that they would not evade the problem of scattering from neighboring tissue layers, as illustrated in Fig. 1. Search of their cited references led to a recent patent by Baer. This instrument operates on the principle of the present instrument and is superior in conception, since, if enough light could be made available, it would allow the optical section of the tissue to be directly observed as well as photographed. No photographs taken with this instrument have been published to my knowledge, however. Goldmann made use of a similar technique to that described here, at a different order of magnification, in his method of photographing the anterior chamber.

I wish to thank Mr. Gunther Kuhn for engineering and constructing the modifications to the microscope, and Miss Betty Cassiman for taking the photographs.

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Key words: scanning microscopy, corneal structure.

REFERENCES


Histopathology of keratopathy in the tyrosine-fed rat. MARGARET E. BEARD, ROBERT P. BURNS, LARRY F. RICH, AND EDWIN SQUIRES.

Young rats fed a diet containing excess L-tyrosine develop a reproducible and reliable keratopathy. This keratopathy clears spontaneously, although the initiating dietary stimulus is maintained. In less than 24 hours, edema of the central corneal epithelium develops, first in the basal cells and then in focal full-thickness areas. These areas of epithelial disease enlarge to form full-thickness "snowflake" opacities with cellular separa-
Blood vessels enter from the limbus at about seven days, reaching the center of the cornea after two to three weeks. As the blood vessels grow in, the epithelium, which may have ulcerated in the central part, regenerates from corneal or conjunctival epithelium. The corneal stroma clears and thins, vessels shrink, the PMNL's are replaced by macrophages, and the endothelium resumes a more normal appearance. Although the 5 per cent tyrosine diet is continued, and serum tyrosine remains high, the corneas of some animals become histologically clear by 21 days. However, abnormalities are still visible on light microscopy. Occasional recurrence of the corneal opacity is associated with stromal edema and PMNL invasion.

Corneal ulceration and opacity occurs in a rare genetic disease, tyrosinosis (Oregon) in which tyrosine aminotransferase deficiency results in tyrosinemia. In the rat, a nutritional imbalance which mimics this unusual human disease is produced by the feeding of excess amounts of tyrosine. Keratopathy with characteristic biomicroscopic appearance develops during this induced tyrosinemia. This is a study of the light microscopic changes occurring in the cornea during this keratopathy.

Materials and methods. Female albino rats (Simonsen Laboratories, Gilroy, Calif.), weighing 150 to 180 grams, were housed in individual cages and received food and water ad libitum. Experimental diets were modified from the formulation of Schweizer. Control animals received the potato-base diet lacking added tyrosine; the experimental animals were fed the base diet supplemented with 5 per cent tyrosine by weight.

Eyes were examined with a Haag-Streit 900 slit lamp before starting the control or tyrosine-containing diets. Thereafter, daily biomicroscopic examinations were made and the gross corneal lesions recorded as previously described.

Enucleated eyes, opened at the limbus on opposite sides by a razor blade, were fixed in 2 per cent glutaraldehyde-1 per cent paraformaldehyde fixative in 0.067 M cacodylate buffer. One cornea from each animal was postfixed in 1 per cent osmium tetroxide in 0.067 M cacodylate, pH 7.4, dehydrated in a graded series of ethanol or acetones and embedded in an epon-araldite mixture; the other cornea was processed in paraffin by standard procedures.

Paraffin sections were stained with Weigert's iron hematoxylin and eosin, Mallory's trichrome, or periodic acid-Schiff (PAS) reagent for polysaccharides. Sections of epon-embedded tissue were either stained directly with 1 per cent toluidine blue with 1 per cent borax or deplastified and stained with Hale's colloidal iron to

Fig. 1. Five per cent tyrosine diet for 24 hours. Stage II, dot-like corneal opacity characterized by disruption of cellular architecture with both pale and dark staining cells. Paraffin, Weigert's hematoxylin-eosin, x240.

Fig. 2. Five per cent tyrosine diet for two days. Corneal epithelium biomicroscopically described as having a full-thickness epithelial opacity and stromal swelling. Further aggregation of PMNL's is apparent beneath a focus of epithelial erosion and adjacent to normal epithelial cells. Plastic, toluidine blue, x450.
For the 3 per cent tyrosine-fed rat is an extremely reproducible and reliable model for tyrosine keratopathy. Older animals do not consistently develop the disease. All of the young animals develop keratopathy. In the 700 rats observed, the degree of disease is remarkably similar, rarely varying by more than one stage between animals in a group or between right and left eye of the same animal.

We have graded biomicroscopic changes into six consecutive stages. Stage 1 is a faint, barely discernible corneal epithelial haze occurring in 12 to 24 hours. By 12 hours after the 5 per cent tyrosine diet is started, before biomicroscopic changes are visible, there is loss of staining in some basal epithelial cells.

Within 24 hours after initiating the diet, Stage 2 develops. It consists of tiny epithelial dots which progress into larger "snowflake" type of opacities in the center of the cornea. These measure 60 to 70 microns across and involve three to five cells. Such lesions are characterized microscopically by cellular separation and disruption of stratified architecture throughout the full-thickness of the epithelium (Fig. 1). Some cells stain densely and others appear pale. Heterochromatin becomes clumped and nucleoli are prominent. The basal lamina loses its strong reactivity with PAS and no PAS-positive material is visible in the focally affected epithelial cells. Granules staining darkly with toluidine blue are apparent in the more superficial cells. By the third day, polymorphonuclear leukocytes (PMNL's) migrate from the limbus into the superficial stroma. These are not present before the epithelial changes. At first, PMNL's leave an acellular zone beneath the epithelium (Fig. 2), then this is invaded (Fig. 3). PMNL's are present in the anterior chamber by the third day, and may collect in the lower chamber angle (Fig. 3, inset).

By the third to fourth day after initiation of the tyrosine diet, Stage 3 develops. This consists of both epithelial and stromal thickening and opacity, and develops first in the center of the cornea. The intercellular space dilates, first in the intermediate, and then in the basal layer of the epithelium. A flocculent material is present in this space, part of which may be an acidic mucosubstance, since it stained with Hale's colloidal iron.

The epithelial cellular architecture becomes extremely distorted. Basal cells elongate, epithelial cells become round and separate, though some desmosomes remain. Cells vary in stainability. Nucleoli are prominent. Mitoses continue to occur. PMNL's invade the stroma, principally in its superficial layers, and the epithelium. PMNL's

Fig. 3. Five per cent tyrosine diet for five days. Larger Stage II epithelial snowflake opacity with cellular disruption and migration of PMNL's into the superficial stroma and epithelium. Paraffin, periodic acid-Schiff, x240. Inset: 5 per cent tyrosine diet for three days. Lower part of anterior chamber angle and trabecular meshwork infiltrated by clumps of polymorphonuclear leukocytes. Paraffin, Weigert's hematoxylin-eosin, x175.

demonstrate acidic mucopolysaccharides, a modified periodic acid-Schiff reagent, or celestine blue-eosin.
Fig. 4. Section of central cornea from rat fed 5 per cent tyrosine diet for nine days. Blood vessels have invaded one-fourth of the way across the cornea. Epithelium disarrayed and eroded. Anterior stromal fibrils loosened and disorganized. PMNL's layered on endothelium. Paraffin, Weigert's hematoxylin-eosin, ×160.

Fig. 5. Five per cent tyrosine diet for 10 days. Blood vessels (arrow) have invaded to the center of the cornea. Epithelium lifted off by collection of PMNL and macrophage-like cells in "pustule" formation. Paraffin, Mallory's trichrome, ×175.

also collect on the endothelium, sometimes in localized plaques. The iris may adhere to these PMNL plaques on the endothelium and the endothelium appears degenerated (Fig. 4).

The epithelial, stromal, and endothelial opacity starts centrally in the cornea, spreads peripherally, and lasts until approximately seven to 10 days after initiation of the diet when blood vessels begin to invade the peripheral cornea. We have classified beginning vascular invasion as Stage 4. This stage progresses for approximately another 10 days when the blood vessels reach completely to the center of the cornea, called Stage 5. The cornea becomes so thickened and opaque the iris is often not visible. The corneal opacity is maximal at the center, less at the periphery, and clears at the periphery as the blood vessels migrate in. The epithelium may be desquamated. The remaining epithelium is completely disorganized, infiltrated with PMNL's, and may slough, resulting in ulcers (Figs. 4 and 5). The stroma is tremendously thickened, edematous, and infiltrated with PMNL's which are later replaced by macrophages. The iris may adhere to the endothelium. PMNL's begin to disappear from the endothelium and anterior chamber.

By Stages 4 and 5, regeneration is beginning. Corneal clearing correlates with the presence of new blood vessels in all layers of the stroma (Fig. 5). The cornea begins to clear from the periphery toward the center, with the deeper layers of epithelium regenerating first causing desquamation of the superficial leukocyte-laden layer. The stroma becomes more compact and less cellular. The endothelium appears more normal, but may show reduplication with extra layers of Descemet's layer or drusen. The epithelium regenerates partly from the cornea and partly from the conjunctiva, which can be identified by goblet cells and different structure of epithelium from that of the cornea. The epithelium becomes more compact. Regenerating cells stain deeply. Chromatin is dispersed. Desmosomal attachments become visible and intercellular spaces less dilated. PAS reactivity is again demonstrable in the basal lamina, as well as in the goblet cells. However, the stratified organization of the normal corneal epithelium is not perfectly regained. The epithelium contains more cell layers than normal, and the many squamous cells are less flat than normal.

In Stage 6, the cornea is biomicroscopically clear, or almost so. At times yellowish droplets, possibly consisting of goblet cells, can be seen in the corneal epithelium. No blood vessels are visible though a faint stromal haze may persist. However, by light microscopy the epithelial cells appear abnormal, and the stroma still contains more cells than normal, and open capillaries.

Occasionally, a rat may have a biomicroscopic recurrence of corneal opacity after the cornea has become almost clear after three to four weeks of diet. Increased stromal cellularity, primarily PMNL's, as well as reopening of capillaries is visible with light microscopy, although the epithelium remains intact.
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. Immigration of PMNL's 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.

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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 IKUI.

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