New method for studying immature retinal vessels in vivo

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The young kitten has proved to be a suitable experimental animal for studying in vivo effects of oxygen on immature retinal vessels. Visualization of these vessels by ophthalmoscopy, however, is unsatisfactory. A method is presented which affords good visualization of the retinal vessels by fluorescein angiography and which permits precise control of the intraocular pressure by use of an artificial anterior chamber.

There has been renewed interest in the effects of oxygen on immature retinal vessels with recent studies pointing to the necessity of determining exact arterial oxygen tensions (PO₂) associated with retinal damage. The arterial PO₂ level at which retinal lesions occur in the immature human eye has not yet been determined, and pediatricians treating premature infants having the respiratory distress syndrome are frequently faced with the risk of administering oxygen beyond clinical needs.1 One method of determining critical PO₂ levels now under investigation by the present authors is direct visualization of the incompletely vascularized retinas of full-term newborn kittens while arterial PO₂ levels are monitored.

Ophthalmoscopy of the full-term newborn kitten is unsatisfactory; this is due principally to a dense tunica vasculosa lentis and secondarily to a faint corneal haze. A method has been developed which permits long-term, high resolution visualization of the immature kitten retina while maintaining the subject in as near normal a physiological state as possible. This method utilizes an artificial anterior chamber (AAC) which has the same optical properties as the “limbal window” of Ashton and Cook,2 but has the principal advantage of permitting control of intraocular pressure.

Methods

Artificial anterior chamber (AAC). The upper portion of the AAC (Fig. 1) consists of a Lucite supporting ring into which a 10 mm. diameter cover slip is glued with Eastman No. 9-10 Adhesive. The lower portion is an annular Lucite ring having water inlet and outlet vents to which No. 20 disposable needles are attached by several inches of flexible hose; it has a beveled underside to match the contour of the scleral surface of the eye. When the two halves of the AAC are mated, the proximity of the underside of the cover slip and the vent holes is such that when water is introduced, air bubbles cannot become trapped.

The kitten is anesthetized with intravenous Nembutal. A tracheotomy is performed, and the
kitten's breathing is controlled by a Harvard small-animal respirator. A catheter is inserted cephalad into the carotid artery on the same side as the operable eye, and the kitten is placed in a restraining bed. After excision of the eyelids, the intraocular pressure of the eye (normally about 18 mm. Hg) is read on a tonometer. A peritomy is made at the limbus, allowing the conjunctiva to retract and exposing the smooth episcleral surface for application of the lower ring.

Eastman No. 9-10 Adhesive is applied to the base of the ring, which is then placed on the eye (Fig. 2A). Dinthermy is applied to the limbus, which also causes coagulation of the vessels at the root of the iris. The cornea, iris, anterior lens capsule, and anterior vascular tunic are excised. Lens substance is removed by gentle irrigation, and the posterior capsule and posterior vascular tunic usually remain undisturbed. (In very young kittens with a dense posterior tunica vasculosa lentis, the posterior capsule and vascular tunic are carefully excised.) Eastman No. 9-10 Adhesive is applied to the undersurface of the upper AAC supporting ring, which is then gently mated to the lower portion already on the sclera (Fig. 2B).

Isotonic saline at about 37° C. is slowly injected to fill the newly created anterior chamber. The intraocular pressure is raised to the desired level and is read on a manometer attached to the outlet hose of the AAC. The AAC pressure is maintained for about one hour to allow for stabilization of retinal circulation and to permit the kitten to recover from deep anesthesia; the animal is then maintained on light sedation.

**Microscope and positioner.** Observation of retinal vessels is accomplished by use of a standard Zeiss operation microscope having a modified light source. Because of the "off vertical" limitations imposed by the light source and the bulk of the microscope, orientation of the kitten eye with respect to the microscope objective is made by moving the animal. The microscope is mounted on a lathe bed (Fig. 3) along with a system of fine-motion positioners and a ball-and-socket vise which holds the kitten's restraining bed. The fine-motion positioners allow for movement of the eye along each of three mutually perpendicular axes, and the ball-and-socket allows for rotational motion. This configuration provides for maximum flexibility with a minimum of vibration, as is necessary for application of time-lapse cinematographic techniques.

**Light source and cameras.** The incandescent-lamp housing of the Zeiss microscope has been replaced by a Leitz No. 250 lamp housing which contains a 150 W XBO xenon lamp and a selection of interchangeable filters. Light from the xenon lamp passes through the existing Zeiss optical system, producing a beam approximately
parallel to the optical axis of the microscope. Light reflected from the retina passes through the microscope and a beam splitter which diverts the image to either a 35 mm. Zeiss still camera or a Bolex 16 mm. movie camera. The ratio of object size to image size on 35 mm. film at greatest magnification is 1:10, and on 16 mm. film it is 1:1.5.

Fluorescein angiography. In order to visualize capillary details of the retina, injections of 5 per cent fluorescein are given via the carotid artery catheter and the subsequent filling of the retinal vasculature is photographed. When fluorescein is being used, a No. 47 Wratten gelatin filter is inserted between the xenon light source and microscope, and a No. 15–G Wratten gelatin filter is inserted between the microscope and camera. Kodak Plus-X Pan film is used in the 35 mm. still camera, and Kodak Tri-X Reversal film is used in the 16 mm. movie camera.

Fluorescein dye is injected in quantities of 0.01 c.c. at a time. Since the fluorescein is introduced close to the eye, it reaches the retinal vessels still concentrated enough that the dye front may be photographed. About 40 such injections may be made over a period of several hours before the concentration of fluorescein in the systemic blood is sufficiently high to make successive dye injections indistinguishable.

Monitoring physiological parameters. The following parameters are monitored at the intervals indicated: (a) EKG, monitored continuously; (b) respiration, controlled continuously; (c) arterial PO2 and PCO2, monitored every 20 minutes; (d) arterial blood pressure, monitored continuously; (e) intraocular pressure, controlled continuously; (f) rectal temperature, monitored continuously; and (g) hematocrit, measured at beginning and end of procedure. Most of the equipment used to monitor the foregoing parameters during a typical procedure is shown in Fig. 3.

Results

Fig. 4 shows the retina of an 11-day-old kitten as seen through the AAC with an intraocular pressure of 0 mm. Hg; Fig. 5 shows the same retina (as seen through the AAC) at 18 mm. Hg intraocular pressure. The vessels (especially the veins) in Fig. 5 under pressure appear narrower and less tortuous than those in Fig. 4; these differences are presumably the result of...
the difference in intraocular pressures. Fig. 6 shows the nasal branch vessels after a fluorescein injection (18 mm. Hg intraocular pressure); visualization of the capillary bed is now possible. This method has permitted clear visualization of the retinal vessels in kittens for as long as 6 hours while the animals are maintained under light sedation.

Discussion

Ashton and Cook² viewed the retinal vessels of young kittens after removing the cornea, iris, anterior lens capsule, and anterior vascular tunic, removing the lens substance, and leaving the posterior capsule and posterior vascular tunic intact. A cover slip held in place by a limbal ring sutured to the sclera provided a
flat optical surface permitting excellent visualization of the retinal vessels. The "limbal window" presented two limitations, however: (a) inability to produce measured and controlled intraocular pressures, and (b) necessity of maintaining the kittens at a relatively deep level of anesthesia to prevent retinal detachments, which tend to eventually occur in the absence of intraocular pressure as extraocular muscle tone returns in lighter stages of anesthesia.

The artificial anterior chamber was developed to overcome these limitations. It has the principal advantage of allowing control of intraocular pressure, including maintenance at normal values. With a normal intraocular pressure, the animal can be allowed to recover from the surgical plane of deep anesthesia and be maintained on light sedation for long periods. Thus undesirable alterations in blood pressure, blood flow, and other physiological parameters accompanying deep anesthesia are eliminated, and retinal vessel response data are collected under physiological conditions closer to normal.

Consideration was given to the possibility that the surgical procedure might alter retinal vessel dynamics. Examination of serial fluorescein photographs taken at time intervals varying from 1/64 to 1 second revealed no abnormal retinal vascular flow characteristics or leakage. Electron microscopic sections of these same retinas taken 30 minutes after intravenous Thorotrast injection showed no abnormal vascular permeability.

Fluorescein angiography improves the resolution of retinal vessels and makes possible dynamic studies of the retinal circulation. Stabilization of the physiological parameters (including the intraocular pressure) has made this method useful in studying immature retinal vessel response to elevated arterial PO2 levels, as will be reported in detail later. The control of these factors may permit application of data from these animal studies to the human problem with a greater degree of confidence.

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REFERENCES