either group of patients. It should be noted that one of the patients with POAG, whose lymphocytes were studied, is the same individual whose trabecular meshwork cells in culture metabolized cortisol predominantly to DHF. These data indicate that the alteration in cortisol metabolism in the TMPOAG cells is not found in all cortisol metabolizing tissues.

Thus, the TMPOAG cells exhibit two alterations in cortisol metabolism. One is a marked increase (greater than 100-fold) in Δ⁴-reductase activity and the other is a decrease (4-fold) in 3-oxodoreductase activity. The relevance of this observation to the pathophysiology of POAG will depend upon the prevalence of these defects when a larger number of cells lines cultured from trabeculectomy specimens (and trabecular meshwork tissue) derived from POAG patients are evaluated. Future studies will examine the effect of age, sex, and time of processing of the specimens, as well as the identification of the TMPOAG cell lines using electron microscopy. The finding that the enzyme defects persist through many generations demonstrates its heritability in culture. Whether the heritable defect is caused by an inborn genetic error or results from effects of chronic ocular hypertension or prior drug therapy remains to be determined. Studies with cells cultured from trabecular meshwork specimens from patients with secondary glaucoma may help to resolve this question.

This study is the first demonstration of a defect in cortisol metabolism in cells obtained from human outflow pathway tissue in POAG.

Key words: cortisol metabolism, trabecular meshwork cells in culture, primary open angle glaucoma, dihydrocortisol

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References


Episderal Venous Pressure: A Comparison of Invasive and Noninvasive Measurements

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Noninvasive (pressure chamber) measurements of episcleral venous pressure were followed by invasive (direct cannulation) measurements at the same venous site in seven eyes of four anesthetized rhesus monkeys. There were three definite effects on the vein caused by the pressure chamber that could be used as endpoints: (1) slight indentation; (2) intermittent collapse; and (3) sustained collapse of the vein lumen. The mean pressure in the chamber corresponding to these endpoints was 9.9 ± 0.9, 23.5 ± 2.9, and 31.4 ± 4.0 mmHg (±SE), respectively. After the chamber was removed, the pressure in the veins determined by cannulation measurements at the same sites was 11.3 ± 0.5 mmHg (±SE). Therefore, the first endpoint with the pressure chamber (slight indentation) correlates most closely and slightly underestimates the cannulated pressure. Endpoints defined by partial or complete venous collapse overestimate the venous pressure. Simultaneous measurements with the chamber and a cannula show a rise of local venous pressure caused by the chamber. Invest Ophthalmol Vis Sci 24:1417–1422, 1983.

It is widely accepted that episcleral venous pressure is within the range of 9–11 mmHg in almost all normal
human eyes, as summarized elsewhere. Similar values have been obtained in rabbits. Primary open-angle glaucoma, and its treatment, appear to have little effect on episcleral venous pressure.

Several endpoints can be used during noninvasive measurements to determine episcleral venous pressure. The purpose of the present study was to ascertain in monkeys which endpoint best corresponds to the actual episcleral venous pressure measured at the same site in the same vein by cannulation and to determine the effect upon the local episcleral venous pressure of its noninvasive evaluation with a pressure chamber.

Materials and Methods. Instruments. Non-invasive venous pressure measurements are performed with either the operating microscope or slit lamp using a pressure chamber that fits a Goldmann® (Bern, Switzerland) applanation tonometer prism holder. This chamber has been described previously. The front wall of the chamber is a thin, pliable, polyurethane membrane. A saline-filled monometer is connected to the chamber. The height of the fluid column in the monometer can be raised with an attached screw syringe. The front membrane of the chamber is applied to the vein which is observed through the operating microscope or a monocular observing tube. Three endpoints were easily discernible as the pressure was varied in the pressure chamber (Figs. 2A-C). First, the initial, slightest narrowing of the vessel could be detected. Second, intermittent collapse of the vessel could be seen. Third, total, sustained collapse of the vessel occurred. When both observers verbally agreed the endpoint was reached, the corresponding manometric pressure was recorded by an assistant. Two to four sites were measured in each eye, and for each the determinations were repeated three to five times for each endpoint by reducing the chamber pressure to zero after each measurement, then raising the chamber pressure again. The observers were not informed of the result of previous determinations until all measurements at the venous site were completed. A mean value was used for analysis.

Next, for one of the measured sites a small nick was made in the wall of the branch vein near the limbus with sharp pointed scissors. A heparinized, saline-filled, tapered, polyethylene microcannula was introduced directed away from the limbus ("downstream") and advanced until it was within mm of the "Y-junction" (Fig. 3). The pressure in the vein at the junction was determined by the "stopflow" method with the cannula attached to a pressure transducer and fluid reservoir. The reservoir height was adjusted until blood refluxed slightly into the cannula and the front of the column stopped, except for slight oscillations back and forth with the venous pulse. It was observed that this endpoint was sharply defined and reproducible.

The pressure chamber then was adjusted so that the manometer pressure was almost zero, and barely enough to cause the membrane to protrude. This was applied again to the episcleral veins at the junction site, with the cannula still in place. The tip of the cannula could be seen with the microscope through the pressure chamber. The "stop-flow" pressure in the vein was redetermined by observing the cannula tip. The pressure within the chamber was raised in increments of 5 cm of saline. After each increment, the
Fig. 2A. Endpoints used in the present study for determination of episcleral venous pressure with pressure chamber. Endpoint 1: First, barely detectable, indentation of vein wall.

Fig. 2B. Endpoint 2: Initial intermittent collapse of vein.

Fig. 2C. Endpoint 3: Sustained collapse of vein with disappearance of blood column.
"stop-flow" pressure within the vein was redetermined using the cannula manometric system. In all, seven steps of chamber pressure were employed, the highest being 35 cm of saline (25.7 mmHg).

Results. Variation of the episcleral venous pressure was seen in this study when several sites on the same eye were measured noninvasively with the pressure chamber. For endpoint 1 the measurements had a range of 2-3 mmHg. For endpoint 2 and 3, the range was larger, being 3-8 and 4-12 mmHg, respectively. There was more variation when the different eyes were compared (Table 1).

Successful noninvasive determinations of the pressure corresponding to each of the three endpoints and subsequent invasive, cannulated pressure at the same site were obtained in seven eyes of four monkeys. The results of the noninvasive measurements clustered within 1-3 cmH<sub>2</sub>O for each endpoint at each site examined. A mean value was used for analysis. Noninvasive and invasive measurements are summarized in Table 1. For the sites that were subsequently cannulated, the mean pressure in the chamber was 9.9 ± 0.9 (±SE) mmHg, 23.5 ± 2.9, and 31.4 ± 4.0, respectively, for each of the three observable endpoints. The mean pressure at the same sites determined by cannulation was 11.3 ± 0.5 mmHg.

The relation of the pressure in the vein measured directly by cannulation and the simultaneous pressure in the chamber applied to the vein was studied successfully in five eyes of three monkeys. Even when the pressure within the chamber was nearly zero, simply applying the chamber to the vein caused a small rise of the pressure within the vein. The pressure measured with the cannula as a function of chamber pressure has an "S" shape (Fig. 4). During the first increments of pressing upon the vein there is little change of the pressure within the vein. At the higher chamber pressures the vein nears collapse and the curve flattens. For most of the curve the pressure in the vein was considerably higher than the pressure within the chamber, but the two pressures tended to approach one another at the higher levels. For endpoint 1, in the measurements before cannulation of the site, the pressure in the chamber was 9.9 mmHg, but when this was applied to the vein, the pressure within the vein was slightly more than 15 mmHg. Thus, the pressure being measured is affected (raised) by the measurement. At the higher chamber pressures the vein progressively narrowed and the pressure difference between the chamber and inside the vein became smaller.

Discussion. The evaluation of episcleral venous pressure by the noninvasive (pressure chamber) method always has been complicated by the absence of a single, clearly defined endpoint. Several authors have noted
The technique of applying the chamber to the vein then slowly raising the pressure within it until the endpoint is identified has been widely used in human studies. This was the technique used in the present study. The determinations of Phelps and Armaly and the air jet measurements of Krakau and colleagues were done, for the most part, with intermittent applications. Phelps and Armaly noted that "... there may be a difference of 2 to 3 mmHg between the pressure required to slightly indent the blood column and that required to completely obliterate the vessel." Our experience with young, normal, sitting humans indicates a spread of 5 to 7 mmHg between slight indentation and obliteration. In the present study of reclining, anesthetized monkeys, the spread was considerably more.

Phelps and Armaly reported a mean episcleral venous pressure of 9.0 mmHg when using an endpoint between slight indentation and complete obliteration. This agrees with the values from most other studies with humans. The higher values obtained by the airjet technique with a similar endpoint (12.8 to 17.5 mmHg) may reflect the different technique. It is most useful when noninvasive episcleral venous pressures are reported with the endpoint of the determination clearly defined, and preferably with the pressures corresponding to several endpoints. The present study indicates that the first detectable change in venous caliber is the endpoint that most closely estimates the local venous pressure; a similar conclusion was reached by Leith nearly 20 years ago.

**Key words:** episcleral venous pressure, non-invasive measurement, venous microconanulation, normal eyes, monkeys

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**References**

The Influence of Calcium on Protein Synthesis in the Rabbit Lens

Kenneth R. Highrower

Biosynthesis of lens proteins, as assessed by the incorporation of $^{14}$C-histidine, was investigated in young rabbit lenses cultured under conditions designed to specifically elevate lens calcium. While such lenses rarely were obtained without small degrees of Na/K imbalances, experiments with ouabain-treated lenses over comparable times indicated that changes solely in Na/K levels did not alter synthesis of lens crystallins. On the other hand, with far smaller changes in Na/K levels, excess calcium accumulation obtained by exposing lenses to A23187 or high levels of medium calcium invariably led to diminished synthesis of lens proteins. An increase in lens calcium from 0.2 mM to 0.6 mM led to a small but statistically insignificant decline in protein synthesis, while an increase to 1.4 mM or 1.9 mM resulted in a decline to values 50% and 11%, respectively, of the control. The results indicate that calcium may be important in influencing protein synthesis in the lens. Invest Ophthalmol Vis Sci 24:1422-1426, 1983

Since abnormally high concentrations of calcium often accompany cataract formation, it is important to consider the variety of physiologic processes that may be affected by alterations in lens calcium. For instance, inactivation of the Na/K-ATPase transport system recently was reported in opaque rabbit lenses in which membrane-bound calcium was elevated experimentally fourfold. Another potentially important role for calcium might be that of regulating protein synthesis, particularly in view of the recently reported role for calcium in biosynthesis of lens proteins. Since the role of calcium in bio-synthesis of lens proteins has received little attention, this fraction following precipitation of 10% trichloroacetic acid (TCA) and washing (three times in TCA). The precipitate of a 0.1-ml Sn sample was dissolved in 0.5 ml of 2 N NaOH for several hours. Hyamine was not used because of variable degrees of quenching. A 0.1-ml sample was assayed for radioactivity in 5 ml scintillation fluid, and 0.1 ml was assayed for protein content by Bio-Rad assays, using standards of bovine serum albumins in NaOH or buffer. Typical protein


Materials and Methods. To assess the influence of calcium on protein synthesis, lenses were precultured for 15–20 hours in a variety of media (Table 1) at 21° C to facilitate calcium accumulation in the lens. Pre-culture media, buffered with HEPES (5 mM), pH 7.3, contained either 20 mM or 1 mM CaCl$_2$ in addition to 5 mM glucose, 2 mM MgCl$_2$, 5 mM KCl, and 100 to 120 mM NaCl to achieve an osmolality of 300 mOsm. Following preculture, all lenses were transferred to TC199 at 37° C. Except where indicated, TC199 was a complete culture medium containing bicarbonate (2.2 g/l) and pregassed with 5% CO$_2$, balanced with oxygen and nitrogen. When the concentration of calcium normally present in TC199 (2 mM) was increased to 10 mM, bicarbonate and phosphate were replaced by HEPES (5 mM) to prevent calcium precipitation. The pH was maintained at 7.3. Lens culture at 37° C was limited to 5 hours, a period sufficient to measure amino acid incorporation.

To measure the extent of incorporation of $^{14}$C-histidine in 4- to 5-week-old rabbit lenses, lenses were incubated at 37°C for 5 hours in 2.5 ml TC199 media containing between 10 and 50 µCi labeled histidine at a specific activity of 343 Ci/mmol. After culture, lenses were blotted on water-moistened filter paper and homogenized in 1 ml of Tris/Ion buffer solution: KCl (120 mM), NaCl (20 mM), MgCl$_2$ (5 mM), CaCl$_2$ (0.2 mM), and Tris (50 mM) at pH = 7.3. Homogenates were centrifuged at 37,000 g for 20 min at 4° C on a Sorvall® RC-5B (Newtown, CT) centrifuge, and the supernatant (Sn) decanted for analysis. In the majority of experiments, radioactive histidine was assayed in this fraction following precipitation of 10% trichloroacetic acid (TCA) and washing (three times in TCA). A small sample (0.1 ml) of the TCA extract was assayed for radioactive histidine by adding to 5 ml of Dimilune-30® scintillation fluid and counting on a Hewlett-Packard® Liquid Scintillation System, Model 300C. The precipitate of a 0.1-ml Sn sample was dissolved in 0.5 ml of 2 N NaOH for several hours. Hyamine was not used because of variable degrees of quenching. A 0.1-ml sample was assayed for radioactivity in 5 ml scintillation fluid, and 0.1 ml was assayed for protein content by Bio-Rad assays, using standards of bovine serum albumins in NaOH or buffer. Typical protein