Permeability of blood-ocular barriers of neonatal and adult cat to sodium fluorescein

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The permeability of the ocular blood vessels to sodium fluorescein (NaFl) was evaluated in neonatal and adult cats by fluorescence microscopy. The iris, ciliary body, and choroidal vessels were markedly permeable, whereas the mature and immature retinal vessels were impermeable. Since there is no apparent barrier to NaFl at the level of the iris vessels, the role of those vessels in aqueous formation is possibly significant. The fact that the immature retinal vessels are impermeable suggests that abnormal permeability to NaFl in retinal neovascularization is a consequence of pathology rather than immaturity.

Key words: cat, ocular development, aqueous humor formation, permeability, blood-retinal barrier, blood-aqueous barrier, vessels, sodium fluorescein, fluorescence microscopy, neonatal kitten

The adult cat has been utilized in many studies concerning ocular physiology, and the neonatal kitten has been particularly important to the study of retrolental fibroplasia. Studies of the permeability of the ocular blood vessels in relation to the blood-aqueous and blood-retinal barriers in the cat, however, are few. Cunha-Vaz et al. demonstrated a free permeability of the iris, ciliary body, and choroidal blood vessels to trypan blue, but the retinal blood vessels were impermeable. They also found that choroidal blood vessels were permeable to colloidal carbon, but not blood vessels of the iris or retina. Rodriguez-Peralta reported the cat retinal blood vessels, ciliary body epithelium, and the retinal pigment epithelium to be impermeable to acriflavine neutral, a vital dye that stains nuclear RNA and DNA; the blood vessels of the iris, ciliary body, and choroid, however, were permeable. We demonstrated by angiography the impermeability of the cat's retinal blood vessels to sodium fluorescein (NaFl), but the vessels of the uveal tract were not evaluated.

We have begun investigating further the permeability of the blood-ocular barriers in the adult and neonatal cat, with NaFl used as a marker dye.

Materials and methods

A total of 14 kittens, ages 5, 13, 17, and 21 days, and three adult cats were anesthetized (sodium pentobarbital, 40 mg/kg, intraperitoneally). After injection of 0.1 ml/200 gm body weight of a 0.5% NaFl solution, the eyes were enucleated at 30 sec and 1, 2, or 4 min after injection and immediately immersed for several minutes in isopentane cooled to approximately -105° C by a liquid nitrogen bath. The eyes were stored in liquid nitrogen and subsequently freeze-dried for 14 days at -35° C in a molecular sieve apparatus. After the enucleations, the kittens were killed with an overdose of sodium pentobarbital.

The dried eyes were opened, and the retinal blood vessel pattern was viewed through a dissect-
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Fig. 1. Fluorescent micrograph of 5-day-old kitten (30 sec after injection), showing (1) marked fluorescence adjacent to iris pigment epithelium (PE), (2) decreased fluorescence surrounding large vessel (V) and between vessel and anterior chamber (AC), (3) band of fluorescence on posterior wall of large vessel, and (4) fluorescence apparently streaming (arrows) from iris stroma into AC. (Epi-illumination, paraffin-embedded; ×240.)

Results

Tissue autofluorescence of paraffin-embedded sections when viewed by transmitted illumination was a bluish green, as previously described for other species. It was a faint greenish tan when epi-illumination was used.

Anterior segment. In eyes removed 1 min or less after NaFl injection, there was profuse penetration of dye into the iris and ciliary body stroma. In the iris, the fluorescence was greater in the stromal tissue adjacent to the pigmented epithelial layers (Figs. 1 to 3), an area having many small vessel profiles. In some eyes there appeared to be diffusion of NaFl from the stroma into the anterior chamber (Fig. 1). In addition, there were areas of stroma between large vessels and the anterior iris surface that had reduced fluorescence, and some large vessels had an intense band of fluorescence along the wall that faced toward the pigment epithelial layers. As such, there was an apparent gradient of fluorescence, suggesting a movement of dye...
**Fig. 2.** Fluorescent micrograph of 5-day-old kitten (1 min after injection), showing gradient of fluorescence from posterior iris stroma (P) toward anterior chamber (AC). Fluorescence is present in nonpigmented epithelium (arrow) and stroma (S) of ciliary process. (Epi-illumination, paraffin-embedded; ×240.)

**Fig. 3.** Fluorescent micrograph of adult cat iris (30 sec after injection), showing fluorescence in stroma adjacent to pigment epithelium (PE) and in vessels. Marked presence of pigmented stromal cells having brownish tan autofluorescence masks to some degree the yellowish green NaFl fluorescence in this black and white print. (Epi-illumination, wax-embedded, ×90.)
Fig. 4. Fluorescent micrograph of retina and choroid of 5-day-old kitten (1 min after injection), showing wide-lumened immature retinal vessels (V) and choriocapillaris (C) connected (open arrow) to choroidal stromal vessels (closed arrow). Note horizontal "layering" of NaFl between tapetal cells (T). (Epi-illumination, wax-embedded; x90.)

through the iris stroma to the anterior chamber. This gradient of fluorescence was not evident in eyes removed 2 or 4 min after injection of NaFl, but rather the iris stroma was diffusely and evenly fluorescent. The overall evidence of NaFl was more pronounced in the kitten iris tissues than in the adult ones, and this could suggest a greater permeability of the kitten iris vessels. However, the adult cat has many fluorescent pigment cells (chromatophores) in the iris stroma (Fig. 3), and their autofluorescence may reduce the visibility of the NaFl fluorescence. The pigmentation of the kitten iris is sparse compared to that of the adult.

The stroma of the ciliary processes and the ciliary body fluoresced markedly in all eyes, regardless of circulation time, and fluorescence was evident within the ciliary body nonpigmented epithelium at all time intervals (Fig. 2).

In eyes enucleated 1 min or less after injection, the corneal stroma and sclera adjacent to the limbal area showed some fluorescence, whereas the remaining corneal tissues did not. In eyes enucleated 2 and 4 min after injection, fluorescence was increasingly evident in the corneal stroma adjacent to the endothelium, and the scleral fluorescence was more diffuse.

Posterior segment. The retinal vessels were filled with NaFl, but fluorescence was not present within the neural tissue (Fig. 4). When viewed as a gross specimen by low-power (4x or 10x) microscopy, the leading edge of the developing vascular beds appeared as an ill-defined area of fluorescence (Fig. 5), almost as if there were leakage. Microscopic evaluation of the tissue sections, however, showed no leakage but rather numerous wide-lumen vessels (Fig. 4). Apparently, hyperfluorescence within the immature vessel beds obscured the vessel outlines when the vessels were viewed as gross specimens by low magnification. The choroid and the tapetum showed marked fluorescence throughout, and in the tapetum the fluorescence could be observed in between the individual cells (Fig. 4). After 1 min or less, the sclera was fluorescent only in the layers immediately adjacent to the choroid and retrobulbar tissues. After 2 and 4 min after injection, the sclera became diffusely (albeit faintly) fluorescent.

Fluorescence was evident in the optic nerve tissue adjacent to the scleral foramen.
in the eyes enucleated 2 and 4 min after injection, but not in those enucleated at 1 min.

Good localization of NaFl was possible when the plastic sections were viewed, and more precise localization was possible in many instances when sections of 2 to 4 μm were viewed. At higher magnifications, slight wrinkling of the sections interfered at times with photography but usually not with interpretation. The degree of fluorescence markedly decreased as the sections were cut thinner than 8 μm, and thus direct correlation with wax embedded tissues was not feasible regarding quantification of NaFl.

There were no apparent differences in these permeability findings in the kittens of various ages nor between kittens and adult cats, except for the possible iris difference noted earlier.

Discussion

The marked permeability of the iris vessels of the kitten and adult cat to NaFl was a striking finding in this investigation. However, since NaFl is approximately 80% bound to serum proteins, it is not known whether the free NaFl molecule with an effective diffusion radius (EDR) of 5 Å passed into the stroma or if the protein-bound molecule (EDR 35 Å) passed through the vessel walls also. Cunha-Vaz et al. observed the presence of trypan blue in the iris stroma of cats and kittens, and since trypan blue binds 100% to serum protein, that work implies that proteins can pass through the iris blood vessels into the stroma. We are currently studying the blood-ocular barriers of adult and neonatal cats with NaFl-labeled dextran molecules of selected molecular weights in order to evaluate that question more fully.

This present study suggests that the cat iris blood vessels differ from those of certain other species. In man and rat and in monkey and rabbit, NaFl does not pass through the iris blood vessel wall into the stroma, whereas we have shown that it does in the
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Yet, if NaFl and possibly protein-sized molecules pass from the iris blood vessels into the stroma, it is interesting that the aqueous humor protein content of the cat is approximately 11 to 30 mg/dl, a figure certainly no higher than that reported for man or rabbit. The possibility exists, though, that NaFl could enter the iris stroma, not from the vessels but from (1) the ciliary body via the iris root, (2) the posterior chamber via the pigment epithelium, and/or (3) the anterior chamber. If (1) or (3) were instrumental, the short circulation times employed in this study should have demonstrated movement of marker from the iris root and/or the anterior chamber toward the deep iris stroma. No such gradients, however, were evident. The only gradient was from the iris stroma adjacent to the pigment epithelium toward the anterior iris stroma. Maurice hypothesized that NaFl might pass from the posterior chamber through the iris pigment epithelium into the iris stroma. This could explain the gradient observed, and critical examination of the kitten iris pigment epithelium did show evidence of fluorescence within those layers. However, this present study did not discern whether the intraepithelial fluorescence was evidence of through-and-through passage of dye from the posterior chamber to the stroma, or vice versa. A study in progress has provided electron microscopic evidence of fenestrae in the endothelial cells of the iris capillaries in kittens. These morphologic data are highly suggestive that those iris vessels near the pigment epithelial layers should be permeable to NaFl, and further studies of the morphology and permeability of the feline iris blood vessels are also in progress to help understand this fascinating phenomenon. From the present NaFl and the preliminary morphologic data, it is apparent that the iris vessels of the cat may have a significant role in the formation of aqueous humor. A previous investigation brought forth that thought also. Sheie et al. reported that the anterior chamber volume and the intraocular pressure was reduced in totally iridectomized eyes of cats, and they suggested that the iris vessels were important to aqueous formation. Conversely, the iris vessels of the monkey (which are impermeable to NaFl) have little if any role in aqueous formation, as shown by studies in both normal and iridectomized eyes. Further inquiry into the role of the cat's iris vessels in aqueous formation is necessary.

Rodriguez-Peralta demonstrated the presence of intravenously administered acriflavine neutral in the nuclei of iris vessel endothelial cells, iris stromal cells, and ciliary body epithelial cells; however, he found none in the aqueous humor. The finding was interpreted as evidence of a blood-aqueous barrier against acriflavine neutral. The present study showed NaFl present in the iris vessels and stroma, in the ciliary body epithelium, and also in the aqueous. The marked dissimilarity of circulation times could account for the differences observed regarding presence or absence of dye in the aqueous. Our time was maximal for only a few minutes, and thus it is understandable that a high concentration of NaFl would be in the aqueous and iris tissues. Grayson et al. reported that after 1 hr of circulation, fluorescence (NaFl) in the iris stroma was no longer demonstrable. Because of the free movement of NaFl between the anterior chamber aqueous and the iris stroma, the lack of NaFl in the aqueous at that time would be expected as well. The inability therefore to detect acriflavine neutral in the aqueous after 1 hr of circulation could represent a “washout” of dye, with only the stained nuclei of the tissue remaining as foci of fluorescence. In this manner, dye could have crossed the blood-aqueous barrier and subsequently not have been detected in the aqueous. This explanation does not take away from the fact that a real dissimilarity could exist in the permeability of the blood-aqueous barrier to acriflavine neutral and NaFl. For example, Rodriguez-Peralta demonstrated a lack of permeability of the pigeon pecten (an intra-vitreal organ of birds) to acriflavine neutral, whereas we have shown this same structure to be permeable to NaFl but not to fluorescein-labeled dextrans.

The present study also demonstrated by
the critical technique of fluorescence microscopy that developing retinal vessels are impermeable to NaFl. Clinically, leakage of NaFl is noted in a number of ocular diseases in which neovascularization is one of the manifestations. As such, neovascularization itself, i.e., the immaturity of the vessels, has been considered a basis for leakage. However, Cunha-Vaz et al.4 found the immature kitten retinal vessels impermeable to trypan blue, Nomura21 demonstrated the immature rabbit retina vessels impermeable to ferritin and horseradish peroxidase, and this present study showed the same for NaFl in the kitten. Thus it is evident that a pathologic alteration of new vessels (as for old vessels) must occur in order for there to be abnormal permeability. In experimental diabetic retinopathy22 a widening of the intercellular junctions was demonstrated to be a mechanism of leakage, whereas in urethan-induced and phototoxic rat retinopathies23, 24 the development of fenestrations in retinal vessels has been identified as a probable cause for leakage. Because this present study showed that under certain conditions (i.e., a low-power magnification view of gross specimens) the wide-lumened immature capillary meshwork could simulate leakage of NaFl, care must be taken in interpreting angiographic data concerning neovascularization. A recent clinicopathologic study of retrolental fibroplasia in the human depicted angiograms of a large mesenchymal arteriovenous shunt that appeared to represent leakage of NaFl.25 However, the histopathology showed those shunts to consist of numerous wide-lumened vessels,26 and it is possible that leakage per se did not occur. In that same study,25 it is also interesting that no leakage was noted in vessels that were actually identified as neovascularization.

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