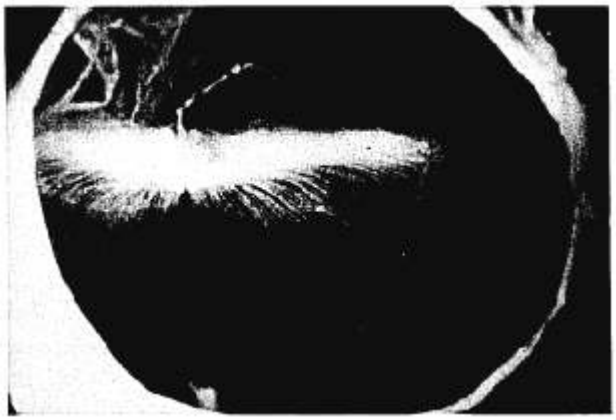

Fig. 4. Rabbit eye 2 months after vitrectomy and intraocular injection of perfluorooctane, with almost all remnants of the vitreous body covered by white precipitates. Gross examination shows no changes of the retina.

taken. In two cases, membranes of the remaining vitreous body containing white precipitates were removed. Smears were taken from these structures, stained with methylene blue, and studied immediately with a conventional light microscope. Additionally, the membranes were fixed in 4% glutaraldehyde-phosphate buffer and embedded in resin. Sections with a thickness of 0.5 μm were prepared for examination by light microscopy.

The eyes were left in the fixative overnight. The following day, retinal tissue, including the choroid and the sclera, was taken from the equatorial area of both the inferior and superior halves of the globe. The samples were cut with a razor blade into segments measuring 1.5 mm × 6 mm. The tissue was postfixed in 1% buffered osmium tetroxide for 4 hours. After dehydration with solutions of increasing concentrations of ethanol, the tissue was embedded in Araldite. Sections with a thickness of 0.5μm were stained with methylene blue–azure II and examined by light microscopy. Thin sections were

stained with uranyl acetate and lead citrate. The specimens were examined with a Zeiss EM 10 electron microscope (Zeiss, Oberkochen, Germany).

Results

Clinical Observations

After surgery, 11 eyes showed 2+ cells and flare in the vitreous cavity above the perfluorocarbon level. This inflammatory reaction was the same in the three perfluorocarbon groups. In 5 cases, a minor-to-moderate vitreous hemorrhage that resolved completely within 10 days was demonstrated. In two eyes, a rhegmatogenous retinal detachment was seen in the temporal or nasal superior quadrant. Retinal reattachment occurred within 7 days without any further treatment. One eye developed a traumatic cataract, but the lens stayed clear in other eyes. Intraocular pressure did not rise during the follow-up period.

When examined by slit-lamp microscopy, all eyes revealed an emulsification of the perfluorinated liquids. During the first week after surgery, eyes injected with perfluorooctane showed the highest degree of droplet formation (Fig. 1). In eyes injected with Hostinert 245, droplet division occurred later, but by the fourth week after surgery was similar to that in perfluorooctane injected eyes. Eyes injected with Hostinert 130 demonstrated the smallest degree of droplet division during the examination period (Fig. 2). At the interface of the perfluorochemical and the remaining vitreous, white flakelike precipitates could be observed (Fig. 3). In some eyes, such precipitates were also present in vitreous bands or occurred like small patches on the surface of the retina. Such changes developed as early as 4 days after surgery in 1 eye injected with Hostinert 245. In the other eyes, the first precipitates occurred 2 to 3 weeks after treatment. The amount of precipitate varied in some eyes, but did not show any dependence on the different perfluorochemicals injected.

Gross Microscopy and Histopathology

All globes dissected 1 month or longer after surgery showed white, flakelike precipitates. The opacities were stored inside membranelike structures surrounded by vitreous remnants, and could be found mainly in the lower periphery (Fig. 4). In some eyes, similar precipitates could be found lying in the residual cortical vitreous, which was now detached from the retina but had obviously been pressed against it by the perfluorochemical. The retina was attached in all eyes, even in those two eyes that had developed a rhegmatogenous retinal detachment during the first days after surgery. In four eyes, the lower retina that had been in permanent contact with the perfluorocarbon had a whitish appearance. These eyes had been filled with perfluorooctane (2 cases) or with Hostinert 245 (2 cases) for 2 months. No gross abnormalities could be detected in the other eyes.

Light microscope examination of semithin sections of the vitreous smear revealed the precipitates as being amorphous material without any cells. Light microscope examination of histologic sections of eyes that had been injected with perfluorooctane or Hostinert 130 for 8 hours occasionally showed some epiretinal macrophages and a slight vacuolization in the nerve fiber and ganglion cell layer. In some eyes, electron microscope examination revealed moth-eaten defects of the outer photoreceptor segments. These changes could be found in the upper and lower retina, and were also observed in the control eye. No further abnormalities could be found.

Apparently, the upper retina maintained its normal appearance even as long as 1 month after surgery. However, close examination of the Müller cells, which were identified by their localization, shape, and cytoplasmic structure, revealed the presence of low, scattered protrusions into the photoreceptor layer, inducing short discontinuities of the outer limiting membrane (Fig. 5). Similar changes were seen 2 months after surgery. In addition,

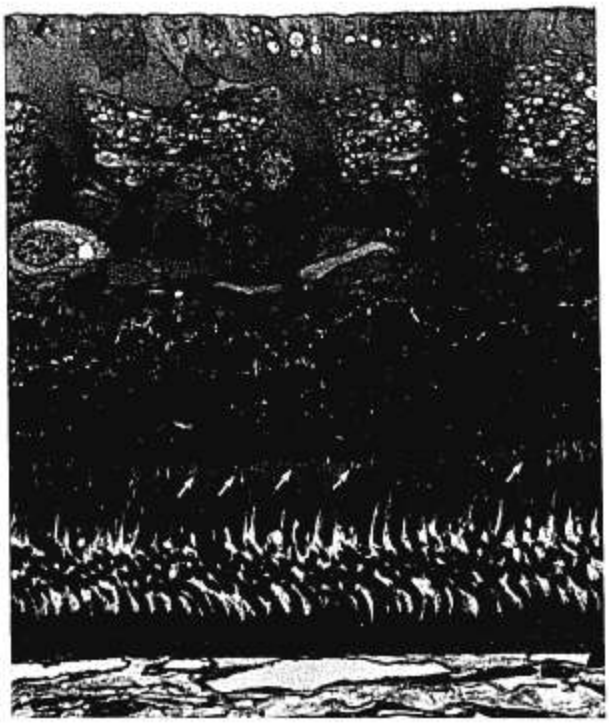

Fig. 5. Upper retina 1 month after injection of perfluorooctane. The ganglion cell layer and the inner plexiform layer present a slight vacuolization, but this was already seen in the retina 8 hours after perfluorooctane injection and in the control eye. The Müller cells show low protrusions (arrows) into the photoreceptor layer. (Original magnification ×810.)

these eyes showed irregularities and a slight rarefication of the outer photoreceptor segments. Macrophages did not occur in the interphotoreceptor space of the upper retina. The eyes that had developed retinal detachment showed some disorganization of the photoreceptors and proliferation of the retinal pigment epithelium (RPE).

In contrast, the lower retina, which had been in permanent contact with the perfluorocarbons, demonstrated remarkable morphologic changes that increased with the duration of the tamponade (Table 3). Light microscope examination 6 days after surgery revealed bumplike protrusions of the Müller cell cytoplasm through the outer limiting membrane (OLM) into the interphotoreceptor space, as well as vacuolization of the outer plexiform layer (OPL) and of the outer segments of some photoreceptors. Frequently, isolated cells with large nuclei and blue-stained cytoplasmic granules, probably macrophages, were present above the pigment epithelium (Fig. 6). Electron microscope examination showed that the granules contained remnants of engulfed outer segments of the photoreceptors (Fig. 7). These changes were the same in all eyes, regardless of the type of liquid injected. The ultrastructure of some of the photoreceptor outer segments showed defects, such as the moth-eaten aspect. In correspondence of the macrophages, the outer seg-

Table 3. Major Morphological Findings of the Lower Retina of Perfluorooctane and Perfluoropolyether-injected Eyes

6 days	1 mo.	2 mos.
hypertrophy of MC	increasing hypertrophy of MC	increasing hypertrophy of MC
bumplike MC-protrusions	larger droplike protrusions of MC	very large droplike protrusions of MC
regular straight arrangement of retina	localized foldings of outer retinal layers except RPE	generalized folding of outer retinal layers except RPE
macrophages in IPS	macrophages in IPS	macrophages in IPS
irregularities of OS	loss of OS	loss of IS and OS
regular arrangement of retinal layers	rarefication of nuclei in nuclear layers	increasing rarefication of nuclei in nuclear layers
	drop down of photoreceptor nuclei	drop down of photoreceptor nuclei
regular RPE cells	beginning hypertrophy of RPE with drusen towards MC-protrusions	increasing hypertrophy of RPE with drusen towards MC-protrusions
no unusual material deposits in IPS	occasional vesicles in IPS	varying amounts of vesicles in IPS

MC, Müller cells; RPE, retinal pigment epithelium; IPS, interphotoreceptor space; OS, outer segments of photoreceptor(s); IS, inner segments of photoreceptor(s).

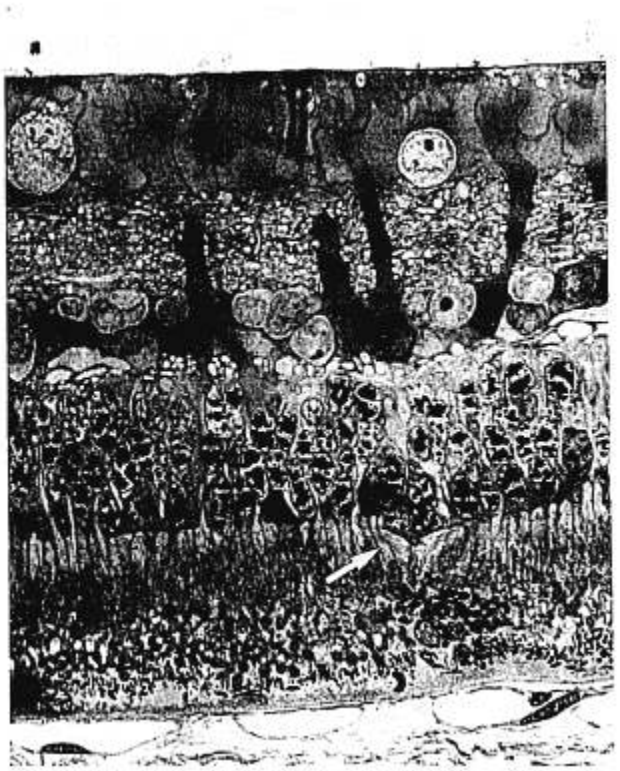

Fig. 6. Lower retina 6 days after injection of perfluorooctane. The external limiting membrane is crossed by a bumplike structure (arrow) extending between the inner segments of the photoreceptors. Some irregularities of the inner segments of the photoreceptors can be seen. A few cells with large nuclei and roundish granules of different size, most probably macrophages, lie internal to the pigment epithelium. (Original magnification ×810.)

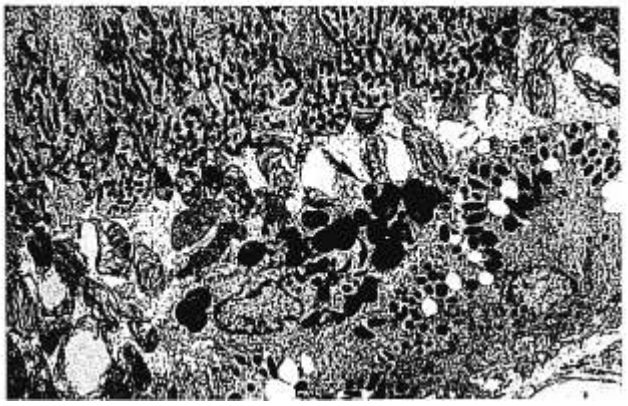

Fig. 7. Electron micrograph of the same eye as Figure 6. The cell in the interreceptor space (probably a macrophage) shows numerous lamellated bodies (possible remnants of engulfed outer segments of the photoreceptors) of different size (arrow). The outer segments of the adjacent photoreceptors are largely destroyed, but the inner segments and the pigment epithelium are well preserved. (Original magnification ×1500.)

ments of the photoreceptors were notably few, abnormally short, and deformed. In contrast, the inner segments of the photoreceptors and the pigment epithelium both showed well-preserved cytologic details, especially mitochondria (Fig. 7). The external limiting membrane of the lower retina was crossed by larger droplike cell protrusions, extending between the photoreceptor inner segments, 1 month after surgery (Figs. 8,9). When examined by electron microscopy, these droplike structures could be identified as a continuous part of hypertrophic Müller cells. These areas also showed a rarefication of nuclei in the nuclear layers. In some of these cells the mitochondria were swollen or destroyed, whereas the mitochondria of the photoreceptor inner segments were well preserved. The droplike structures were covered by short, scattered microvilli, which were probably remnants of the fiber baskets of the Müller cells (Fig. 10).

The protrusions of the Müller cells were frequently seen together with the moth-eaten appearance and shortening of the photoreceptors, the drop down of photoreceptor nuclei into the interphotoreceptor space, the presence of macrophages in the interphotoreceptor space, and large drusen in the RPE. These protrusions were also associated with large foldings of the external retinal layers (OPL, outer nuclear layer, OLM, and receptors) (Figs. 8,9,11–13).

In eyes injected with fluorochemicals for 2 months, light microscope examination of the lower retina occasionally revealed localized hypertrophy of the RPE in areas facing the folds of the outer retinal layers and Müller cell hypertrophy. These eyes showed a reduced thickness of the OPL as well as a rarefication of perikarya in the inner and outer nuclear layer in the affected areas. Both phenomena may only be relative, and due to the hypertrophy of the Müller cells (Figs. 11,13).

In other eyes or in other regions of the same eye, roundish, membrane-covered structures about the size of the drusen were seen arranged in one, two, or more layers along the border between pigment epithelium and photoreceptors (Fig. 12). Even with electron microscope examination, it was impossible to establish whether these structures were generated by the pigment epithelium or by the photoreceptors. Occasionally, similar roundish structures could also be found in eyes enucleated 1 month after surgery.

When examined by electron microscopy, the normal microvilli of the Müller cells (fiber baskets) that extend beyond the external limiting membrane were consider-

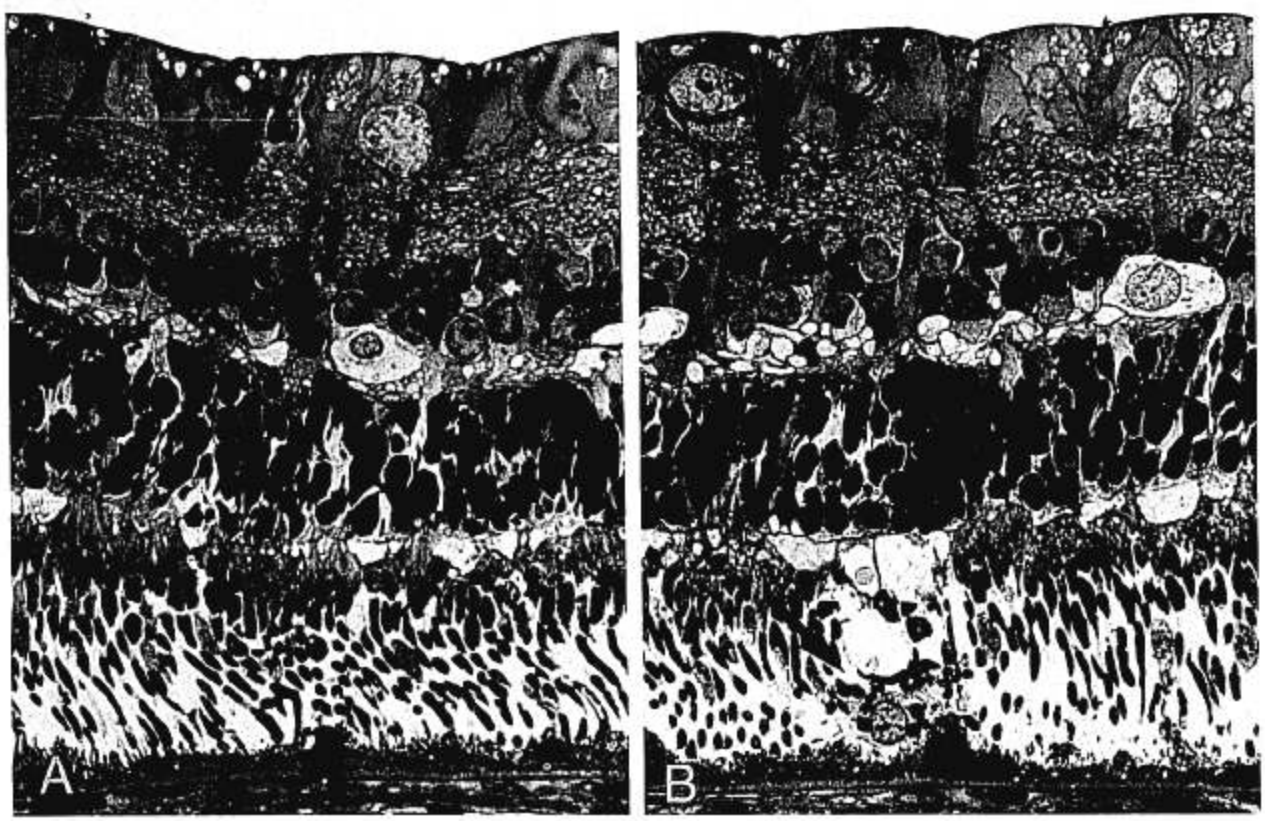

Fig. 8. The retina of an eye 1 month after injection of Hostinert 130. **A.** Larger droplike structures (arrows) are protruding through the external limiting membrane between the inner segments of the photoreceptors. They are associated with localized foldings of the external retinal layers. **B.** Cell with large granules (arrow) in the interphotoreceptor space close to the retinal pigment epithelium. The cell is located directly external to large droplike expansion outside the external limiting membrane; drusen in RPE. (Original magnification ×810.)

Fig. 9. Lower retina of an eye 1 month after injection of perfluorooctane. Arrow indicates drop down of a photoreceptor nucleus. In the same location and close to the right border of the figure (arrowhead), the external limiting membrane is interrupted and forms a slight fold towards the interreceptor space; drusen in RPE. (Original magnification ×810.)

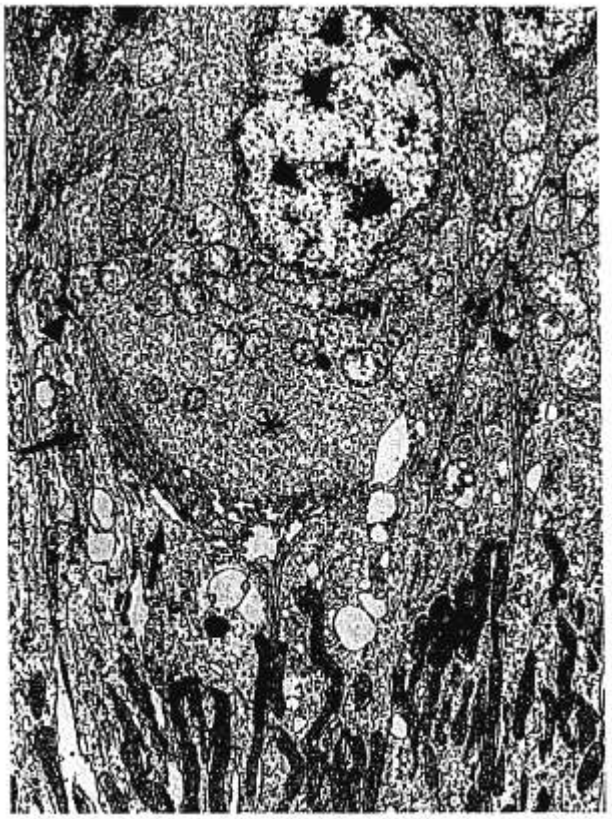

Fig. 10. Electron micrograph showing the outer limiting membrane (arrowheads) of the lower retina of an eye 1 month after injection of perfluoroctane. Cytoplasm of Müller cell (asterisk) containing a large number of altered mitochondria protrudes in a bumplike shape outside the outer limiting membrane. The mitochondria of photoreceptor inner segments are well preserved. The arrows indicate remnants of the basket fibers. (Original magnification ×4000.)

ably damaged or even lost in areas with droplike processes (Fig. 14), as had already been observed in retina 1 month after surgery. In comparison to eyes enucleated 1 month after surgery, an increased disorganization and vacuolization of the photoreceptor outer segments could be observed. The pathologic changes, including the Müller cell hypertrophy, did not show any dependence on the respective perfluorinated liquid injected. In all eyes, Müller cells of the upper retina that had not been in contact with the liquids did not show any hypertrophy.

Pathologic changes of the inner surface of the retina were confined to epiretinal macrophages (Fig. 13), which were numerous and occasionally formed large clusters (Fig. 12). There was no epiretinal proliferation of Müller cells or any breaks in the inner limiting membrane.

Discussion

The use of liquid perfluorocarbons in the treatment of complicated retinal detachment has attracted much interest among vitreoretinal surgeons. Due to the high specific gravity of approximately 1.8 g/cm^3 of these liquids, reattachment of the retina can easily be achieved without the need for a posterior retinotomy for internal drainage of subretinal fluid. A similar effect can be achieved using fluorosilicone oil,[11–13] but it's lower specific gravity of 1.29 g/cm^3 does not provide the same excellent stabilization of the retina that perfluorocarbons can provide. Furthermore, long-term intraocular tolerance to fluorosilicone oil is still controversial.[7,14–16] Perfluorocarbon liquids are used only as an intraoperative tool for reattachment of the retina. In most cases, they are in contact with the retina for no longer than 30 minutes. Long-term tolerance has to be studied, however, because of the possibility of intraocular droplets that remain after surgery.

Several authors have examined tolerance to different perfluorinated liquids in the rabbit eye. Miyamoto et al.[6] observed a gliosis on the optic disc and retina 1 month after injection of the perfluoropolyether Flombin H (Montedison, Milan, Italy). Using the highly purified polyether Freon E15 (Du Pont, Wilmington, DE), the same authors found an intercellular edema of the outer retina 1 to 3 months after injection, and a preretinal membrane formation 6 months after surgery.[7] Chang et

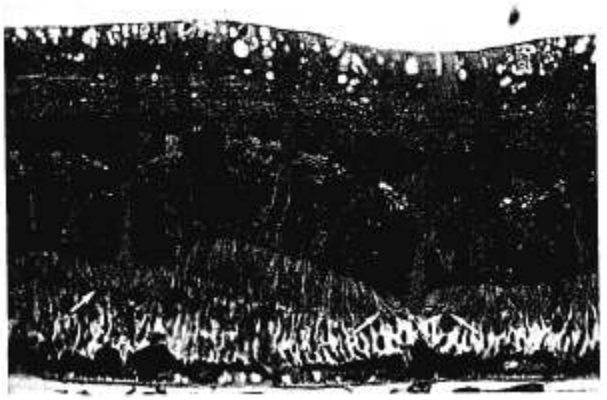

Fig. 11. Lower retina 2 months after injection of perfluorooctane. Extensive folds of the outer retinal layers with the outer limiting membrane toward the pigment epithelium in correspondence of two areas with Müller cell protrusions (arrows). Localized hypertrophy of the retinal pigment epithelium beneath the Müller cell hypertrophy. On the left, a cell (presumably a macrophage) is interposed between pigment epithelium and bumplike processes of the Müller cells; on the right pigment epithelial cells and bumplike processes are almost in contact. The RPE contains drusen. Note the thinning of the outer plexiform layer as well as the rarefication of cells in the nuclear layers. (Original magnification ×400.)

al.[10] reported moth-eaten defects of the photoreceptor outer segments as early as 2 days after injection of perfluorotributylamine. The same investigators found a preretinal gliosis and a decreased number of photoreceptor nuclei 3 to 4 months after surgery. In contrast, no pathologic retinal changes were seen 6 weeks after vitreous replacement with perfluorophenanthrene[17] or 30 days after intravitreous injection of the perfluoropolyether Afflunox (PCR, Gainesville, FL).[8]

The differing results of these studies may be due to the different chemical structures and physical properties of the perfluorochemicals examined. Other factors that may play an important role are the differences in purity and uniformity levels of the liquids. In addition, the results could be affected by differences in the volume injected into the vitreous cavity and by variation in the amount of remaining vitreous protecting the retina.

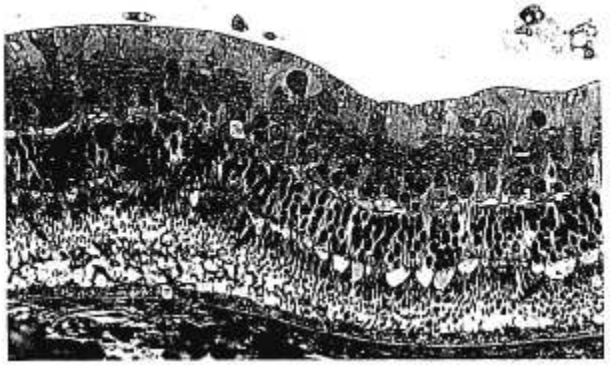

Fig. 12. Lower retina 2 months after injection of Hostinert 130 shows droplike processes between photoreceptor inner segments. Roundish membranebound structures occur in the subretinal space between the photoreceptor outer segments and close to the retinal pigment epithelium (RPE). Some of these roundish structures are also comparable to the drusen of the RPE. Note that the distribution of the roundish structures is very irregular, being numerous (arrows) and in several layers in some regions and absent in others. Numerous epiretinal macrophages are present. (Original magnification ×400.)

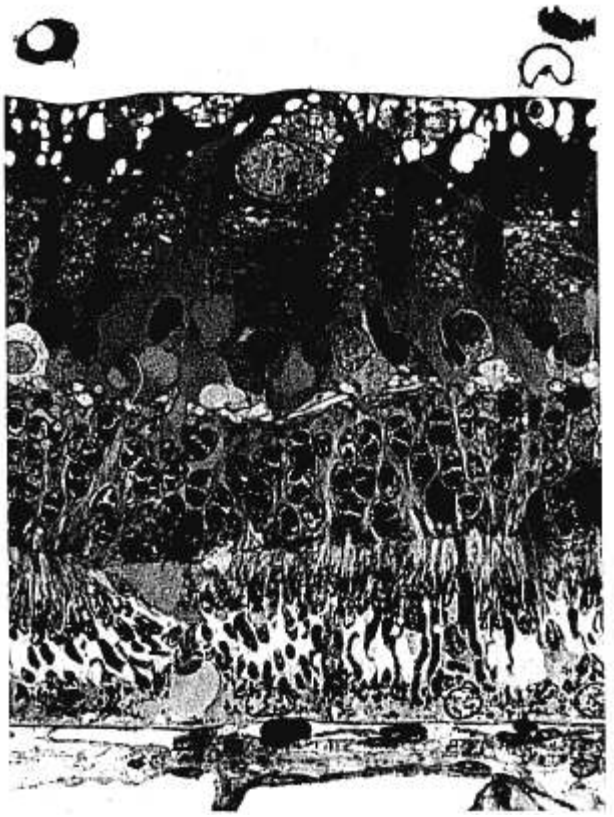

Fig. 13. Lower retina 2 months after injection of Hostinert 245 shows disorganization of the photoreceptor outer segments and a localized drusen formation facing a fold of the outer retinal layers with a droplike process. Epiretinal macrophages are present. (Original magnification ×810.)

In the present study, we have compared the intraocular tolerance of three perfluorinated liquids. While perfluorooctane has been used clinically for some years,[2–4] the polyether liquids Hostinert 130 and Hostinert 245 were examined as vitreous substitutes for the first time in this study. The main differences in the physical properties of these three perfluorinated liquids were in vapor pressure and viscosity. Perfluorooctane had the lowest viscosity (0.8 mpa · s) and showed the highest degree of droplet formation during the first 4 weeks after injection. Surprisingly, although Hostinert 130 had a lower level of viscosity (2.1 mpa · s) than Hostinert 245 (16.9 mpa · s), it demonstrated the smallest degree of droplet division.

In both perfluorooctane- and perfluoropolyether-filled eyes, the most prominent finding during slit-lamp microscope examination was the appearance of white, flakelike precipitates, which occurred in the vitreous cavity in structures of the residual vitreous and in the outermost vitreous cortex that remained attached to the retina. Similar precipitates have already been observed by other investigators after injection of perfluorooctane

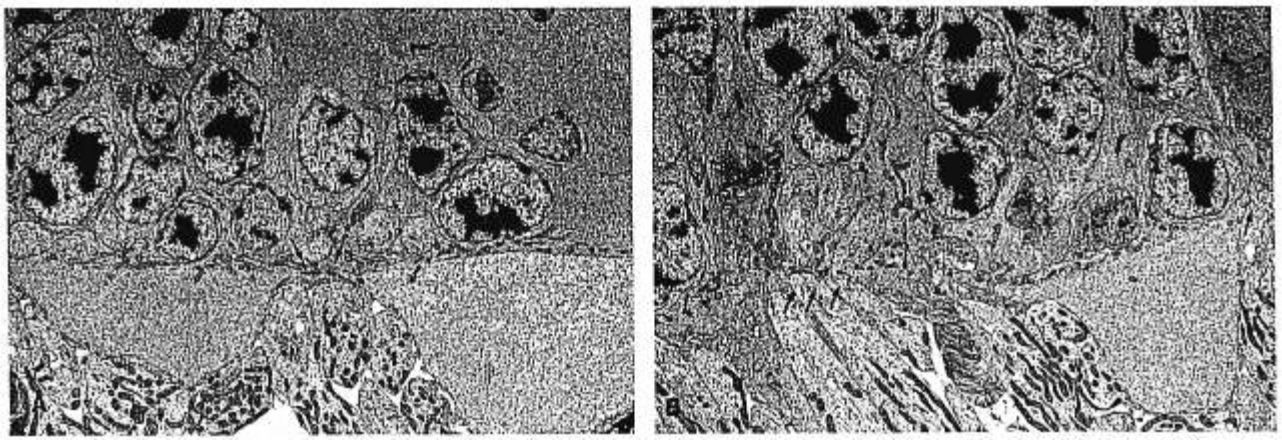

Fig. 14. Electron micrographs showing Müller cell protrusions of different size (bumplike and droplike) 2 months after injection of perfluorooctane (A) and Hostinert 245 (B). The arrows indicate the outer limiting membrane. The cytoplasm filling the bumplike processes has a composition morphologically different from that of the Müller cells inside the outer limiting membrane. (Original magnification ×5300.)

(Chang S, personal communication, 1990) and other kinds of perfluoropolyethers.[6,7] Our preliminary results show that these precipitates are not composed of aggregated inflammatory cells, but consist of amorphous material, possibly precipitates of vitreous proteins that may result from mechanical forces of the perfluorochemicals. In an experimental study (unpublished data), we observed similar precipitates *in vitro* when pig vitreous mixed with either type of the perfluorinated liquids was placed in a rotary shaker for 6 to 12 hours. Further analysis of these precipitates continues in our laboratory.

Regarding retinal changes, there was no difference in tolerance to the three perfluorinated liquids. Short-term use of these liquids, in which the retina was in contact with the liquid for 8 hours, did not cause significant retinopathy. Longer-term exposure of 6 days to 2 months, however, resulted in increasing damage to the lower retina. The most prominent histopathologic finding was the presence of Müller cell hypertrophy with bumplike cytoplasm protrusions extending across the external limiting membrane into the interphotoreceptor space. This reaction of the Müller cells has not yet been reported either after injection of perfluorinated liquids or after injection of other vitreous substitutes, such as silicone oil. A similar Müller cell hypertrophy has only been known to occur following retinal detachment[18] or cryopexy,[19,20] but in such cases the shape of the hypertrophic processes extending into the subretinal space is more irregular than those observed in this study.

Morphologic alterations of the lower retina after 6 days of exposure to perfluorocarbons consist of a marked hypertrophy of the Müller cells, which started to show bumplike protrusions extending into the interphotoreceptor space. Macrophages with remnants of photoreceptor outer segments were also observed in this location. A distinct folding of the external limiting membrane and the adjacent outer nuclear layer and increasing disarrangement in the interphotoreceptor space occurred 1 month after surgery. Loss of photoreceptor nuclei and receptor segments and early hypertrophy of the RPE with drusen were also seen. Hypertrophic cells of the RPE with increased occurrence of drusen arising toward extensive Müller cell protrusions, and a subsequent narrowing the interreceptor space, were seen 2 months after surgery (Fig. 13). Interposed macrophages were often seen in the remaining space (Fig. 11). Furthermore, there were large deposits of vesicular-covered material lying on the pigment epithelium, which showed an electron density similar to the Müller cell protrusions and the drusen.

This hypothetical course might indicate that the injected perfluorocarbons, which are lying on the internal limiting membrane, indeed cross the whole retina, involving the Müller cells and causing them to protrude into the interphotoreceptor space. The perfluorocarbons could then initiate the other changes described here of the more external parts of the retina as they pass further and are finally stored in the RPE drusen. In addition, some alterations of the outer retina could also be induced by changes of the Müller cells. On the other hand, the early and strong macrophage migration into the interreceptor space 6 days after surgery implies that some material may already have reached this area. The macrophages are absent from the upper retina and the control eye. In the later stages of treatment (2 months after injection) the macrophagic reaction remains the same, with more pronounced morphologic alterations of the outer retina.

These aspects suggest that the alterations have a toxic nature. A toxic effect seems unlikely, however, since one would expect that, due to the vapor pressure of the liquids, toxic damage would occur to the same degree in the upper retina, but retinal lesions were mainly confined to the lower retina in this study.

It remains unclear whether the Müller cell hypertrophy and the other retinal lesions, such as loss of photorecep-

tors and cells in the nuclear layers and thinning of the outer plexiform layer, can also be attributed to mechanical damage. A pure mechanical lesion might very well be expected, because of the high gravity of the liquids and the location of the alterations in the lower retina. However, similar changes did not occur after injection of a considerably larger volume (up to 1.5 ml), despite the higher pressure on the lower retina.[8,10,17]

Some of the Müller cells of the specimens examined 1 month after surgery showed swollen, destroyed mitochondria, while the mitochondria in the surrounding inner segments of the photoreceptors remained normal. Therefore, a relationship between fixation and Müller cell alteration could not be clearly established. Protrusions of the Müller cells are usually devoid of organelles. All these are retained inside the Müller cells by a discontinuous border that is formed by electron-dense material and occasionally aligned in the plane of the outer limiting membrane. This borderline is indicated by the light staining of the droplike protrusions in light microscope examination (Fig. 11) and by the rather homogeneous aspect in electron microscope examination (Fig. 14). The mitochondria of the Müller cells were well preserved if the borderline was present (Fig. 14), while they were altered if the border line was absent (Fig. 10). This observation suggests that the mitochondrial damage in some Müller cells could be associated with other, as yet unidentified (general) alterations, and does not depend on the quality of the fixation.

The moth-eaten aspect, however, that was observed in some of the photoreceptor outer segments could be fixation-dependent, since this phenomenon was also noted in the control eye. In contrast, the dropdown of photoreceptor nuclei seen here (Fig. 9) seems to be a basic, and possibly pathologic, reaction of the retina in various circumstances and is reported in vertebrates by other investigators.[21,22]

The results of this comparative experimental study demonstrate that perfluorooctane and the perfluoropolyether liquids Hostinert 130 and Hostinert 245 appear to be tolerated equally well upon intraocular injection. Perfluorooctane and Hostinert 130 were well tolerated when they were in contact with the retina for 8 hours, but all three liquids produced the same considerable retinal lesions when injected as a long-term vitreous replacement. It remains unclear whether the liquids have a similar effect on the human retina.

Key words: complicated retinal detachment, Müller cells, liquid perfluorocarbons, photoreceptors, vitrectomy, vitreous replacement.

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