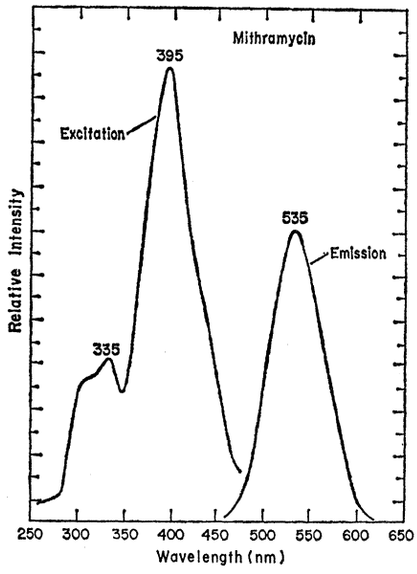
Fig. 2. Excitation and emisson spectra of mithramycin-stained Chinese hamster cells (line CHO) measured with an Aminco-Bowman spectrophotofluorometer.



Although the CV were somewhat larger in the mithramycin-treated cultures, the two staining protocols yielded essentially equivalent values for the fraction of cells in various phases of the cell cycle. The CV for suspension cultures of CHO cells treated with mithramycin or acriflavine were 5.8 and 4.0 percent, respectively; the fractions of cells in G_1, S, and G_2 or M were 51.4, 41.2, and 7.4 percent, respectively, in mithramycin-treated samples and 48.4, 44.4, and 7.2 percent, respectively, in cultures treated with acriflavine (not shown).

Initial experiments indicated that stable fluorescence patterns were obtained only after 15 to 20 minutes of exposure of cells to mithramycin solution; patterns remained stable for at least 1 hour thereafter. Lower concentrations of mithramycin than the 100 μg/ml used in the experiment shown in Fig. 1 could be employed, but the amount of fluorescence was decreased and the time required for drug equilibration was greatly increased. Treatment of cells with mithramycin in phosphate-buffered saline or in water was unsatisfactory because of an apparent inability of the cells to incorporate sufficient quantities of drug to yield reproducible fluorescence patterns. Subsequent experiments indicated that cells stored in cold 70 percent ethanol for a least 1 week prior to staining yielded distribution patterns comparable to those of cells stained immediately after harvesting. Treatment of fixed cells with ribonuclease (1 mg/ml) for 30 minutes at 37°C had no effect on the fluorescence pattern, while treatment with deoxyribonuclease (1 mg/ml) for 2 hours at 37°C prior to exposure to mithramycin rendered the cells virtually nonfluorescent.

Figure 2 shows the excitation and emission spectra for mithramycin-stained Chinese hamster cells. The lowest available wavelength for exciting the drug with the laser employed in the FMF (argon ion, Coherent Radiation model 52) is 457 nm. Although the drug is suboptimally excited at 457 nm, this wavelength was superior to other available laser lines of higher wavelength (461, 476, 488, and 514 nm). In particular, the fluorescence distribution patterns obtained for mithramycin-treated CHO cells excited at 488 or 514 nm proved unacceptable for cell-cycle analysis. We would expect a significant improvement in CV values if laser lines of substantial power were available for excitation of mithramycin in the 390- to 400-nm range.

The fluorescence patterns in mithramycin-treated cells agree well with patterns obtained with acriflavine-Feulgen–stained cells, and staining by the latter method has been shown to correlate with DNA content by standard cell-cycle analytical techniques with [^{3}H]thymidine (*1*). We therefore conclude that mithramycin is bound and fluoresces in direct proportion to the amount of cellular DNA. The lack of fluorescence in deoxyribonuclease-treated cells exposed to mithramycin solution is further support for the specificity of interaction between mithramycin and cellular DNA.

Among the advantages of this technique is its applicability to the following kinds of studies: (i) populations of cells devoid of DNA-synthesizing or mitotic cells; (ii) slowly or nonprogressing populations; and (iii) isotopically labeled cells in which standard [^{3}H]thymidine autoradiographic techniques are difficult or impossible. However, the unique features of the mithramycin technique are rapidity and simplicity, which permit monitoring of population kinetic properties in ongoing experiments, with the added option of altering an experiment in progress in response to a population change.

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8. Mithramycin (NSC 24559) was obtained through the Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland.
9. Supported by contract NIH-CR-(71)-56 from the Division of Cancer Treatment, National Cancer Institute, under interagency agreement with the Atomic Energy Commission.

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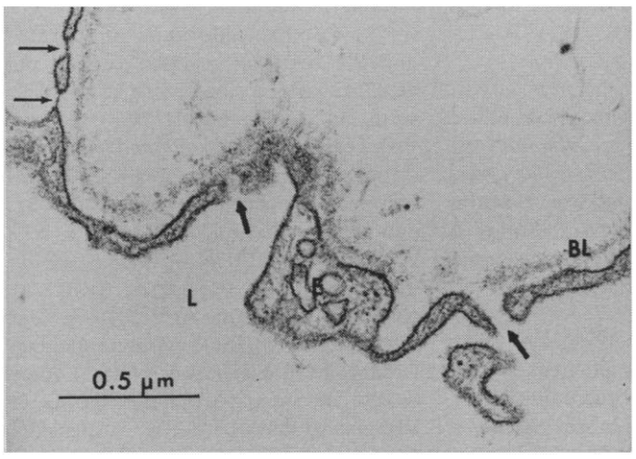
Selective Destruction of the Pigmented Epithelium in the Ciliary Body of the Eye

Abstract. *Perfusion of the internal carotid artery with hypertonic solution selectively destroys most of the pigmented epithelium of the ciliary body of the monkey eye, converts fenestrated endothelium of capillaries to continuous endothelium, and transiently breaks down the blood-aqueous barrier. The nonpigmented epithelium regenerates and the intraocular pressure returns to normal even though the pigmented epithelium is permanently destroyed.*

The mechanism and site of aqueous formation remain major questions in eye physiology and are important to understanding the pathogenesis of glaucoma. It has been proposed that aqueous humor is secreted by the ciliary epithelium into the posterior chamber of the eye after plasma ultrafiltration through fenestrated capillaries of the ciliary body (*1*). The ciliary epithelium is double-layered, having a pigmented layer facing the stroma and blood vessels and a nonpigmented layer whose basal surface faces the posterior cham-



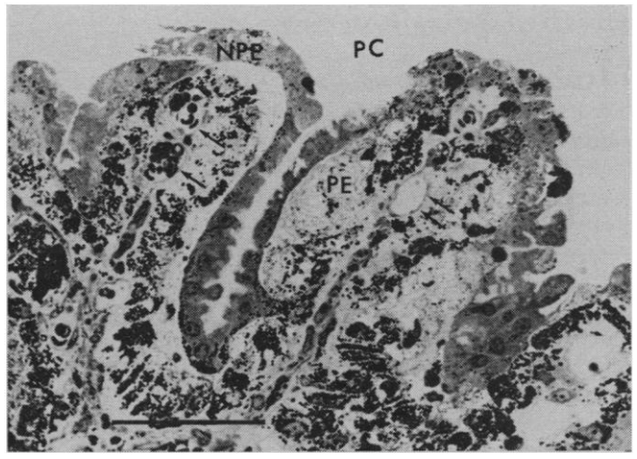


Fig. 1 (left). Capillary wall in ciliary body 1 minute after lactamide perfusion. Diaphragms are absent from fenestrae (large arrows), although some are still intact (small arrows); *L*, lumen of the capillary; *BL*, basal lamina; *E*, endothelium of the capillary. Fig. 2 (right). Ciliary epithelium 1 day after urea perfusion. The pigmented layer (*PE*) is severely damaged and occupied by necrotic cells and macrophages. The nonpigmented epithelial layer (*NPE*) appears to be intact. The scale indicates 100 μm. Arrows demonstrate capillaries; *PC*, posterior chamber.

ber of the eye. The junctional complexes connecting these cells restrict protein diffusion and are a site of the blood-aqueous barrier (*2*). Active transport of sodium and aqueous secretion have been ascribed to the nonpigmented layer (*3*).

We now present a method that selectively damages the pigmented epithelium without irreversibly damaging the nonpigmented epithelium. This selective damage should make it possible to evaluate experimentally the separate roles of these epithelial layers in regulating flow and composition of aqueous humor.

Solutions of either 2*M* urea or 2*M* lactamide were perfused into the left internal carotid and ophthalmic arteries of 15 rhesus monkeys via the left lingual artery. The left external and common carotid arteries were clamped for the 20-second perfusion, and intravascular Evans blue–albumin was used as a protein tracer for determining the integrity of the blood-ocular barriers (*4*).

Intraocular pressure was measured after perfusion, and eyes were enucleated from some animals at 1 minute and from others at later times, up until 3 months thereafter. The ciliary epithelium was examined with light and electron microscopy.

The intraocular pressure of the left (perfused) eye fell from about 12.5 mm-Hg before perfusion to about 2 mm-Hg within 1 hour after perfusion, remained at this low level for several days, and then slowly increased until it became normal 3 to 6 weeks later. After perfusion, the aqueous and vitreous humors, the iris, and the ciliary body became stained with Evans blue–albumin, an indication of breakdown of barriers in the eye that normally exclude protein (*1*, *5*).

One minute after hypertonic perfusion, diaphragms were absent from fenestrae of the capillaries of the ciliary body, and the diameters of the fenestrae had increased from normal values of 300 to 400 Å to 400 to 600 Å (Fig. 1). Endothelial cells were vacuolated. At 10 minutes, disrupted fenestrae were filled with a fine granular substance and still lacked well-defined diaphragms. One day later the fenestrae were obliterated entirely, and the endothelium of the capillaries appeared continuous. The capillaries did not regain their normal appearance even after 3 months.

The ciliary epithelium was swollen 1 minute after perfusion, and intercellular spaces were expanded between the pigmented and nonpigmented layers and between adjacent cells of nonpigmented epithelium. From 10 to 30 minutes later the pigmented and nonpigmented layers had separated partially from each other, with tearing of the junctions that connect them.

The necrotic change of the pigmented epithelium progressed until, after 1 day, macrophages had invaded the pigmented layer to take up the cellular debris. At this time, however, nonpigmented cells appeared normal except for some widening of the basal infoldings and of adjacent cells (see Fig. 2).

At 3 to 7 days, when intraocular pressure started slowly to rise, macrophages occupied much of the pigmented layer, especially at the pars plana region near the retina, and the pigmented epithelium continued to degenerate.

As intraocular pressure approached normal levels at 3 to 6 weeks after perfusion, macrophages receded from the pigmented layer, and connective tissue (collagen and elastic fibers) filled the sites they had occupied. It could be seen that the pigmented cell population was reduced by 50 to 80 percent below normal, particularly at the pars plana. Remaining pigmented cells were abnormally small and often were separated from nonpigmented epithelium. The nonpigmented cells appeared entirely normal, except for accumulation of material in the widened basal infolding resembling basement membrane (*6*).

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5. Breakdown of blood-equeous barrier after hypertonic urea perfusion was also shown with sodium fluorescein as a tracer (A. Laties, personal communication).
6. We thank H. Thompson, K. Matthews, and D. W. Player for technical assistance.

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