Applanation and Schiøtz tonometer standardizations for the owl monkey eye with a new technique for measuring episcleral venous pressure

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A Schiøtz P, calibration by open manometer was performed on 18 owl monkey eyes cannulated in situ. P, values for the owl monkey corresponded closely to those of the 1955 Friedenwald nomogram. The average ocular rigidity measured in 16 additional eyes with the use of clinical techniques was 0.0173 (± 0.0015). A manometric calibration for a 4 mm. diameter applanation face was done similarly on 21 eyes. The following standardization formula was generated:

Pressure in mm. Hg = 0.641 (tonometric scale reading) + 2.56 mm. Hg.

An attempt was made to compare the two tonometric pressure standardizations. In 16 intact eyes, Schiøtz P, pressures, corrected for ocular rigidity, averaged 0.77 ± 0.47 mm. Hg higher than corresponding applanation pressures. An improved method for measuring episcleral venous pressure, with the use of a new synthetic polyurethane pressure chamber membrane, is also reported.

Key words: applanation tonometer standardization, intraocular pressure of owl monkey, Schiøtz tonometer standardization, episcleral venous pressure, Aotus trivirgatus.

Several investigators have found the owl monkey (Aotus trivirgatus) to be a useful model for the study of primate (including human) ocular physiology. Smith and associates found the vascular anatomy of the owl monkey fundus quite similar to that of man and pointed out that the large anterior segment of the owl monkey made them advantageous for anterior segment experiments. Kalvin and co-workers and Zimmerman and colleagues used this species to study optic nerve and intraocular vascular changes in experimental glaucoma. These investigators noted that the axial length of the eye was 18 to 20 mm. and the corneal diameter was 13 to 15 mm. They assumed the 1955 Schiøtz scale would be sufficiently accurate for their purposes. However, no standardization experiments have been reported. Other in-
vestigators who have used the owl monkey include Hamasaki and Fujino, Machemer and Norton, and Kroll and Machemer.

Members of the genus Aotus are higher primates (Simiae). Their trabecular meshwork has the same three basic parts as the meshwork in all other higher primates, but the greater amount of connective tissue anterior to the ciliary muscle, the relatively undeveloped reticular structure of the meshwork, and the absence of lamellae and differentiated trabeculae are characteristics found also in the lower primates. Lower primates have a less differentiated chamber angle than higher primates; their chamber angle is similar to that of the rabbit. The trabecular meshwork of higher primates such as the rhesus monkey is histologically the most nearly identical to that found in the human being, but the anterior chamber of those higher primates which are widely available for study is generally small. Because the owl monkey has reverted to a nocturnal life typical of lower primates, it has the large anterior chamber seen in many lower primates. This unusual combination of a well-differentiated chamber angle with an anterior chamber of dimensions approximating the human eye makes the owl monkey eye especially suitable for the study of aqueous dynamics.

Recent experiments in our laboratory required Schiotz and applanation pressure standardizations of the owl monkey eye. Also, episcleral venous pressure measurements were done. The purpose of this paper is to present these standardizations and an improved pressure-chamber method of measuring episcleral venous pressure. In addition, a few experiments done to check the correlation of the standardizations will be described.

Methods

Schiotz P, standardization. Eighteen eyes were cannulated in situ with a 25 gauge needle fired from a needle gun. The animals were anesthetized with intravenous Nembutal and held supine in a mechanical holder. After the eyes had been used for perfusion outflow-facility measurements, the needle in the anterior chamber was connected via silastic tubing to an open manometer filled with saline. The level in the manometer was raised to the equivalent of 40 mm. Hg and the Crescent electronic tonometer head with a 5.5 Gm. weight was placed on the eye and stabilized with a mechanical holder. After one minute for pressure stabilization with the stopcock open, the pressure in the manometer was recorded and then lowered in small (about 2.5 mm. Hg) increments. The tonometer was allowed to stabilize at each new pressure. The procedure was then repeated with the 7.5 and the 10 Gm. weights.

Applanation standardization. After the preliminary experiments indicated a need for modification, the doubling prism in the Draeger11-12 hand applanation tonometer was set so that the applanation face was 4 mm. in diameter. The applanation doubling prism was calibrated with a metal cylinder machined with precision to a diameter of 4 mm. This enlarged applanation face had been recommended for use in animals by Schmidt.13 In 21 eyes prepared as above, the manometer was raised to the equivalent of 40 mm. Hg and five applanation measurements with the modified Draeger tonometer were made. The pressure was then lowered in small increments (about 2.5 mm. Hg) and five additional applanation measurements were made at each new pressure. For each eye, a least squares regression line was calculated and the equations for these lines were used to generate values from 5 to 40 mm. Hg at 2.5 mm. Hg intervals. A least squares regression line for the mean of all experimental eyes was calculated.

Correlation experiments. Clinical type measurements were made in 16 intact eyes of monkeys anesthetized with Nembutal. Five applanation measurements were made within five minutes prior to Schiotz measurements. A total of approximately fifty of these paired measurements were made. Applanation pressures were obtained from the regression line generated by the applanation standardization. Ocular rigidity was experimentally determined on each of these eyes on at least five previous occasions by placement of 5.5, 7.5, and 10 Gm. weights in quick succession on the tonometer head. A least squares computer program was used to determine the ocular rigidity of each eye. The experimentally determined average ocular rigidity for each eye was used to calculate the Schiotz estimate of $P_o$ according to the tables and the formulas presented by Becker and Shaffer14 for the human eye.

Applanation and Schiotz measurements were made in seven eyes treated previously with various drugs in another study. These eyes were...
cannulated and the intraocular pressures were measured with a P23BB Statham transducer. Applanation pressures were obtained as above. Schiötz P₄ values in this small series were obtained directly from the 1955 human scale without further corrections.

Episcleral venous pressure. A pelote pressure chamber for determination of the episcleral venous pressure was made available by Richard F. Brubaker. The pressure chamber was connected to a P23BB Statham transducer. The second port of the transducer head was connected to a 500 μl Hamilton syringe attached to a micrometer. Adjustment of the micrometer made it possible to change the pressure in the saline-filled system from 0 to 40 mm. Hg. A special transparent polyurethane membrane, of low modulus and high elasticity, produced for this purpose by Gulf South Research Institutes, Inc., New Orleans, La., was placed over the front end of the chamber.

The chamber was mounted on a Zeiss slit lamp with the front membrane in focus. The preselected episcleral vein was observed under 40x magnification. Slowly and precisely the pressure was increased by turning the micrometer. The pressure at which the flow within the vessel was diminished to 50 per cent of its original level was taken as the endpoint. That area of the vessel used for the endpoint was always placed at the center of the membrane and care was taken to use the same point on the vessel at each examination. In the owl monkey, this point was 1 to 1.5 mm. from the limbus. During the measurements the animal was anesthetized with Nembutal and held supine in a mechanical holder.

Results and discussion

Schiötz P₄ standardization. The solid dots in Fig. 1 represent the average scale readings with brackets enclosing ± 1 S.E. The open circles are the 1955 human P₄ standardization. Although differences between the Aotus and the human P₄ calibrations are evident, the differences are small enough so that the 1955 human Schiötz calibration may be taken as a good approximation for the owl monkey. This is not unexpected as the general dimensions of the Aotus anterior chamber are similar to those of the human anterior chamber. The average radius of corneal curvature of Aotus was 7.32 (± 0.09) mm., as measured by keratometry.

Schiötz, himself, used eyes in situ, cannulated with a needle, for his "final graphs." Friedenwald used enucleated eyes but placed them in an artificial orbit designed to imitate the eye in situ. In this important monograph, Friedenwald discussed the reasons why the stopcock to the manometer should be left open and a P₄ calibration done, rather than a closed stopcock P₄ calibration. The main problems with the closed stopcock measurements were the large variations due to ocular rigidity and excessive outflow of fluid via the usual routes after the stopcock was closed.

Applanation standardization. A regression line for the mean experimental values obtained as described above can be seen in Fig. 2. This line is described by the following equation:

Tonometric scale reading =
1.56 (intraocular pressure in mm. Hg). -4.00 mm. Hg

Simple manipulation puts it in a more useful form:

Pressure in mm. Hg =
0.641 (tonometric scale reading) + 2.56 mm. Hg.

The equation in this second form is remarkably similar to those given by Schmidt for other animals.

Goldmann and Schmidt and Schmidt have stated that in the human eye, the elastic forces which must be overcome to initiate corneal applanation are neutralized by oppositely acting forces of capillary attraction when a circular area of about 3 to 3.5 mm. diameter is flattened. This fortuitous balance does not occur in any animal eye presently standardized. Schmidt recommended a 4 mm. diameter applanation face for cats, dogs, and rabbits because intraocular pressures below about 8 mm. Hg could not be measured with the more convenient 3.06 mm. diameter face in these animals. Similarly, a standardization done with the standard 3.06 mm. diameter face for the owl monkey produced a line somewhat to the right of that seen in Fig. 2. This would have been unusable for the lower pressures. The
present standardization line in Fig. 2 does not go through the origin, indicating that the elastic forces opposing applanation do not precisely equal the forces of capillary attraction acting upon the 4 mm. diameter face used with this animal.

It was not necessary to close the stopcock during the application of the applanation tonometer because of high resistance in the small gauge needle cannula. Application of this tonometer for 15 seconds caused a change in the manometer level equivalent to no more than 0.1 mm. Hg.

The primary purpose of this study was not to provide detailed insight into the variability of a single applanation measurement; however, some of the results may be of interest. Five applanation measurements were made on each eye at each experimental pressure. The standard deviations of these five measurements were calculated for each set each time they were done. The average of these standard deviations was 0.64 (± 0.22) mm. Hg. There was no obvious increase in these standard deviations with increasing pressure in any given animal.

However, when all applanation measurements for the 21 eyes were averaged for each manometric pressure, the standard deviations of applanation measurements (made by a single observer) tended to increase with higher pressures. (For example, at 10 mm. Hg manometric pressure the S.D. of the applanation measurements was 1.90 mm. Hg. At 20 mm. Hg pressure the S.D. was 1.97 mm. Hg, at 30 mm. Hg the S.D. was 2.83 mm. Hg, and at 40 mm. Hg the S.D. was 3.94 mm. Hg.)

Goldmann and Schmidt have presented applanation standardization data on freshly enucleated human eyes and on cadaver eyes graphically. The variability
Fig. 2. Manometric applanation standardization for applanation face 4 mm. in diameter for 21 owl monkey eyes. Dots are average (± 1 S.E.) of scale reading values generated as described in text. Least squares regression line equation is $Y = 1.56X - 4.00$. A more useful form of the equation is:

$$\text{Pressure mm. Hg} = 0.641 \times \text{(tonometric scale reading)} + 2.56 \text{ mm. Hg.}$$

of measurements reported here in eyes of live animals, taken with the hand-held Draeger tonometer, is somewhat greater than that of the Goldmann measurements on freshly enucleated eyes. The variability reported here is about the same as that of Goldmann's cadaver measurements.

**Correlation studies.** In the 16 eyes in which clinical-type measurements were done, Schiötz $P_0$ pressures averaged higher than the corresponding applanation pressures. The average differences was 0.77 (± 0.47) mm. Hg. Average ocular rigidity in these eyes was 0.0173 (± 0.0015). Corrections for variation in ocular rigidity among different eyes were significant and changed the corrected values by 0.4 to 3.6 mm. Hg. Precision required multiple determinations of ocular rigidity.

The average Schiötz $P_0$ pressure mea-
measurement in these 16 uncannulated eyes from live animals was 15.7 ± 1.7 mm. Hg. Ten of these eyes were also measured again by applanation as controls prior to a drug study and had an average pressure of 13.2 ± 2.3 mm. Hg. The correlations of this small series are generally comparable to those reported between human applanation and Schiötz $P_e$ values, except that in the human studies the Schiötz values were generally lower than the applanation values. One reason for this difference may be the lack of correction for ocular rigidity in the clinical studies.\(^1\)\(^9\)\(^-\)\(^2\)\(^2\)

In the seven eyes measured manometrically, Schiötz $P_e$ measurements averaged 1.6 (± 1.6) mm. Hg higher than the transducer measurements, and applanation measurements averaged 1.1 (± 2.3) mm. Hg lower than the transducer pressures.

Pressures in the cannulated eyes were measured after the animals had been under Nembutal anesthesia for one hour. Pressures in the intact eyes reported above, were taken within ten minutes after the onset of anesthesia. Probably longer duration and greater depth of anesthesia in the cannulated animals were the major reasons that pressure measurements in the cannulated eyes were generally slightly lower.\(^2\)\(^3\)\(^-\)\(^5\)\(^\)

**Episcleral venous pressure ($P_v$).** In three eyes (used as controls in other experiments\(^1\)\(^\)\(^9\)) the $P_v$ averaged 10 (± 1.6) mm. Hg. Other values reported for higher primates with the use of pressure chamber methods were similar. These include 12.1 (± 1.0) mm. Hg for the Rhesus monkey\(^2\)\(^6\) and 11.6 (± 0.22).\(^2\)\(^7\) 8.3 (± 0.2),\(^2\)\(^8\) and 10.4 (± 0.21)\(^2\)\(^9\) for the human being.

The point on the vein selected for measurement of $P_v$ was 1 to 1.5 mm. from the limbus due to the difficulty of rotating the owl monkey eye. Previous investigators have chosen points more distant from the limbus in other animals in order to avoid any influence of intraocular pressure on the blood flow and pressure in the episcleral vessel at the point measured. In a series of 33 measurements made near the limbus with this system and reported elsewhere,\(^2\)\(^9\) no correlation was found between intraocular pressure and episcleral venous pressure. The owl monkey, however, is not the ideal primate for experiments concerning episcleral venous pressure due to the small area of exposed sclera in the owl monkey eye. The method described here has worked well also in rabbit experiments in our laboratory in which the point of measurement was 2 to 3 mm. from the limbus.

Previous nondestructive measurements of $P_v$ have made use of either a pelote or a flat surface to indent or obstruct the episcleral vessel.\(^2\)\(^7\)\(^-\)\(^2\)\(^9\) Brubaker\(^1\)\(^5\) compared these two methods with direct cannulation and found that the endpoints with the pelote method were easier to reproduce, corresponded well with the cannulation pressures, and had considerably less variances. The polyurethane membrane used in our investigation is more transparent than the frog pericardium used by Brubaker and Kupfer\(^2\)\(^6\) and facilitated our measurements.

Other pelote techniques have involved increasing the pressure by hand bulb,\(^1\)\(^5\)\(^,\)\(^2\)\(^6\) foot pedal,\(^2\)\(^9\) or air pump\(^3\)\(^1\) and stopping the rising pressure "at the very instant" the observer "saw the vein collapse."\(^2\)\(^7\) With the use of our method, the pressure could be more precisely adjusted, up or down, by turning the micrometer knob.

This precision of adjustment made a more exact observation of the endpoint possible. Leith\(^2\)\(^9\) has pointed out that using the obliteration point as the endpoint may give values 0.25 to 2.0 mm. Hg higher than using that point where flow is just altered. In several of our experiments with the use of the polyurethane membrane, the obliteration point was observed to be higher than the intraocular pressure. This indicated that the obliteration point was somewhat above the true episcleral venous pressure. The endpoint used in these experiments, a 50 per cent decrease in blood flow, was a compromise between "the instant when the vessel wall was first ob-
served to yield to the externally applied force" used by Leith29 and the "obliteration point" used by Brubaker.15

The Imbert-Fick law states that the pressure in a sphere filled with liquid and surrounded by an infinitely thin membrane is measured by the minimum counterpressure required to flatten the membrane to a plane.13 The pelote pressure measurement is based upon the Imbert-Fick principle. Technically it is not possible to see the vessel wall well enough to judge when it is just beginning to flatten. What is more easily seen is a change in blood flow. In our experience it is easy to overestimate the episcleral venous pressure by obliterating the blood column completely because continued application of increasing pressure will show no change after obliteration. With the polyurethane membrane, the most satisfactory and reproducible technique to reach the endpoint has been to compress the vessel with the pelote until there is approximately a 50 per cent reduction in blood flow. By exceeding this pressure until the flow is stopped, and then by lowering pressure until no change in flow is present, one may easily go just above and just below this endpoint. A graphic record of these super- and sub-threshold pressures makes it possible to select the midpoint which corresponds to an approximate 50 per cent reduction in blood flow.

Ten Pₐ measurements were made on each of six monkey eyes with the use of the obliteration point as the endpoint. The standard deviations of these measurements averaged 1.71 (± 0.32) mm. Hg. Two weeks later, ten measurements were made on each of the same eyes with a 50 per cent reduction in blood flow as the endpoint. The standard deviations of these latter measurements averaged only 0.85 (± 0.71) mm. Hg.

A similar experiment was done on six rabbit eyes. The obliteration endpoint produced an average standard deviation of 1.18 (± 0.28) mm. Hg, and the 50 per cent reduction in blood flow as an end-point produced an average standard deviation of only 0.50 (± 0.35) mm. Hg. The latter is similar to the 0.57 standard deviation reported by Brubaker15 using this same pressure chamber with the frog pericardium as the membrane.

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REFERENCES