Bacteriologic studies of eyes at various stages of keratopathy showed no consistent changes and three viral isolation attempts in various cell cultures were negative.

**Discussion.** There is excellent correlation between the biomicroscopic appearance of the corneas of the tyrosine-fed rat and the histopathologic changes by light microscopy. The first changes noted with light microscopy were not yet visible by the lower magnification of slit lamp biomicroscopy. Early cellular degeneration in the epithelial cells is first shown as loss of cytoplasmic staining followed by cytoplasmic condensation. The degeneration in the basal epithelial cells seems to initiate a focal rapid piling up of cells, resulting in full-thickness epithelial opacities by the second day. Dilation of intra- and intercellular spaces makes visible the focal areas of edema characteristic of the biomicroscopic lesions.

During the degeneration of the epithelial cells it is notable that mitoses continue to occur. The rapid loss of PAS-positive stainable material from the epithelial cells in the focal lesions and the underlying basement membrane may reflect changes in the metabolic processes of the affected epithelial cells. Immersion of PMNL's follows the epithelial disease. Endothelial disease from leukocytic invasion and decreased aqueous outflow from leukocytic obstruction of the trabecular meshwork may contribute to stromal edema. The stroma biomicroscopically becomes opaque, due to thickening, fibrillar disorganization, and cellular increase. Eventually, vascularization and clearing ensues.

The stimulus for epithelial repair is not understood. Regeneration commences and proceeds until the corneas are almost clear by biomicroscopy; yet the animals are still being fed excess tyrosine and serum and aqueous humor levels of tyrosine remain higher than normal. The precise role of neovascularization in clearing the cornea is not known. When the epithelium is fully reformed no blood vessels are discernible by biomicroscopy but by light microscopy "ghost" vessels devoid of circulating cells are seen. Recurrence of the biomicroscopically visible opacity following the reparative process is associated with infiltration of leukocytes migrating from the open "ghost" vessels in the central cornea. The epithelial cells themselves do not seem to develop the degree of disease in the recurrent lesions which is typical of the early keratopathy.

Tyrosine-induced keratopathy in the rat is blocked by large doses of systemic adrenal glucocorticoids. Furthermore, tyrosine-induced keratopathy is prevented in both eyes by administration of potent steroid eyedrops in only one eye. This also suggests that systemic absorption of steroids and induction of hepatic tyrosine transaminase may be a factor in amelioration of keratopathy. Partial lessening of tyrosine-induced keratopathy by intramuscular phenobarbital, a well-known inducer of hepatic microsomal enzymes, would agree with this hypothesis.7

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**Key words:** cornea, corneal ulcer, corneal opacity, cytology inflammation, keratopathy, leukotaxis, tyrosine tyrosinemia.

**REFERENCES**


**Fine structure of Müller cells in the human retina as revealed by ruthenium red treatment. Shigekazu Uga and Hiroshi Ikul.**

*Müller cells in the human retina were labeled darkly, by treatment with ruthenium red, and their tall columnar outline, lateral fine branches, and expanded end-feet were clearly demonstrated. In addition, the structural relationship between the
retinal capillary and Müller and accessory glial cells are also shown. This dye is useful for analysis of the cytoarchitecture of the glial cells in the human retina.

Silver staining and histochemical stain techniques employed by light microscopy have been recently introduced into electron microscopy procedures to clarify the cellular organization of various tissues, including the retinal tissue. The technique applied to the retina succeeded in the intracellular marking of the neural cells and the dense staining of the extracellular space between the neural and glial elements, but a method to specifically delineate the glial elements has as yet been unexploited.

In the present study, the application of ruthenium red to the human retina has modified the preservation of glial Müller cells and revealed structural details so far not appreciated. It was thus possible to examine the fine structure, the distribution, and the relationship of this cell to other glial elements and the vascular system.

Material and method. The retina was obtained from the surgically enucleated eye of a 63-year-old man. It was normal ophthalmoscopically. The tissue was fixed for two hours in 4 per cent glutaraldehyde buffered with 0.1 M sodium phosphate solution containing 500 p.p.m. of ruthenium red at pH 7.4, and postfixed for two hours in 1 per cent osmium tetroxide in 0.1 M phosphate buffer containing 500 p.p.m. of ruthenium red at pH 7.4. After fixation, the tissue was dehydrated in a series of ethanol, treated briefly with propylene oxide, and embedded in Epon 812. The embedded tissue was oriented for vertical or horizontal sectioning of the retina. Thin sections were cut on a Porter-Blum MT-2 ultratome, doubly stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-11 DS electron microscope.

Observations. After treatment with ruthenium red, the cytoplasmic matrix viread to the nuclei of the Müller cells was seen to be of selectively enhanced contrast. Their lateral fine branches and expanded end-feet were also labeled (Fig. 1). On the other hand, the processes of astrocytes, which lay in the nerve fiber layer and the ganglion cell layer, remained consistently unlabeled. Taking advantage of the different labeling of these two glial elements, one can distinguish between the Müller cell processes and those of astrocytes which contact the surface of the retinal capillaries. The outer rim of the capillary, shown in Fig. 2, consists of only the dark cytoplasm, which illustrates a complete envelopment by the Müller cells. In Fig. 1, dark broad processes, which are easily classified as those of Müller cells by their density and pale, closely aggregated processes can be seen around the capillary. The latter are parts of astrocytes, which are characterized by the presence of fine filaments, glycogen particles, and a small number of mitochondria within their cytoplasm. However, the lighter appearance of the astrocyte cytoplasm may result from the less numerous cell organelles it contains. These processes were closely packed together, and were variable in diameter, ranging from 0.2 µ to 1 µ. In the horizontal section of the deep retinal layer, a lattice network of the bundled processes of the astrocytes was observed. No single process of the astrocyte which branched or separated from the bundle was found. The inner (vitread) processes of the Müller cells, extending toward the vitreous across the retinal layer were often interrupted and shifted by the bundled processes of the astrocytes.

In the horizontal section of the retina at the level of the inner plexiform layer, the dark-staining processes and branches of the Müller cells were seen to be dispersed and distributed among nerve fibers with pale density (Fig. 4). Each process of the Müller cells has a relatively uniform diameter measuring approximately 2 to 3 µ, but the lateral, walls were pushed in by intricated nerve fibers, so that the Müller cells showed a polyhedral-foam. Occasionally, their neighboring processes were grouped in a mass, producing a linear or a circular (Fig. 3) appearance.

In the untreated retina of the same specimen the Müller cells have never been labeled.

Discussion. Electron microscopic techniques for staining the cell surface with ruthenium red was initially introduced by Luft. The exact nature of the substances produced from the reaction with this inorganic ionic coordination complex is unknown, but it is generally thought that the ruthenium red-positive substances are acid mucopolysaccharides which are present as an extraneous surface coat, called "glycocalyx." This staining method has been applied to the mouse and rabbit retina, in which the reactive material was present on the inner limiting membrane and in the intercellular spaces between the Müller and accessory glial cells as well as the neurons. The present study revealed that in the human retina, ruthenium red is predominantly available for the increased density of the cytoplasmic matrix of the Müller cells. It is questionable, however, whether the dye penetrates intracellularly during the immersion of the specimen into the fixative, since ruthenium red does not cross the undamaged cell membrane. It is, therefore, inferred that the dark-staining property of the Müller cells may be due to hypertonic osmolality of the fixatives modified by blending with ruthenium red, by virtue of the fact that the cells observed showed a slight shrinkage. We have no further morphologic evidence for this peculiar phenomenon but an in-
Fig. 1. Electron micrograph of the human retina treated with ruthenium red. The inner processes vitread to the nucleus of Müller cells (NM) are darkly stained. A cross-sectioned retinal capillary (cap) located in the nerve fiber layer is enveloped by both the Müller cell processes and astrocytes (As). ilm, inner limiting membrane; nf, nerve fiber. x4,000.
Fig. 2. Electron micrograph showing a capillary (cap) which is completely enveloped by the inner processes of the Müller cell (M) with dark cytoplasmic matrix. Gc, ganglion cell; nf, nerve fiber. ×4,000.

Fig. 3. A horizontal section through the inner plexiform layer of the retina. Müller cell processes (M), which are grouped in the form of a circle are shown. nf, nerve fiber. ×4,600.

Fig. 4. A horizontal section through the inner plexiform layer of the human retina. The processes of the Müller cells (M) are seen to be diffusely distributed among neurons (nf). ×3,200.
vestigation applying this method to the experimentally damaged retina (monkey) is in progress in our laboratory.

In the present study, the Müller cells, seen in the horizontal section of the retina, were often found in a small mass in close contact with the neighboring cells. This tendency is more noticeable in the posterior than in the peripheral part of the retina. Larger masses of the Müller cell processes have been observed in the extremely thickened nerve fiber layer in the juxta-optic nerve region of the human retina. These facts may represent functional aspects of the Müller cells such as mechanical support of the retina. The present observations also showed quite clearly that there is a close relationship between the retinal capillary and the Müller and accessory glial cells and are in accordance with those of earlier investigators. The astrocytes, terminating on the surface of the capillary, always showed an end-foot structure comprising bundled processes. The light microscopic studies of the retinal glia have revealed the presence of an additional two types of glial cells, perivascular glia and lemmocyte (cell of Remak), but these glial elements have not been pursued in this study.

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REFERENCES

Estimation of the ratio of cones to neurons in the fovea of the human retina. L. Misotto

The ratio of pedicles over neurons has been determined in thin sagittal and flat sections of the rod-free central area of the human retina. The real amount of neurons has been calculated from the number of transections of nuclei. Results show that for each pedicle two to three bipolar cells are found, ± 0.6 horizontal cells, ± 0.7 amacrine cells, and 0.9 ganglion cells.

The central rod-free area of the fovea is especially well-suited for a study of the synaptic contacts of the cones. Only one class of receptor cells is present in this area and the neurons of the second and third echelon are small and, for this reason, easier to investigate by electron microscopy. In order to translate the findings of the analysis of single cells in a general schema, it would be helpful to know the ratio between the different neurons and the receptor cells.

A comparison of the data available from the literature on the number of cones and the number of ganglion cells does not give the answer, because the inner layers of the retina are shifted centrifugally in respect to the visual cells (Fig. 1).

In front of the central bouquet of cones, in the foveola, the inner layers of the retina are absent. No synaptic connections are found in this area. The synaptic pedicles of these cones are located in an annular zone surrounding the central area; they are connected to their parent cells with long fibers "Henle's fibers." The centermost pedicle is usually found at 100 microns distant from the center of the fovea. The area between 100 and 300 microns contains pedicles at irregular intervals; from about 300 microns outward the pedicles form a continuous and uniform layer to about 800 to 1,000 microns from the center, where rod spherules are found in increasing numbers.

The centrifugal shift of the neurons connected to the cones of the fovea hinders the correlation of the numbers of receptor cells and neurons, because the layers are displaced in respect to each other, and because the area available to the neurons is much larger than the surface occupied by the cones, as is shown in Fig. 1.

The neurons of the inner layers are oriented