chambers of all eyes was not greatly different from that of the anterior chambers, the difference between the two chambers was significant ($P < 0.001$). Similarly, in rhesus monkeys anesthetized with pentobarbital sodium, the concentration of ascorbic acid in the posterior chamber exceeded that in the anterior chamber; however, the magnitude was greater. The posterior chamber concentration was 11 per cent higher than the anterior chamber (24.41 mg. per cent versus 21.76 mg. per cent). The difference between the two chambers was significant ($P < 0.005$).

The ascorbic acid concentration in the posterior chambers of monkeys anesthetized with pentobarbital sodium exceeded that of monkeys sedated with phenacyclidine hydrochloride (24.41 mg. per cent and 22.60 mg. per cent, respectively). However, the concentration in the anterior chambers was the same no matter which anesthetic agent was used (21.76 mg. per cent with pentobarbital and 21.12 mg. per cent with phenacyclidine). These results are in general agreement with those reported by Becker, Krupin, and Podos for rabbits anesthetized with phenobarbital sodium. They noted that one hour after phenobarbital anesthesia the ascorbic acid concentration in the posterior chamber increased from 27.3 mg. per cent to 30.9 mg. per cent. The anterior chamber concentration remained essentially the same, 20.4 mg. per cent before phenobarbital and 21.2 mg. per cent one hour after. They associated the increased posterior chamber ascorbic acid concentration after phenobarbital with a reduction in aqueous humor formation. It has been observed that rhesus monkeys anesthetized with pentobarbital sodium also have a reduction in aqueous humor formation. The higher posterior chamber ascorbic acid concentration found in the present study after pentobarbital, as compared to that after phencyclidine, may also be associated with a reduction in aqueous formation.

If the ascorbic acid concentration in the posterior and anterior chamber is known, as well as the time required for 100 per cent turnover of aqueous humor, then an ascorbic acid diffusion coefficient can be calculated. Using the posterior and anterior chamber concentrations found in this study and the turnover time previously reported by Cevario and Macri, the ascorbic acid diffusion coefficient was calculated for rhesus monkeys anesthetized with pentobarbital sodium and sedated with phenacyclidine hydrochloride. Monkeys given pentobarbital had an ascorbic acid diffusion coefficient of 0.0010 min.\(^{-1}\); monkeys given phenacyclidine had a coefficient of 0.0012 min.\(^{-1}\). These numbers are not significantly different ($P > 0.400$). The time required for 100 per cent turnover of aqueous humor in monkeys anesthetized with pentobarbital is twice that in monkeys given phencyclidine (114.19 minutes versus 56.72 minutes). Therefore, even though the aqueous remains in the anterior chamber longer after pentobarbital administration than after phencyclidine, the diffusion coefficient of ascorbic acid between plasma and anterior chamber remains the same.

Becker reported an ascorbic acid diffusion coefficient for the rabbit of $0.0046\text{ min.}^{-1}$. This is higher than found for the rhesus monkey and probably represents a species variation. The knowledge of the ascorbic acid diffusion coefficient for rhesus monkeys is useful, in that it provides a method for calculating either the posterior or anterior chamber ascorbic acid concentration for those experimental conditions where the concentration in only one chamber can be determined directly.

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Key words: rhesus monkey, posterior chamber, anterior chamber, ascorbic acid concentration, diffusion coefficient, phencyclidine, pentobarbital.

REFERENCES


Experimental tonography on enucleated human eyes. II. The loss of intraocular fluid caused by tonography. J. Hetland-Eriksen and Tor Odberg.

In clinical work, the loss of intraocular fluid caused by tonography ($dV$) is usually calculated according to the formula of Grant and Frieden-
woald. The validity of this formula has been tested by comparing the dV calculated from known factors in the formula with the dV measured directly on enucleated eyes. Our results show a good correlation between the two methods, especially when the rigidity coefficient at the beginning of tonography is used in the calculations. It is concluded from our experiments here and in our previous paper that the theoretical foundation of tonography in principle is valid.

In a previous investigation, we have tested the validity of the tonography formula by means of experimental tonography and, under particular assumptions, the formula was found valid. The loss of intraocular fluid due to tonography (dV) was directly determined, and not calculated according to Grant, and Friedenwald. In the present investigation, we want to obtain an experimental control of dV when this value is calculated according to the modified Friedenwald's formula:

$$dV = dV_1 + dV_2 = (V_n - V_{i1}) + \frac{1}{K} \left( \log P_{i1} - \log P_{n1} \right).$$

Here dV1 corresponds to the change of corneal and posterior bulbar deformation during tonography, and dV2 represents the change of volume caused by scleral contraction as the pressure falls.

Friedenwald, worked out tables for the corneal indentation (Vc) based on measurements on excised corneas mounted in a special chamber. However, Vc hardly expresses the total deformation of the eye during tonometry, as there is also a deformation of the posterior part of the eyeball. One of us (H-E) has therefore worked out a method for determining the total deformation of the eyeball during tonometry, Vt. Tables for Vc show somewhat higher values than tables for Vc. Using a slightly modified technique, and at room temperature, Moses and Grodzki found approximately the same Vc values as Friedenwald.

So far as is known, Friedenwald’s formula for calculating dV has not been confirmed experimentally. To do this, it is essential that the absolute values for all factors are known. We decided, therefore, to use the same model system for experimental tonography on enucleated eyes as described previously. Thus, dV can be calculated by Friedenwald’s formula, and compared with the dV determined directly.

**Materials and methods.** The material consisted of 10 of the 14 enucleated human eyes previously described. Three eyes were omitted due to technical failure. Eye No. 12 was rejected as the Vc measurements were considered inaccurate because of great corneal deformation.

The eyes were perfused at a constant rate through a needle in the anterior chamber. The intraocular pressure was continuously recorded by a transducer via a needle in the anterior chamber. When a steady-state was reached, a conventional tonography was performed. The loss of fluid during the procedure (dV) was measured directly. The constant-rate perfusion was continued unaltered, and it was checked that the intraocular pressure remained at a steady-state level. The system was then opened to the fluid reservoir which was used for the dV measurements. This was set to P1 level, i.e., the intraocular pressure immediately after starting the previous tonography. Determinations of Vt were then performed according to the technique described by Hetland-Eriksen, using the same Mueller electronic tonometer as in the tonography. The measurements were repeated until at least three consecutive readings did not diverge by more than 0.5 µl (except for eye No. 3). The fluid reservoir was now set at P0, i.e., the intraocular pressure at the end of the previous tonography, and Vt measurements were determined in the same manner.

**Results and discussion.** In Table I the measurements of dV, Vc, and Vt are shown together with the corresponding values for P0, P1, and P2, as well as P0 and P0 (the intraocular pressures immediately before and after tonography). All these values are clearly defined. The pressures could be read from the transducer recordings during the tonography. Each Vt measurement had to be repeated three to six times in order to obtain consistent values. This was not unexpected, knowing that at each tonometry there is a deformation and a slight displacement of the eyeball. In eye No. 3, four measurements were done: 22.5-24-21-21 µl, and Vt was determined to 22 µl.

Based on the data in Table I, it is now possible to calculate dV according to the formula of Grant and Friedenwald. The question which
then arises how to determine the rigidity coefficient to be used in the calculations? K may be determined by three different formulas using the values in Table I:

\[ K_1 = \log \frac{P_{t1}}{V_{t1}} - \log \frac{P_{o1}}{V_{o1}} \]
\[ K_2 = \log \frac{P_{t2}}{V_{t2}} - \log \frac{P_{o2}}{V_{o2}} \]
\[ K_3 = \log \frac{P_{t3}}{V_{t3}} - \log \frac{P_{o3}}{V_{o3}} \]

Table II shows the values of measured dV compared to the calculated dV, + dV, using data in Tables I and II. The coefficients of correlation between dV and dV, + dV, were 0.93 when K1 was used in the calculations, and 0.80 when both K2 and K3 were used. Thus, the best coefficient of correlation, 0.93, was obtained when the tonometric rigidity coefficient calculated at the beginning of the tonographic period was used. This correlation must be considered as a remarkably good result.
Our experiments in this and the previous investigation with controlled tonography show that the facility of outflow can be determined by the way postulated by Grant\(^2\) and Friedenwald.\(^3\) Our controlled experiments have included the same assumptions regarding unchanged intraocular blood volume and stable production of aqueous as put forward by those authors more than 20 years ago. It is evident that these factors will influence the results of tonography in vivo. We are, however, concluding that the theoretical basis of the controlled tonography is in accordance with our practical experiments and, therefore, in principle is considered correct.

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**Key words:** tonography, experimental tonography, ocular volume displacement, ocular rigidity, fluid loss by tonography, enucleated eyes.

**REFERENCES**


**Analysis of ascorbate concentration in the aqueous humor by high-pressure liquid chromatography. Kwok-Wai Lam and Pei-Fei Lee.**

A high-pressure liquid chromatography instrument has been used to establish a method for the analyses of ascorbate concentration in aqueous humor. The method detects only the reduced form of ascorbate. The values obtained by this method are very similar to those of the colorimetric method. The ascorbate value in a group of random samples obtained from human glaucoma eyes varied between 3.5 to 31 mg. per cent, independent to blood ascorbate concentration.

Investigations of the biochemical changes associated with eye disease have been a difficult task. A major difficulty is due to the small amounts of pathologic samples, which can be obtained from the eye for biochemical analyses. Therefore, it is important to fully exploit modern technology of microanalysis in this area of investigation.

High-pressure liquid chromatography has become increasingly important for microanalyses of a wide variety of biological compounds.\(^1\) Recently, this laboratory explored the possible application of this instrument for microanalyses of the biochemical content of aqueous humor. During the initial study it was found that the modern liquid chromatography instrument can be used to develop a very sensitive and rapid procedure for the analysis of ascorbate concentration in aqueous humor. The detailed procedure established for the analysis of ascorbate and the preliminary analyses of eight aqueous humor samples obtained from glaucoma eyes are discussed in this report.

**Methods.**

Aqueous humor collection. Aqueous humor samples were obtained under an operating microscope at the time of glaucoma surgery in all eyes. No eye drop medication was given for at least 14 hours before the operation. After the routine preparation, a sliding peripheral corneal incision was made with a Wheeler knife. A No. 30 gauge or No. 27 gauge needle cannula attached to a disposable tuberculin syringe was used to aspirate aqueous humor without totally collapsing the anterior chamber. Usually, about two-thirds the amount of total aqueous humor with withdrawn without injury to the iris or corneal endothelium by the tip of the needle cannula. Repeated withdrawal of aqueous humor was not attempted in any case in order to avoid the contamination of the secondary aqueous humor. If the anterior chamber was lost following the corneal incision, or during the cannula insertion into the anterior chamber, the aqueous sample collection was abandoned. The anterior chamber was then re-