Mitotic activity in the cortical vitreous cells (hyalocytes) after photocoagulation

Balder P. Gloor

After photocoagulation of the rabbit retina wound healing occurs with a marked increase in mitoses not only in choroid, pigment epithelium, and retina, where mainly Müller cells are proliferating, but also in the cortical vitreous cells, the hyalocytes. The rate of mitosis in the hyalocytes was investigated by administration of systemic colchicine. The rate of mitosis is highest around the third to the fifth day post photocoagulation. In localized areas near the region of photocoagulation and in more diffuse areas in the zonular region corresponding to the equatorial band of coagulation the rate of mitosis reaches values of up to 100 mitoses per 1,000 cells, when colchicine is in effect for four hours. This is at least five or more times higher than in control eyes. The rate of mitosis is high enough to explain, in large part, the increase in the cell population in the vitreous. The possible relationship between proliferation of hyalocytes after photocoagulation and epiretinal fibroplasia and massive preretinal retraction is discussed.

Key words: Cortical vitreous cells (hyalocytes), complications following photocoagulation, epiretinal fibroplasia, massive preretinal retraction, cytology, histology, photocoagulation, wound healing, mitosis, hyalocytes, choroid, retinal pigment epithelium, retinal Mueller cell, ciliary body epithelium, colchicine, pharmacodynamics, histopathology, time factors, rabbits.

From the Department of Ophthalmology, Washington University School of Medicine, St. Louis, Mo. 63110.

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Reprint requests to: Dr. Gloor, Universitäts-Augenklinik, Bern, Switzerland.

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The source of the cortical vitreous cells, or hyalocytes, after embryological development is unknown. It was assumed that after the embryological stage the hyalocyte population is replaced by cells penetrating from the ciliary body into the vitreous.1-3 Gärtner4 has suggested that the vitreous border cells are involved in proliferative processes. Previous observations of vitreous whole mount flat preparations showed an increased number of cells in the cortical vitreous after photocoagulation.5-6 The increase took place locally around the area of photocoagulation as
well as in the neighboring region of the zonula overlying the ciliary body and at the pars plana. A large number of these cells which not only appeared to be hyalocytes, but also demonstrated mitotic activity, were observed in these preparations. Thus it appears that the hyalocyte is able to multiply. In this study, the question of the multiplication of hyalocytes after photoocoagulation was further investigated in the rabbit by using colchicine. The initial experiments served to determine the time interval after photoocoagulation in which the highest rate of mitosis could be expected. In a second series, the relationship between the colchicine effect, the rate of mitosis, and time were investigated in more detail.

Materials and methods
In the initial experiments, 28 pigmented rabbits of a mixed breed, weighing from 1.2 to 2.4 kilograms, were used. The animals were fed a Purina laboratory chow diet. The pupil was dilated with Neosynephrine 10 per cent and Cyclogyl. Photoocoagulation was performed with the Zeiss-Xenon arc photoocoagulator. Two to three rows of lesions containing 100 marks, all together, were placed in the lower half of the globe, covering a field of 120° to 150° equatorially. The intensity was chosen in order to get a gray to white mark in an exposure time of approximately 0.2" (green 1, intensity diaphragm 2, focus diaphragm 3° to 4.5°). The coagulation was performed under general anesthesia (Dialural, 20 to 30 mg. per kilogram of body weight). At 8:00 A.M., six hours before death, 0.1 mg. of colchicine per 100 Gm. of body weight (10 mg. of colchicine in 1 c.c. of saline) was injected subcutaneously. The first rabbits were killed by air injection into the heart 6 hours post coagulation, then at daily intervals until the ninth day.

Whole mount flat preparations of the vitreous and sagittal sections of the eye were performed as in previous experiments. The vitreous flat preparations were fixed with ether-alcohol and stained with PAS-He. The meridional slices of the eyes with or without remaining vitreous were fixed in Heidenhain's SUSA for three hours, embedded in paraffin and stained with hematoxylin and eosin, Mallory, PAS-He, and May-Grünwald-Giemsa.

The second series consisted of three litters of pigmented rabbits: the first litter of 4 rabbits, two months old, weighed 1.1 to 1.4 kilograms; the second litter of 6 rabbits, 2½ months old, weighed 1.7 to 2.1 kilograms; the third litter of 4 rabbits, three months old, weighed 2.1 to 2.2 kg. Only one eye of each animal was photoocoagulated. Each animals received 85 lesions in a double equatorial row. The colchicine experiments were started at midnight, 83 hours after photoocoagulation, in litters 1 and 3, 107 hours after photoocoagulation in litter 2. All of the animals, except for the control animals, received 0.2 mg. of colchicine per 100 Gm. of body weight. The animals were killed at intervals of approximately 90 minutes. Vitreous whole mount flat preparations were made from both eyes, fixed with ether-alcohol and stained with PAS-He or hematoxylin and eosin.

The cells in the flat preparations were counted by means of an ocular grid, divided in 10 x 10 fields, covering a field of 0.25 mm.2 with 125 x magnification. Counts of mitoses were performed with the same grid with 312 x or 562 x magnification.

Results
Flat preparations. An increase in the number of cells around the area of photoocoagulation begins on the third day; in the corresponding zonular area it begins one day earlier. As summarized in Fig. 1, the cell proliferation is not restricted to the area of photoocoagulation itself, but it also takes place in the corresponding zonular area. In this area, it fades out toward the upper quadrants. There is a zone between the area of photoocoagulation and the zonular area which lacks cellular proliferation. Also the coagulated area itself is almost cell free around the third day. Fig. 1 shows that a presentation of the average increase of cells and cells in mitosis for the entire preparation is meaningless. Cell counts were performed at random in fields of 0.25 mm.2 over the entire surface of the preparation. Usually, 12 to 40 fields of 0.25 mm.2 were counted. The maximal values found in four neighboring fields of 0.25 mm.2 were selected for Figs. 2 and 3. Sometimes the content of 1 mm.2 was projected from counts in smaller areas. In determining the rate of mitotic activity in the zonular area, a minimum of 2,000 cells, usually 3,000 to 6,000 cells, were counted. In Fig. 2, maximal values of mitoses for one set
Fig. 1. The scheme shows the localization of the increase of cells in vitreous whole mount flat preparation. PC = area of photocoagulation (more or less cell free); Z = zonular area, partially covered by a belt of ciliary epithelium (CE). Cell-free belt in zonular area around the lens. The region of the zonula which shows an increase in number of cells corresponds to the area of photocoagulation, but is separated by a zone of nonproliferation. The marked rectangular areas provided the values for Figs. 2 and 3.

of 1,000 cells are represented. The values of the areas immediately adjacent to the photocoagulation lesion had to be computed from lower counts.

Fig. 3 demonstrates the increase in number of cells in the area of photocoagulation. The values of the control eyes range between 74 and 194 cells per square millimeter (mean 106 ± 40). The cell content is doubled to tripled four days post photocoagulation and tripled to sixfold higher after eight days. On the eighth day, a peak is reached of 984 cells per square millimeter in the area of highest cell content. Often, after the seventh day, small round cells with practically no cytoplasm can be present in between the hyalocytes. Their incidence is shown in Fig. 3.

The increase of cells in mitosis per 1,000 cells is impressive. Maximal values are shown on the fourth day with 70 cells in mitosis per thousand in one area (real count 14 mitoses per 200 cells, in a larger area of 777 cells 46 mitoses, corresponding to 60 cells per 1,000). In the corresponding area of the control eyes the rate of mitosis remains below one division per thousand cells.

Analogous to the events in the area of
photocoagulation, a marked increase in the number of cells is also present in the zonular region (Fig. 2). From an average value of 317 ± 87 cells in the untreated eyes, a rise to a maximal value of 1,474 cells per square millimeter was noted. The presence of arrested mitoses in metaphase (Figs. 4 and 5) reaches its maximum also in this region on the fourth day post photocoagulation (42 cells in mitosis per thousand cells). In control eyes, values from 0.5 to 16 mitoses per 1,000 cells can be observed. In the area of photocoagulation and in the zonular area other phases of mitosis such as ana- and telophases and small cells already divided in pairs close together are present (Fig. 5C).

Fig. 6 shows, in the series of the se-
Mitotic activity after photocoagulation

Sequentially killed animals, that a localized process is going on with a peak in the center of the most affected area. In Figs. 7 and 8 the rate of mitosis is plotted against time for the zonular area and the area of photocoagulation, respectively. Maximal values of mitotic activity in the peak area and the average number of mitotic figures for the entire zone of increased activity are plotted. In the untreated eye, a definite but less substantial rise is noted. The effects of colchicine are first seen 90 minutes after injection. The rate of mitotic activity increases to a maximum five hours post injection and then appears to remain constant.

Sagittal sections. The prominent feature during the healing process of the photocoagulation wound is the proliferation of local cells in the different layers of the globe. This phenomenon becomes apparent by arresting the mitosis with colchicine. Pronounced mitotic activity of the endothelium cells of the choroid vessels and of choroidal mesenchyme cells appears about 24 hours post photocoagulation. At about 48 hours post photocoagulation, mitoses of pigment epithelium cells and of Müller cells start. Müller cells mitoses are very frequent. The Müller cell is easily identifiable because its protoplasmic processes still reach from the internal to external limiting membrane. The mitotic figure may be seen in either the

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**Fig. 3.** Increase in number of cells and mitoses present near area of photocoagulation. The rate of mitosis in the control eyes is below 0.5 mitoses per 1,000 cells.
inner or the outer nuclear layer (Fig. 9). The mitotic activity in all layers reaches its peak on the third to fourth day after photocoagulation and, except in the pigment epithelium, disappears by the seventh day. The different cellular layers remain well preserved. No fusion between choroid and pigment epithelium cells is noted during the first nine days post coagulation. Bruch’s membrane remains preserved as an anatomical barrier.

In sagittal sections the cellular infiltration of the vitreous overlying the photocoagulation lesions is scant. A slight aug-

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**Fig. 4.** Proliferation of hyalocytes near area of photocoagulation. 5 cells are arrested in metaphase (arrows). Not all of these mitotic figures lie in the same focal plane and therefore cannot clearly be shown in the photograph. (Ether-alcohol-periodic acid-Schiff (PAS-He); x562.)

**Fig. 5.** A, Arrested metaphase of hyalocyte near area of photocoagulation, and (B) in zonular area. Note the zonular fibrils in B. C, Dividing hyalocyte in anaphase. (Ether-alcohol-PAS-He; x1,000.)
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Fig. 6. Distribution of number of cells and number of mitoses over different neighboring fields. The counts performed in the same animal are linked. Colchicine was injected 83 hours post photocoagulation, the mitoses were counted from one hour and 20 minutes to 6 hours and 40 minutes after the injection was made.

Starting on the third day, it is not unusual to find pigment granules within the hyalocytes. In a few cases a migration of hematogenous cells, e.g., polymorphonuclear leukocytes, into the necrotic area of the retina is observed only during the first three or four days post photocoagulation. The polymorphonuclear leukocytes do not seem to enter the vitreous.

As long as the major part of the inner layers of the retina remain intact after photocoagulation, the cellular processes taking place in the vitreous and in the
Mitoses per 1 000 cells

Fig. 7. Relationship between the rate of mitosis and the time elapsed after injection of colchicine in the zonular area. Two different litters are represented. Colchicine was applied 83 hours post photocoagulation. A definite increase in number of mitoses begins 1½ hours after the injection and does not last longer than five hours and 20 minutes after injection. The time related increase in number of mitoses is much lower in control eyes than in coagulated eyes.

Comments

Prior experiments demonstrated that the so-called hyalocyte was responsible for the proliferation of vitreous border cells becomes apparent on the third day. Frequent mitoses are seen in sagittal sections also (Fig. 10B). Proliferation is seen in the entire area corresponding to ciliary body, including the ciliary ridge, the ciliary processes, and pars plana, but not in the region of the iris processes. In contrast to the frequent mitoses seen in the hyalocytes, mitoses in the cells of the ciliary epithelium are extremely rare. There is no infiltration of inflammatory cells present.

In the ciliary region, corresponding to the photocoagulation area, but separated from it by a large band of healthy tissue, retina are separate entities. If the inner layers are severely involved, the Müller cells proliferate along the internal limiting membrane, forming a bridge of new tissue over a cavity of necrosis. There is a possibility that other glial cells as well as pigment epithelium cells take part in this process. In the case where the entire inner retina and the internal limiting membrane are destroyed, the cortical vitreous acts as a barrier to migration of cells with its surface serving as the scaffold for repair.
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Fig. 8. Relationship between the rate of mitosis and the time elapsed after injection of colchicine in the area of photocoagulation. Only litter No. 3 is represented. The values of the control eyes are considerably lower in this area than in the zonular area.

rise in cell content in the cortical vitreous after photocoagulation. The finding of mitoses in this cell layer after colchicine treatment confirms the fact that the hyalocytes are able to proliferate wherever they are.

Colchicine by arresting mitosis in metaphase allows the localization of the site of cell proliferation. The photocoagulation wound is repaired by proliferation in the preformed layers, i.e., choroid, pigment epithelium, retina, and cortical vitreous. In the retina, the proliferative potency of the Müller cells is very impressive (Fig. 9). The only migration of cells into the retina occurs in the first three to four days post photocoagulation. This migration consisting of polymorphonuclear leukocytes and eventually hematogenous monocytes is rather unusual. Polymorphonuclear leukocytes can be differentiated from hyalocytes by their smaller size, and by the presence of PAS + granules, which are only found in hyalocytes. Polymorphonuclear leukocytes and hematogenous monocytes do not divide outside of the bone marrow, and therefore cannot be responsible for the presence of dividing cells in the vitreous (Fig. 10A). The so-called macrophages found in the retina in the area of the photocoagulation lesion originate to a large part from pigment epithelium. Macrophages were not seen to pass through the internal limiting membrane in our specimens. These macrophages are found only in the area of the photocoagulation lesion. If the photocoagulation wound is torn away during the making of
Fig. 9. Four arrested mitoses of dividing Müller cells (arrows). The mitotic figures show up in the outer nuclear layer. The protoplasmic process of one of the dividing cells is outlined with India ink. (SUSA, PAS-He; x450.)

From the third day on, the hyalocytes phagocytize pigment granules, which have been dispersed from the photocoagulation wound into the vitreous. These pigment granules can be found even in dividing hyalocytes. The uptake of pigment spindles by the hyalocytes is compatible with the phagocytizing activity attributed to them. The pigment is not an indication that we are dealing with scattered pigment epithelium cells, because the pigment epithelium cells which appear occasionally in flat preparations in the area of photocoagulation itself are larger than hyalocytes and show a finely granular, lightly basophilic cytoplasm.

In the flat preparations the hyalocytes in mitosis in the area of the zonula and pars plana can clearly be localized in a layer internal to the ciliary epithelium. This is confirmed in sagittal sections (Figs. 10A and 10B). In contrast to the high rate of mitosis in these cells, mitotic activity in both layers of the ciliary epithelium is extremely rare (flat preparations and sagittal sections). A few mitoses are present in the stroma of the ciliary body. No cellular infiltration in the form of inflammatory cells can be found. The appearance of cells penetrating through ciliary epithelium as demonstrated by Hamburg is a rare phenomenon. Desquamation of ciliary epithelium cells is also extremely rare, and neither of these events could explain the increase in cell population.

Quantitative aspects. In the first series, rate of mitosis and cell rise are proportionate to some extent in the area of photocoagulation as well as in the zonular area (Figs. 2 and 3). The rate of mitosis rises sharply between the third and fourth day post photocoagulation. From the cell rise curve we would expect the increase in the number of mitoses one day earlier. From the fourth day to the ninth day the rate of mitosis slowly decreases. In the control eyes the rate of mitosis in the region corresponding to the area of photo-
coagulation is below 0.5 per 1,000 cells, in the zonular area it is between 1 and 8 per 1,000 cells. These values are slightly higher than in animals without coagulation in either eye. If this is a significant rise, it could be explained by a tissue-specific chalone effect, transmitted by the blood.

The cell counts performed do not permit any more than an approximate mathematical approach. Considering average rate of mitosis and number of cells of the previous day, the expected increase in number of cells for the next day is only about one third of the real increase. Then also the following assumptions are made: (1) The rate of mitosis does not change during 24 hours, (2) no hyalocytes are dying in this time period, (3) the normal rate of mitosis is negligibly low, and (4)
colchicine arrests all cells in mitosis during six hours and does not influence the rate of mitosis.\textsuperscript{11} The following correcting factors must be considered: In the first series, several cells were seen in anaphase and telophase, suggesting that the dose of colchicine was insufficient. Therefore, the dose of colchicine was doubled for the second series (0.2 mg. per 100 Gm. of body weight). In this series the relationship between colchicine effect and time can be demonstrated. Figs. 7 and 8 show that in the cortical vitreous a clear colchicine effect is not visible before 1½ hours after injection. Until 2 hours and 20 minutes after injection still all phases of mitosis are present. This late colchicine effect corresponds to the delayed penetration of other substances into the vitreous.\textsuperscript{12} The peak in the rate of mitosis is reached between 4 hours and 30 minutes and 5 hours and 20 minutes after injection. Therefore, the time during which colchicine is effective is not longer than approximately 4 hours.

Since the maximal values for cells in mitosis are considerably higher in the second series than in the first one, the higher dose of colchicine and also the possibly higher rate of mitosis during night may be responsible.\textsuperscript{13} If we consider 4 hours to be the time in which colchicine is effective, and that during this time interval 10 per cent of all hyalocytes enter into mitosis, the cell rise in the cortical vitreous in the area of photocoagulation as well as in the zonular area may be roughly explained by self-reproduction of the hyalocytes.

It has been suggested that isolated proliferation of the cortical vitreous cells may occur in inflammatory processes,\textsuperscript{4} but this could not really be proved because a migration of cells from the surrounding tissues could not be ruled out. That the cortical vitreous cell is a microglial cell penetrating through internal limiting membrane and developing into a macrophage, as concluded by Wolter\textsuperscript{14} from a case of melanoblastoma of the ciliary body, cannot be confirmed in our experiments. Furthermore, autoradiographic investigations on cellular response on damage of brain and of spinal cord raise the question of the existence of the microglial cell of Hortega.\textsuperscript{15-18}

Hamburg,\textsuperscript{2} Szirmai and Balazs,\textsuperscript{3} and Balazs and colleagues\textsuperscript{4} concluded that the source of the hyalocytes, which they consider to be mesenchymal cells, must be the ciliary body. They reasoned that this is true because their distribution is densest in this region, and because mitotic activity in the hyalocytes had never been observed before.

Our findings suggest that the layer of the cortical vitreous cells, the hyalocytes, must be considered an independent tissue layer in which the cells are replaced by reproduction. Mitoses arrested by colchicine not only are present after photocoagulation, but also are present in untreated eyes. These facts are in good agreement not only with embryologic findings,\textsuperscript{10} reporting that the hyalocyte enters the vitreous cavity as long as the latter is open, but also with the fact reported that vitreous cells grow in tissue culture.\textsuperscript{19}

**Cell rise and photocoagulation**

Our findings prove that photocoagulation of the rabbit retina stimulates the proliferation of hyalocytes. It is most likely an indirect stimulation secondary to necrosis of the cortical vitreous, of the retina, of the pigment epithelium, and of the choroid. This is followed by a reparative process which usually ends after a certain period of time, the rate of mitosis decreasing to the ninth day post photocoagulation. The increase of mitotic activity in all layers from choroid to cortical vitreous after photocoagulation corresponds to the process of wound healing in other tissues. The rise in mitotic activity is most likely due to the decreased level of mitotic inhibiting substances secondary to the damage of tissue.\textsuperscript{19} In choroid, pigment epithelium, and retina the process is restricted to the photocoagulation wound
and its immediate surrounds. In the vitreous, which is more or less a fluid compartment, the mitosis stimulating effect is less delimited. The proliferation of hyalocytes adjacent to the ciliary body and pars plana is remarkable. There the proliferation is most pronounced in a region corresponding to the area of photoocoagulation. If an overheating of the iris were responsible for this, we would expect an iritis or a cell proliferation in iris and ciliary body.

The proliferation of hyalocytes in response to photoocoagulation may partially explain the so-called macular puckering syndrome (epiretinal fibroplasia), and the massive preretinal retraction, which belongs to the same pathogenetic entity. It could be possible that under certain circumstances the proliferation of hyalocytes does not come to an end but continues until an epiretinal membrane is formed which later shrinks and detaches. If these hyalocytes are really fibroblasts, as suggested by Gartner, Brini and associates, Lerche and Wulle, and Teng in electron microscopic studies, the production of collagen fibrils and membranes would not be unexpected.

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