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# Experimental Tractional Retinal Detachment in Rabbits

## Clinical Picture and Histopathologic Features

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**Abstract.** A simple reproducible model for the creation of tractional retinal detachments in rabbits is presented. This model comprises retinal membranes composed of different cell types derived from ocular tissues, and eliminates the need for lengthy and expensive tissue culturing procedures. The injected cell bolus rapidly migrates to the optic disc and forms surface retinal membranes. These lead to tractional retinal detachment confined to the area of the vascular medullary rays. The rapidity and severity of the clinical process seems to be directly dependent upon the number of cells injected. Good visibility of the posterior pole is maintained throughout the course of the development of detachment.

This model is acceptable for testing agents to suppress intraocular cellular proliferation. It can also serve to teach surgical techniques for the management of vitreo-retinal membranes without endangering human eyes.

**Zusammenfassung.** Es wird ein einfaches, reproduzierbares Modell zur Erzeugung einer Traktionsamotio bei Kaninchen vorgestellt. Dabei werden epiretinale Membranen, die von unterschiedlichen okulären Zelltypen abstammen, gebildet und so die Notwendigkeit langdauernder und kostspieliger Gewebekulturen eliminiert. Der injizierte Zellbolus wandert schnell in Richtung Papille, bildet Membranen auf der Netzhautoberfläche, und führt dann zum Auftreten einer Traktionsamotio, die auf das Gebiet der vascularisierten Markstrahlen beschränkt ist. Die Schnelligkeit und Schwere des klinischen Verlaufes scheint direkt proportional zur Zahl der injizierten Zellen zu sein. Während der gesamten Entwicklung der Traktionsamotio bleibt der Einblick auf den hinteren Augenpol ungestört.

Dieses Modell eignet sich zur Testung von Mitteln zur Unterdrückung intraokularer Zellproliferation. Außerdem bietet es die Möglichkeit, chirur-

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gische Techniken zur Behandlung vitreo-retinaler Membranen zu lernen, ohne dabei menschliche Augen zu gefährden.

## Introduction

In the past, vitreo-retinal membranes and tractional bands have been frequent causes of inoperable retinal detachments. The advent of vitrectomy and techniques for surgical management of such membranes offered hope of visual restoration. However, many eyes which achieve anatomical surgical success may redetach due to the stimulation of remaining membrane tissue or the formation of new membranes. In an effort to reduce the redetachment rate, Tano et al. (1980) have suggested that, with current vitrectomy techniques, intraocular fibroblastic proliferation may be suppressed by the injection of intravitreal steroids.

This report describes a method for production of a simple reproducible model to create cellular retinal surface membranes with secondary tractional detachment. In addition, its clinical appearance and histopathologic features are presented. The model may serve to test agents to be used to suppress membrane formation, and eliminates the need for lengthy and expensive tissue culturing procedures. This is important when considering the great number of animals required for assessing the effectiveness of pharmacologic agents prior to use in human eyes. The model allows a clear view of the posterior pole throughout the course of development of the tractional retinal detachment. Therefore, evaluation of the effect of pharmacologic agents at any stage of the development of retinal detachment is possible. Furthermore, this model could be used to teach surgical techniques of handling vitreo-retinal membranes without risking human eyes.

## Materials and Methods

Eyes from 40 pigmented Dutch rabbits weighing 1.5 kg were studied. 24 eyes were utilized to duplicate existing models. 8 eyes were used to test our model and 8 eyes served as controls. Further description of the materials and methods, as well as the results, will be confined to comments concerning the 8 test and 8 control eyes.

In an effort not to blind the test animals, cells for injection into the mid-vitreous were taken from a donor animal. The donor animal was sacrificed by intravenous injection of a lethal dose of Pentobarbital. The eyes were enucleated and the anterior segment, including iris and lens were removed under sterile conditions. The vitreous and retina were teased from the eye cup, which was then irrigated with a $^1/_4$% Trypsin solution. The cells liberated in this way were harvested and counted. The cell bolus was suspended in a total volume of 0.1 cc of Hamm's F10 medium. In all cases, the cell bolus was composed of about 90% retinal pigment epithelial cells and 10% intraocular fibroblasts, as determined by light microscopic counting.

While the donor cells were being prepared for injection, the eight recipient animals were sedated with Thorazine and then given intraveneous Pentobarbital for anesthesia. The pupil was dilated with Cyclomydril drops and examined for pre-existing retinal disease. If retinal disease was present, the animal was eliminated from the study. With the aid of the operating microscope the conjunctiva was reflected. 0.1 cc of aqueous was removed from the anterior chamber through

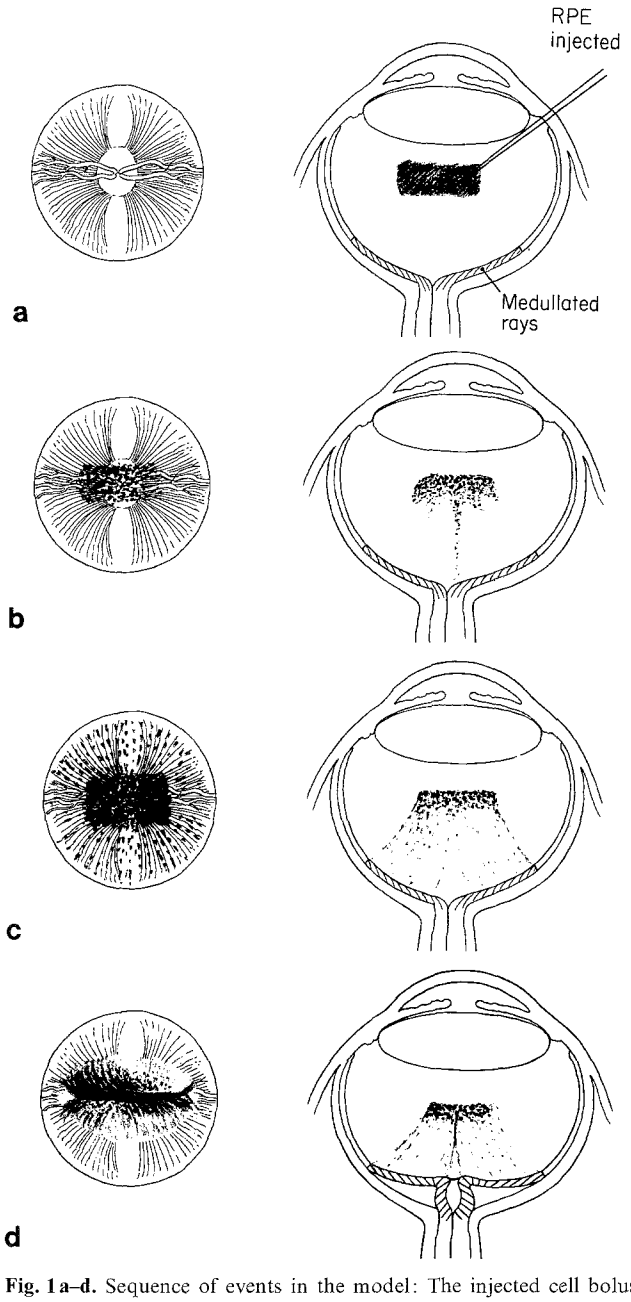


**Fig. 1 a–d.** Sequence of events in the model: The injected cell bolus (**a**) migrates toward the optic disc (**b**), spreads on the retinal surface in the area of the medullary rays (**c**) and pulls off the retina (**d**)

a paracentesis incision using a 25 gauge needle. The cell bolus from the donor animal was then injected through the pars plana avoiding the large rabbit lens. The bolus could easily be seen in the mid-vitreous cavity with both the indirect ophthalmoscope and the slit lamp biomicroscope. Cells harvested from one eye were injected into the remaining eye. The animals were examined daily for 4 days and then weekly until sacrificed. All animals were sacrificed by 12 weeks. Criteria for elimination from the study were bleeding at the time of injection, signs of infection and/or obscurred funduscopic observation.

Control eyes were injected with 0.1 cc of Hamm's F10 medium (3 eyes), 0.1 cc of $^1/_4$% Trypsin solution (3 eyes), or the needle alone was introduced through the pars plana (2 eyes).

For microscopic evaluation the eyes were fixed in 10% formalin or glutaraldehyde 2+2%. The eyes were opened horizontally immediately after enucleation and fixitive was allowed to enter the eye to achieve rapid fixation of the ocular tissue. The eyes were examined with a disecting microscope according to the method of Roth and Foos (1973). Tissue was embedded in Parafin for light microscopy and stained with hematoxylin and eosin as well as Masson Trichrome stains. Tissue for electronmicroscopy was fixed in glutaraldehyde and stained with osmium stain.

## Clinical Picture

Following injection, three stages finally leading to retinal detachment could be observed: (1) migration of cells to the optic disc, (2) development of surface retinal membranes and (3) development of tractional retinal detachment.

After injection of the cellular bolus (Fig. 1 a), all animals showed a rapid migration of cells to the area of the optic disc (Fig. 1 b). The larger the number of cells injected, the more rapid the migration to the optic disc was (Table 1).

Surface retinal membranes developed in all test eyes by one to three weeks. These membranes were confined to the area of the medullary rays (Fig. 1 c). The membranes became less pigmented with time. The larger the number of cells injected, the more rapidly these membranes developed and, clinically, the more dense the membrane appeared (Table 1).

Tractional retinal detachments developed in all test eyes between four to eight weeks (Fig. 1 d). The area of detachment selectively involved the area

**Table 1**

| Animal | Cells injected (× 1,000) | Migration to optic disc (h) | Surface retinal membrane (wks) | Tractional retinal detachment (wks) | Height of detachment |
|---|---|---|---|---|---|
| 1 | 320 | 2 | 1 | 4 | +++ |
| 2 | 200 | 2 | 1 | 4 | ++ |
| 3 | 300 | 2 | Cataract | | +++[b] |
| 4 | 100 | 24 | 2 | 7 | + |
| 5 | 250 | 2 | 1 | 4 | +++ |
| 6 | 300 | 2 | Blepharitis | | +++[b] |
| 7 | 100[a] | 48 | 3 | 8 | + |
| 8 | 300 | | Anesthetic death | | |

[a] Autologous cells

[b] Observed at autopsy

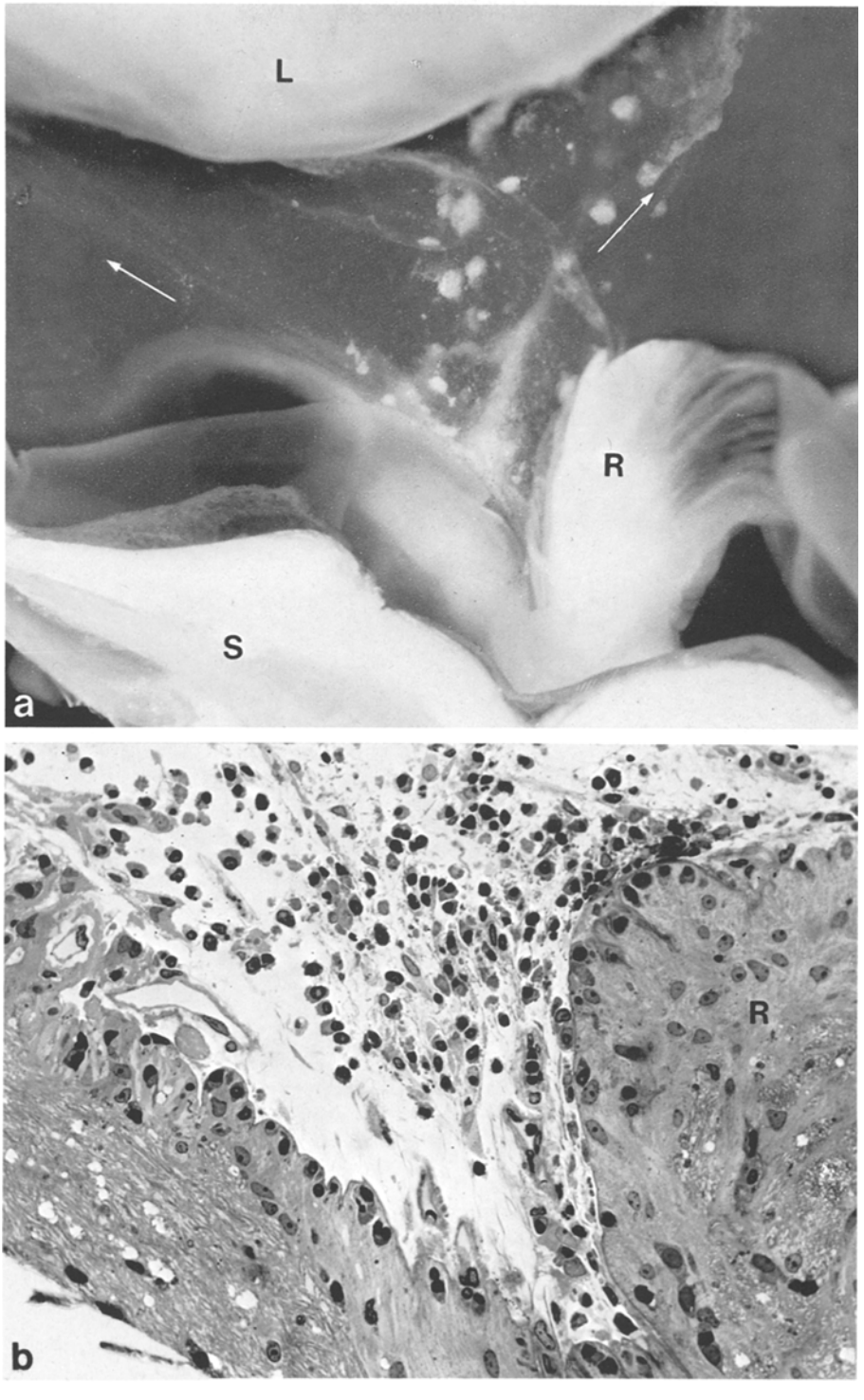


**Fig. 2.** Retinal detachment caused by vitreous traction. **a** Macroscopy. *L*, lens; *R*, retina; *S*, sclera; arrows: tractional bands. **b** Light microscopy. Tractional fold of the retina with epiretinal accumulation of different cell types. *R*, retina. ×450

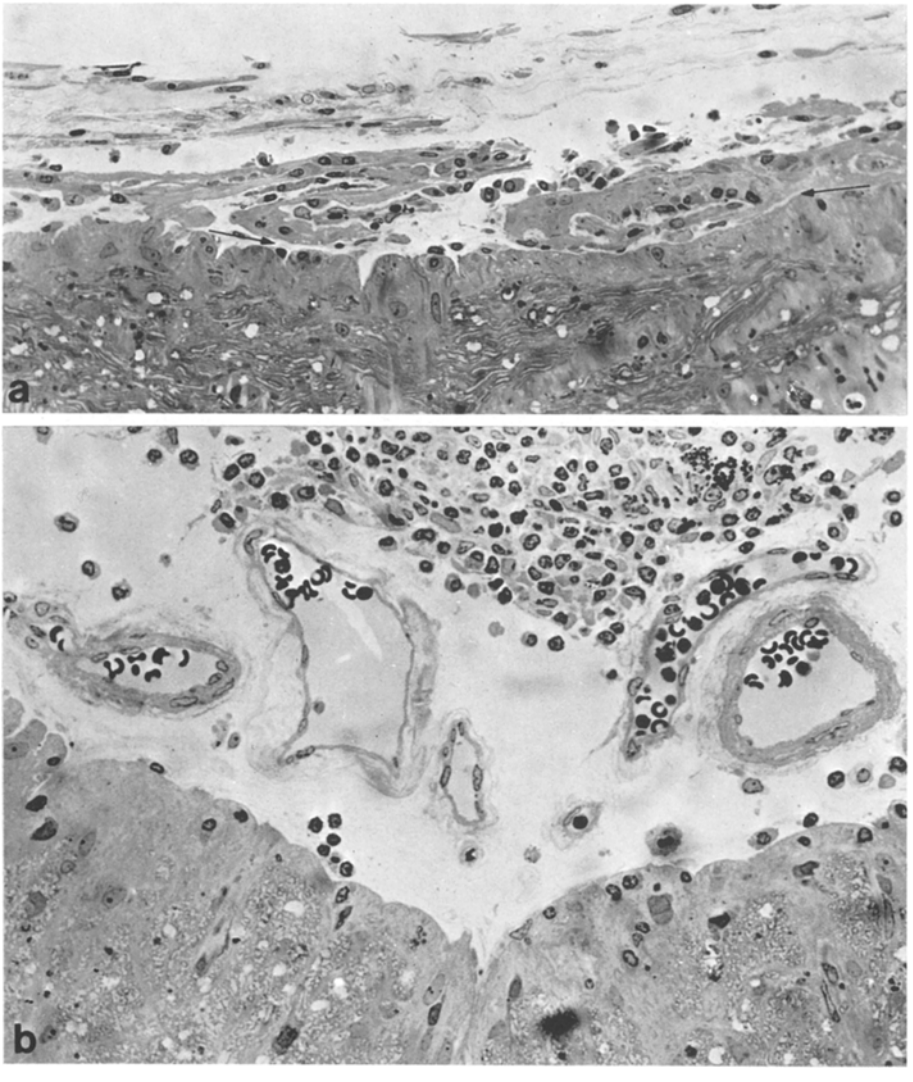


**Fig. 3a and b.** Light microscopy. Epiretinal membranes. **a** The retinal surface (arrows) is covered with a cellular membrane composed mainly of fibroblast-shaped cells. ×200. **b** In the area of epiretinal vessels the membrane has a loose matrix with interspersed collagenous fibrils. The cellular components are clustered near the vessels. ×380

of the medullary rays. Onset of retinal detachment was defined as appreciable elevation of the neurosensory retina upon ophthalmoscopic examination. To determine severity, a grading system of 1+, 2+ and 3+ was devised, a 3+ being the highest detachment and a 1+ being the shallowest detachment. Wrinkling of the retina alone was not considered detachment. In eyes injected with a larger number of cells, the vitreo-retinal bands appeared to be denser and the detachment was higher (Table 1).

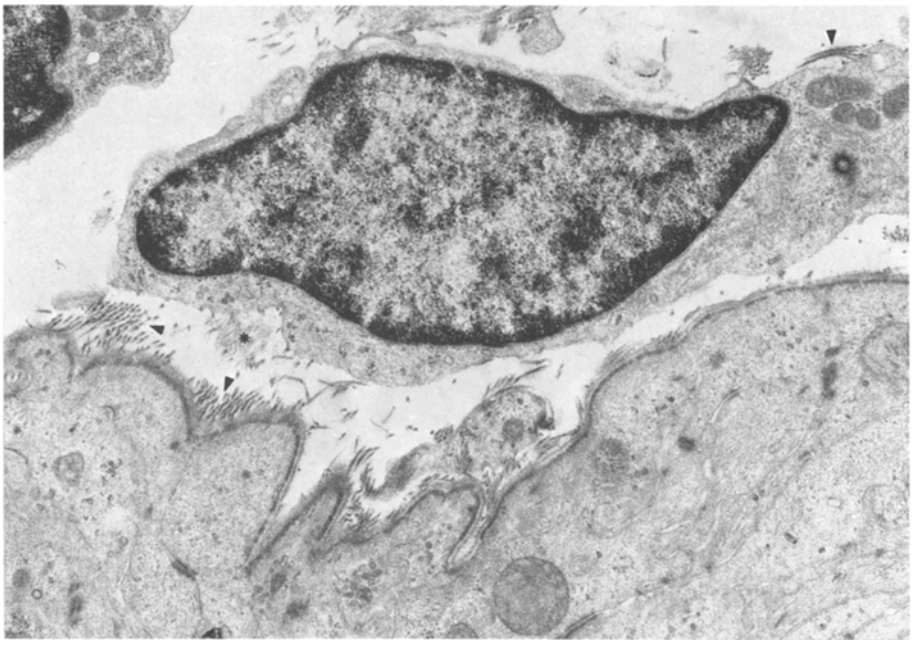

**Fig. 4.** Electron microscopy. Epiretinal fibroblast surrounded by collagen fibrils of both the vitreous (*) and the non-vitreous (arrows) variety. ×8,700

The anterior segment in all eyes remained quiet throughout the course. One eye developed a cataract secondary to lens injury during injection of cells. One animal developed blepharitis, limiting visibility of the development of the detachment. With these two exceptions, the clinical course could easily be followed by indirect ophthalmoscopy. No eye showed remarkable intraocular inflammation. One animal died secondary to anesthesia at the time of procedure.

All of the model eyes, but none of the control eyes developed tractional detachment. The eyes injected with autologous cells behaved similar to the eyes injected with homologous cells.

## Pathologic Features

Macroscopic study 6 weeks following injection revealed an intact anterior segment with the bolus of injected cells and cell debris immediately behind but not touching the lens. When the eyes were cut, the vitreous body was noted to be formed. Clumps of pigmented cells, as well as non-pigmented vitreous strands were observed throughout the vitreous posterior to the cell bolus. A whitish retinal surface membrane containing areas of pigmentation could be seen confined to the areas of fixed folds of detached retina (Fig. 2a).

Light microscopy 6 weeks after injection revealed cellular surface retinal membranes composed of fibroblasts and round pigmented cells consistent with

retinal pigment epithelium or macrophages which have ingested pigment (Figs. 2b, 3a). A few chronic inflammatory cells, as well as a few plasma cells were seen clustered in the area of retinal vessels (Fig. 3b). Masson Trichrome staining revealed these cells to be suspended within a matrix of material which stained positively for collagen. Areas of avascular retina revealed cells which had migrated to the retinal surface yet had not developed into retinal surface membranes.

Electron microscopy was done to determine the character of the collagen matrix within which the cells were seen. Much of the collagen material observed was clearly thicker than vitreous collagen fibrils and showed a faint periodic cross striation of 640 A (Fig. 4). An effort was made to identify intracytoplasmic actin filaments consistent with those seen in myofibroblasts. As yet the intracellular filaments morphologically resemble, but have not been conclusively identified as actin.

## Discussion

Many models have been presented to study intraocular cellular proliferation (Cleary, Ryan 1979; Numata et al. 1975; Algvere, Kock 1976; Topping et al. 1979; Mueller-Jensen et al. 1975; Abrams 1980). These models can basically be divided into two groups. The first group is concerned with cellular proliferation following major ocular trauma (Cleary, Ryan 1979; Numata et al. 1975; Topping et al. 1979). This group generally develops major vitreous hemorrhage and later membrane formation and tractional retinal detachment. Direct clinical observation of the development of detachment is impossible due to the presence of blood in the vitreous. Moreover, vitreous hemorrhage in rabbits tends to liquify the vitreous, thus eliminating the scaffold for vitreo-retinal tractional bands.

The second group is concerned with proving that a specific cell type can result in retinal membrane formation and tractional retinal detachment. Injected skin fibroblasts and retinal pigment epithelium have been most extensively studied (Algvere, Kock 1976; Mueller-Jensen et al. 1975; Abrams 1980). All of these models have utilized tissue culturing techniques. This has two distinct disadvantages. First, it is time consuming and expensive. It can easily take four to six weeks to generate enough cells in tissue culture for injection. Second, retinal pigment epithelial cells in tissue culture can undergo fibroblastic transformation, or bacterial contamination can render the cells unusable. Our model eliminates the need for tissue culturing and yields a bolus consisting of various ocular cell types for injection. It is well documented that human epiretinal membranes are composed of multiple cell types (Machemer 1978; Constable et al. 1974; Machemer, Laqua 1975). Retinal pigment epithelial cells, pigment laden macrophages, glial cells, fibroblasts, and myofibroblasts have been implicated. The myofibroblasts are fibroblastic cells with intracytoplasmic actin filaments (Gabbiani et al. 1972; Peacock, Van Winkle 1970). All of these different cell types may appear in a collagenous matrix. The matrix may be collagen produced by the cells of the membrane itself, or vitreous collagen incorporated

into the membrane. Presumably myofibroblasts and possibly other cells can undergo contraction. This leads to shortening of the retinal surface membrane, resulting in surface folding and subsequent tractional detachment. The collagen matrix is thought to contribute to the fixation of the retinal folds. It has been previously proposed that the collagen itself may contribute to the contraction of the surface membranes. Since only denatured collagen seems to be capable of contracting, this explanation appears unlikely (Peacock 1970).

In our model the membrane is composed of pigmented cells (retinal pigment epithelium, pigment laden macrophages) fibroblasts and a collagenous matrix. This membrane simulates the membranes described in human disease. The vitreous in this model remains formed and therefore maintains the scaffolding for growth of anterior-posterior vitreo-retinal bands. The restriction of membranes to the area of vascularized medullary rays suggests that a growth environment is supplied by these vessels.

Mueller-Jensen (1975) and Mandelcorn and Machemer (1976) in two separate experiments injected pure autologous retinal pigment epithelial cells from tissue culture into animal eyes. These animals did develop retinal surface membranes, but not tractional detachment. Recently, other investigators who injected 250,000 cells found the tractional retinal detachment to develop in a pattern similar to ours (Abrams 1980). In the earlier studies by Mueller-Jensen et al., the number of cells injected was not published, but certainly less than 100,000. Based upon the early work, showing membrane formation without development of retinal tractional detachment and our work, showing increasing severity of detachment with increasing numbers of cells injected, it may be speculated that the tendency to membrane formation in the rabbit model is dose dependent. In other words, the larger the number of cells in contact with the retinal surface, the more marked the membrane formation and resultant tractional retinal detachment seems to be. This concept of dose dependence is consistent with the observation of Tano et al. (1980) who injected skin fibroblasts.

Autologous cells seem to behave in a pattern similar to homologous cells, as indicated by our observations and those of other investigators such as Abrams (1980), who have used solely autologous cells.

In conclusion, we have described a simple reproducible model for the production of tractional retinal detachment in rabbits. This model utilizes ocular cells to form cellular epiretinal membranes and eliminates the need for lengthy and expensive tissue culturing. We suggest that this model be employed to test agents to be used to suppress intraocular cellular proliferation and to teach retino-vitreal surgical techniques without risking human eyes.

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