Uveal blood flow determined by the nitrous oxide method

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By application of the Kety-Schmidt nitrous oxide method to the study of uveal blood flow, the total uveal blood flow in dogs was determined. By use of a 15 per cent N\textsubscript{2}O, 85 per cent O\textsubscript{2} mixture, the uveal blood flow was found to be 0.56 c.c. per gram of uvea per minute.

Extensive investigation of the uveal circulation and ocular blood flow has been carried out since the original work by Waller\textsuperscript{1} in 1856. A dynamic determination of the uveal blood flow without alteration of normal physiologic processes has been hampered by the complexity of the vascular supply and its inaccessibility. Several recent reviews\textsuperscript{2-4} have summarized previous methods used.

The significance of the Kety-Schmidt\textsuperscript{5} nitrous oxide method for determination of regional organ blood flow, including ocular blood flow, was recently emphasized by Walsh.\textsuperscript{2} This method, originally applied to the determination of total cerebral blood flow and subsequently to cardiac, renal, and hepatic blood flow,\textsuperscript{6, 7} is of importance because it allows accurate determination of organ blood flow without operative interference in the organ involved. In addition, the method was the forerunner of many indicator-dilution techniques currently utilized in circulation studies of other organs.\textsuperscript{5, 6}

This article introduces this technique to the study of ocular blood flow and concerns adaptation of the nitrous oxide method for determination of uveal blood flow in the dog. A second report\textsuperscript{10} utilizes these data for quantitative determination of uveal tissue respiration and glycolysis in vivo.

Application of the nitrous oxide technique

The nitrous oxide method is based on the Fick\textsuperscript{11} principle, that the quantity of any substance taken up in a given time by an organ from the blood which perfuses it is equal to the total amount of the substance carried to that organ by the arterial inflow less the amount removed by the venous drainage during the same time period.
Kety and Schmidt\textsuperscript{12} expressed this theory in mathematical terms:

\[ \text{CBF} = \frac{100 \times V_u \times S}{\int_0^u (A - V) \, dt} \]

Where:

- \( \text{CBF} \) = cerebral blood flow in cubic centimeters of blood flow per 100 Gm. brain tissue per minute.
- \( V_u \) = venous blood nitrous oxide concentration at time \( u \) when equilibrium between nitrous oxide concentration in the venous blood draining the organ and that of the brain tissue has been achieved.
- \( S \) = the partition coefficient for nitrous oxide difference from beginning of N\textsubscript{2}O administration (time zero) to the time \( u \) at which equilibrium of the N\textsubscript{2}O concentrations between brain tissue and venous blood draining the organ is obtained.
- \( \int_0^u (A - V) \, dt \) = the integral of the arteriovenous N\textsubscript{2}O difference over time \( u \).

During a suitable period of inhalation of a constant N\textsubscript{2}O (a metabolically inert gas) concentration blood samples were taken from a peripheral artery and from a vein containing venous drainage representative of the organ studied. Before local tissue saturation occurs, the venous samples show less N\textsubscript{2}O than the arterial; after saturation they are nearly equal. The venous blood gas concentration multiplied by the organ tissue-blood partition coefficient \( S \) makes it possible to estimate indirectly the inert gas uptake in the total organ.

Several minor modifications have been introduced in the technique.\textsuperscript{13-15} The continuous sampling techniques of Scheinberg and Stead\textsuperscript{14} and Lambertson and Owen\textsuperscript{15} reduced the number of blood samples necessary, and are important in studying ocular flow. The theory behind the continuous sampling techniques has been described by Lambertson and Owen. If \( \int_0^u (A - V) \, dt \) is the integral of the arteriovenous difference over time \( u \), and if their N\textsubscript{2}O contents may be considered to be automatically integrated with respect to time, the difference between them then represents the mean value of the arteriovenous N\textsubscript{2}O difference for that period. If time \( u \) is considered to be 10 minutes, then Formula 1 reduces to:

\[ \text{CBF} = \frac{10 \times V_u \times S}{(A - V)} \]

Application of these methods to the eye requires that certain basic assumptions be tested: (1) Blood from the region under study must show minimal contamination from other regions, and must be representative venous drainage of the organ under study. (2) The time required for equilibrium between the local tissue under study and its venous blood must not be impractically long. (3) The uveal tissue-blood partition coefficient must be measured as it cannot be assumed to be equal to the brain-blood value \( S \) used in Formula 1 by Kety and Schmidt. (4) All the ocular tissues cannot be assumed to have the same partition coefficient for N\textsubscript{2}O. The various tissues are represented in the blood flow determination in proportion to the ratio of their weight and relative N\textsubscript{2}O solubility. If any have a very slow clearance rate of N\textsubscript{2}O, they will be represented less than in proportion to their weight. Since the eye shows great heterogeneity of tissue and since only a small area is actually perfused with blood, it is essential to determine that N\textsubscript{2}O diffusion is limited to the tissues of the uvea and does not diffuse, during the time period of the experiment, into the vitreous, lens, or sclera. The problem of the aqueous will be discussed subsequently.

Preliminary studies of uveal anatomy, microgasometric techniques, and ocular tissue solubilities for N\textsubscript{2}O were made to examine these foregoing assumptions. Ocular pressure and blood flow effects were studied to determine the physiologic status.
of the eye during the study. It has been found that the 15 per cent N₂O administration does not affect the blood flow measurements, although the supplementary gases may.

Methods

Anatomic considerations. Preliminary to the study, it was necessary to determine that a representative mixed venous sample would be obtained from the eye of the dog. The anatomy of the dog eye was examined by means of casts prepared for us through the courtesy of Dr. Frank Macri, according to the techniques that he has described in which Tygon paint TP-12 is injected into the anterior ciliary vein or vortex vein immediately after enucleation from living animals. These casts were used to show the degree to which the venous systems of the anterior and posterior segments communicated. Photographs of the cast as seen through the transparent sclera were made, and a composite drawing was made from the photographs.

The venous casts of the dog eye are quite similar to those of the cat, which Macri had previously studied, and which were expected from the work of Duke-Elder. Casts prepared by injection into the anterior ciliary vein or vortex vein were found to be identical. Branches from the circle of Hovius gave rise to the anterior ciliary vein, while other branches of the circle anastomosed with the vortex veins. The vessels of the choroid, ciliary body, and iris could easily be discerned, as well as their anastomoses with the circle of Hovius.

As a result of these findings, anatomic evidence is presented to show that blood obtained from the anterior ciliary vein or a vortex vein would be representative mixed venous blood from the uveal tract. Other evidence for physiologic mixing will be presented subsequently (Fig. 1).

Determination of N₂O. The quantitative determination of nitrous oxide in the small blood samples available from the venous drainage of either the vortex or anterior ciliary veins necessitates a microprocedure modifying the generally accepted manometric technique. The procedure used herein is described in detail by Van Slyke and Plazin. Because of the solubility of N₂O in mineral oil, which would distort the actual blood
concentrations measured, all blood specimens were collected under 0.25 c.c. mineral oil in 5 mm.
bore test tubes maintained in a constant ice bath prior to prompt N₂O determination.

The microtechnique utilizes a 10 ml. gas extraction chamber designed for attachment to the
standard 50 ml. Van Slyke-Neill manometric apparatus, which, with needle-tipped micropipettes,
can measure blood gases on 0.2 ml. blood samples to approximately ±0.25 per cent standard
error.

Determination of N₂O solubilities in blood and uveal tissue in vitro. The factor S, representing
the tissue-blood partition coefficient, was obtained by the technique used by Ketley¹⁸ of equilibrating
homogenized tissue and blood with 100 per cent N₂O under constant conditions. Instead of brain
tissue, the entire uveal tract of the dog eye, including choroid, iris, and ciliary body (average
weight, 340 milligrams) were excised after the technique of Macri¹⁹ and used analogously for
the brain tissue.

Freshly shed, heparinized, whole dog blood was used. A sample of about 1 Gm. of uveal tissue
was accurately weighed and homogenized in a glass homogenizer. The homogenate was pressed
through gauze to remove small shreds of connective tissue, and transferred to a 5 mm. bore test
tube covered with 0.25 c.c. mineral oil, as was a similar volume of blood. One hundred per cent
N₂O was equilibrated with these by constant gentle bubbling through the fluids in a 37°C
water bath for 1 hour. The N₂O concentration was then determined on both blood and uveal samples
in the manner previously described.

In four separate experiments the uveal tissue-blood partition coefficient was 1.06, as compared
with the brain-blood coefficient as determined by
Kety of 1.03. As with Kety, the value of S was taken as unity in all experiments.

Diffusion of N\textsubscript{2}O into vitreous and aqueous humor. In order to demonstrate the quantitative, diffusion of inert gas into the vitreous, autoradiographs of the eye made in conjunction with studies of localized brain tissue blood flow were examined. I-131-trifluoroiodomethane is employed as a tracer substance. Its concentration in the blood and tissues can be estimated from the autoradiographs by densitometric analysis.

The theory behind this technique is based on the principle that in the absence of diffusion limitations or arteriovenous shunts, the quantity of a circulating inert, diffusible tracer substance taken up from the blood by a given mass of any homogeneous tissue is a function of: (1) the blood flow to that tissue, (2) the time from the appearance of the tracer, (3) the partition coefficient for the tracer between the tissue and the blood, and (4) the record of the arterial concentration of the tracer since the onset of its circulation. If these variables can be determined, then the blood flow to the tissue can be calculated by the equation derived by Kety.

Typical radioautographs of frozen sections of brains from decapitated conscious cats as prepared by Sokoloff and colleagues showing effects of different concentrations of the inert radioactive gas in the various cerebral structures as well as in the choroid and vitreous are presented in Fig. 2. The circular areas of uniform density represent calibrated I-131-containing gelatin solutions used for quantitative densitometric analysis. It can be noted that the vitreous, which remained in place on the sections, has essentially no optical density, indicating absence of blood flow at a low partition coefficient. Since the I-131-trifluoroiodomethane has diffusional properties similar to those of N\textsubscript{2}O, it reasonably may be assumed that N\textsubscript{2}O diffuses extremely slowly into the vitreous and that such loss is of negligible concern during the time period of N\textsubscript{2}O equilibration with the uveal tissue in our study.

Of additional interest is that the optical density of the choroid is similar to the denser brain
tissue structures in the same section. Although densitometric analysis cannot be performed on an area of this size, from comparison of the tissues it may be seen that the blood flow determined by this technique is quite similar. It must be understood from the theory of this technique described in detail by Sokoloff that the optical density on the autoradiograph represents blood flow and not blood volume. The calculated average blood flow through the denser cerebral tissues was 0.55 to 0.65 c.c. per gram per minute, which is in excellent agreement with values for uveal flow obtained by us subsequently.

To determine the $\text{N}_2\text{O}$ concentration in the aqueous, 0.5 c.c. of aqueous was obtained anaerobically in an oilied syringe in four separate experiments. After 10 minutes of 15 per cent $\text{N}_2\text{O}$ administration, this was then mixed with 0.5 c.c. of deaerated 5 per cent dextrose and water to increase the specific gravity of the solution, which is necessary for analysis in the micromanometric chamber to prevent the solution from rising to the top of the reagents introduced into the chamber and exposing the aqueous solution to the air.

After appropriate correction, the $\text{N}_2\text{O}$ in volumes per cent was obtained in order to correct, if necessary, for the problem of aqueous contamination of the mixed venous blood. The average of the four experiments was 7.5 to 8.0 volumes per cent $\text{N}_2\text{O}$ which is approximately the same $\text{N}_2\text{O}$ concentration as in the venous blood at equilibrium. The aqueous does represent a $\text{N}_2\text{O}$ drainage source from the uveal tract, but, since its concentration is approximately equal to that of the venous blood, it would not change the venous blood $\text{N}_2\text{O}$ concentration significantly by dilution and so alter the arteriovenous $\text{N}_2\text{O}$ concentration difference on which the method is based.

Experimental technique. Male and female dogs of approximately the same age and weighing between 16 and 22 kilograms were anesthetized with pentobarbital sodium (35 mg. per kilogram) intravenously. The trachea was intubated with a cuffed cannula, which was attached to an automatic dog respirator with a tidal volume of 200 c.e. at a rate of 25 respirations per minute. The expiratory arm of the system was deliberately made as short as possible to avoid CO$_2$ accumulation.

The infraorbital artery was selected as the source of arterial blood closest to that of the eye$^{21}$ although this was not necessary to the method. The vessel was then isolated, as was a branch of the femoral vein.

Contra lateral to the side of this isolated infraorbital artery, the conjunctiva was incised close to the superior limbus, and with blunt dissection the prominent anterior ciliary vein was isolated. This vein originates approximately 5 to 6 mm. posterior to the superior limbus and passes medially. Aqueous heparin (5 mg. per kilogram) was then administered via the femoral vein.

The infraorbital artery was cannulated for a 3 cm. distance with a 6 inch siliconized polyethylene catheter (I.D. 0.26 mm.; O.D. 0.58 mm.). The anterior ciliary vein was ligated distally, a 6 inch siliconized polyethylene catheter of the same dimensions was introduced upstream into this vessel with the aid of a biomicroscope, and the catheter was ligated securely into this position with the tip 2 to 3 mm. distal to the origin of the vessel from the outside of the sclera in a manner similar to that described by MacL. Care was taken to avoid constriction of the vessel, and flow from both these vessels was constant, free, and continuous, although the arterial rate of flow was naturally greater than that from the venous catheter. A polyethylene catheter was introduced into the branch of the exposed femoral vein and passed in the direction of flow about 2 inches so as to lie in the inferior vena cava. Arterial and venous blood specimens were obtained from the animal simultaneously.

After approximately 60 minutes' ventilation with room air via the respirator, a mixture of available 15 per cent $\text{N}_2\text{O}$ and 85 per cent O$_2$ was passed at a constant rate into an anesthesia bag attached to the intake valve of the respirator from which the animal breathed. The start of the inhalation of the $\text{N}_2\text{O}$ mixture was taken as zero in all subsequent collections.

In the last 3 animals in the series, 100 per cent O$_2$ at 6 L. per minute was administered for 30 minutes immediately prior to the $\text{N}_2\text{O}$ mixture administration. Since the administration of 100 per cent O$_2$ would eliminate physiologic gaseous nitrogen from the blood, the blood nitrogen correction factor would be changed as described by Van Slyke and Plazin. This was done to determine how a change in the baseline blood nitrogen (blood blank correction) would affect the calculated arterial and venous $\text{N}_2\text{O}$ concentrations. From consecutive 10 minute arterial and venous samples taken during the period of $\text{N}_2\text{O}$ administration, standard curves to determine proper equilibrium time were obtained. Calculations were based on Formula 2. At least a 10 minute sample was required to recover enough blood from the anterior ciliary vein for practical micromethod analysis. The catheters being of equal length, the cut-off point of the arterial and venous collections were identical, and thus no allowance for dead space was required.

Intraocular pressure recordings. In a separate series of animals, intraocular pressure readings were obtained by the introduction of 27G needles into both anterior chambers of dogs not participating in the blood flow determinations. The readings were made in a Sanborn Visocardiometer strain gauge with a Sanborn 467B transducer.
with a sensitivity setting of 4.5 mm. saline. Pressure studies were carried out in animals receiving room air, 100 per cent O₂, and the N₂O mixture, in which one anterior ciliary vein was cannulated and the other undisturbed. The average blood flow was obtained by timed collection of the venous outflow into calibrated tubes. The results are given in Table I. Intraocular pressures recorded in the eye with the anterior ciliary vein catheterized as described above, as well as in the control eye, gave a mean pressure of 22.5 ± 1.0 for both, with an average blood flow of 0.04 ± 0.01 c.c. per minute. This PE 10 catheter was subsequently shortened from 33 cm. to 18 cm. to 3 cm. Subsequently, a larger polyethylene sleeve (PE 50) was placed over the shortened PE 10 tubing, and allowed to drop 23 cm. below eye level. The data are presented in table form. The implications of these findings will be discussed later. The systemic blood pressure measured in the femoral artery averaged 120/80 mm. Hg.

**Results**

The data presented in Table II represent N₂O concentrations in arterial and venous blood at 10 minute sampling periods from time zero at the beginning of inhalation of the gas mixture. Since more frequent analyses would be necessary to obtain the precise time interval required for equilibrium, these values representing automatically integrated 10 minute samples, it appears that approximately a 10 minute period is adequate for equilibrium to occur and that any error so introduced is not significant. Allowing a longer period for equilibrium would introduce additional error of delayed diffusion into regions of the eye having low partition coefficients.

The first five experiments were per-

<table>
<thead>
<tr>
<th>Catheter size</th>
<th>Blood flow (c.c. per minute)</th>
<th>Intraocular pressure (mm. Hg)</th>
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<tbody>
<tr>
<td>PE 10: (I.D. 0.26; O.D. 0.58 mm.) Length: 33 cm.</td>
<td>0.04 ± 0.01</td>
<td>22.5 ± 1.0</td>
</tr>
<tr>
<td>PE 10: Length: 18 cm.</td>
<td>0.08 ± 0.01</td>
<td>21.5 ± 1.0</td>
</tr>
<tr>
<td>PE 10: Length: 3 cm.</td>
<td>0.4 to 1.0 (range)</td>
<td>19.0 ± 1.0</td>
</tr>
<tr>
<td>PE 50: (I.D. 0.4; O.D. 0.8 mm.) Length: 30 cm. sleeve over PE 10</td>
<td>0.4 to 0.5 (range)</td>
<td>19.0 ± 1.0</td>
</tr>
</tbody>
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Normal intraocular pressure in eye in which anterior ciliary vein is not cannulated is 22.5 ± 1.0. Average of four experiments.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Nitrous oxide volumes per cent (corrected for blood blank)</th>
<th>Uveal blood flow (c.c./Gm. of uvea per minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vₐ</td>
<td>Aₐ</td>
</tr>
<tr>
<td>1</td>
<td>5.4</td>
<td>6.7</td>
</tr>
<tr>
<td>2</td>
<td>6.3</td>
<td>7.7</td>
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<tr>
<td>3</td>
<td>5.0</td>
<td>6.1</td>
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<tr>
<td>4</td>
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<tr>
<td>6</td>
<td>6.5</td>
<td>8.0</td>
</tr>
<tr>
<td>7</td>
<td>6.1</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Subnumbers indicate 10 minute period after time zero during which the blood specimen was obtained. Uveal blood flow calculated per gram uvea on the basis of formula 2.
formed by allowing the animals to breathe room air for 30 minutes prior to the inhalation of the N₂O mixture. The last two experiments were performed by administering 100 per cent O₂ as described previously. Although the blood blank values in the two sets of experiments differed, the final corrected results are not different.

Discussion

Indicator-dilution techniques as represented by the Kety-Schmidt nitrous oxide method require satisfactory demonstration of the validity of several criteria inherent in the application of the technique to any particular organ. These criteria have been extensively studied and satisfied in previous applications of the nitrous oxide method to organ blood flow since its introduction in 1945. An attempt is made in this study to satisfy these criteria in its first application to uveal blood flow.

The first criterion is that drainage from the anterior ciliary vein is a source of representative mixed venous blood from the uveal tract of the dog. Anatomic evidence has been presented. In addition, since N₂O is a freely diffusible gas in the blood, its arterial concentration is uniform throughout the arterial blood. Evidence against plasma skimming in the eye has been presented by Cohan, and Bill has presented evidence in rabbits and cats that there is no indication of important local adjustment of the resistance within any part of the uvea and that local blood changes in parallel with the whole uveal blood flow. It must be pointed out that arteriovenous anastomoses from branches of the anterior ciliary arteries to the large intrascleral veins have been described in the dog by one author. As pointed out by Cohan, the evidence for hemodilution in the anterior ciliary vein by aqueous is questionable, and, since the N₂O concentrations of both aqueous and anterior ciliary blood are approximately equal, this factor would be of little significance. The difficulty associated with nontraumatic cannulation of the vortex veins of the dog eye and constant blood collection from them makes comparison of the N₂O concentrations of these veins with the anterior ciliary vein most difficult. Certainly, this will be the ideal way to determine finally that anterior ciliary venous blood is representative mixed venous blood from the dog uvea.

The second criterion is that diffusion of the gas remains essentially limited to the tissue under examination during the time period of equilibrium. This is strongly suggested by the work of Sokoloff. However, at the present time, it cannot be demonstrated that over the entire equilibrium time no diffusion of N₂O occurs into the vitreous. It is felt, however, that this will not significantly affect the results obtained.

The third criterion is determination of the time required for equilibrium of the blood with the uveal tissue. According to Sokoloff, the tissue N₂O concentration never actually equals that of the arterial blood, but reaches a curve plateau at which it remains slightly below that of the arterial concentration. This is demonstrated in our data as well. Since the blood flow is such as to require approximately a 10 minute flow to obtain a sufficient quantity of blood for practical analysis, it is obvious that the 10 minute specimen only approximates the exact equilibrium time, but it also avoids prolonging the equilibrium time which would allow introduction of error by allowing greater time for diffusion of N₂O into other ocular tissues.

The question of equilibrium time is of importance in the application of this method to uveal blood flow. It has been demonstrated in this study that the size and length of the polyethylene tubing introduced into the anterior ciliary vein, when care has been taken to avoid constriction of the vein by artifact, influence both the intraocular pressure and the blood flow rate through that catheter. It is important to maintain as closely as possible the physiologic pressure relationships between the anterior ciliary vein and the intraocular pressure, since, by decreasing the outflow resistance of the anterior ciliary
vein, the intraocular pressure falls and the anterior ciliary vein blood flow increases. It is not clear whether increased flow rate causes or is caused by pressure drop, but there does seem to be a direct association of the two factors. Under no conditions did a cannulation cause elevation of intraocular pressure, except for minor fluctuations at the outset of the procedure.

Since it is obvious that an increase in uveal blood flow will shorten equilibrium time, and, since from observation of the formula utilized it is evident that a shortened equilibrium time causes an increased calculated blood flow, the equilibrium time for each range of uveal blood flow must be determined separately. This has not been necessary in application of the nitrous oxide method to larger organs because of their far greater mass and because only a relatively insignificant amount of the venous drainage was needed for analysis, thereby avoiding the problem of resistance in the collection of the venous drainage. It must be kept in mind that the measured blood flow through the polyethylene catheter represents the drainage of the anterior ciliary vein, not the total uveal blood flow as measured by the nitrous oxide method, although the two may be similar.

The original determinations of Kety showed cerebral blood flow to be 0.55 c.c. per gram per minute, which is close to our average figure of 0.56 c.c. per gram uvea per minute for uveal blood flow. Not only would this seem reasonable, it is somewhat supported by the autoradiographic determinations cited earlier. Thus, it may be concluded that blood flow through the restricted uveal channels is actually greater than cerebral blood flow on a weight basis. These values for uveal blood flow may be compared with the 0.32 c.c. per gram per minute as determined by Levene and the 1.20 ± 0.41 c.c. per minute per eye as determined by Bill. Both of these latter figures, for which different methods were used, were obtained on rabbits and cats, respectively.

Recently, Cohan and Cohan have described a technique for determining the drainage of the anterior ciliary veins in the dogs, obtaining an average flow of 0.67 c.c. per minute through that vein using a larger catheter than that used in our study (I.D. 0.86 mm.; O.D. 1.6 mm.) tapered at the point of cannulation to about half the internal diameter. Estimating the weight of the tissue drained by the anterior ciliary vein to be 0.7 Gm. (scleral band in which the intrascleral plexus is situated, outer portion of the ciliary body, and whole cornea), they estimated a flow of 0.7 c.c. per gram per minute.

These data may be compared with data for other organs obtained by the nitrous oxide method. Coronary blood flow in the dog has been estimated at approximately 0.71 c.c. per gram per minute and renal blood flow in man at 4.0 c.c. per gram per minute.

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