Measurement of the Rate of Aqueous Humor Flow

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Techniques which estimate the rate of aqueous flow generally require the use of tracer substances. Determination of the distribution of the tracer in the relevant body compartments permits calculation of the rate of flow within the limits of accuracy of the method used. The underlying theory, as well as the advantages and limitations of methods employing systemic, topical, intracameral, and intravitreal administration of tracer substances are reviewed. Since these methods all assume that the rate of aqueous secretion is constant, yet the presence of a diurnal rhythm of flow has been demonstrated in both rabbits and humans, a compartmental model of a circadian system based upon the vitreous depot technique is presented. This model estimates the degree to which a continuously changing rate of aqueous flow limits the ability to determine aqueous flow rate accurately by this particular method and illustrates this limitation, which is common to all tracer methods.

The ability to estimate the rate at which aqueous humor is secreted by the ciliary epithelium has been a necessary tool in gaining knowledge about ocular physiology and has been important in the development of effective agents in the treatment of glaucoma. Techniques which estimate the rate of aqueous flow generally require the use of tracer substances. Determination of the distribution of the tracer in the relevant body compartments permits calculation of the rate of flow within the limits of accuracy of the method used. Several methods have been developed, some requiring systemic and others requiring local (topical, intracameral, or intravitreal) administration of the test substance. Still others make use of endogenously produced substances as tracers.

Techniques exist by which the facility of outflow from the anterior chamber can be measured. These measurements, in conjunction with measurements of intraocular pressure and episcleral venous pressure, have been used to calculate the rate of aqueous flow. They provide an indirect estimate of aqueous production, however, and will not be included in this discussion of methods permitting direct measurement of flow.

SYSTEMIC TRACERS

Early techniques employed a systemically administered tracer which enters the aqueous from the blood either by an active secretory process, by diffusion, or both. The tracer is administered by intravenous and intraperitoneal injection or by continuous intravenous infusion so that plasma tracer levels remain essentially

"Abbreviations": FITC: fluorescein isothiocyanate  IOP: intraocular pressure

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constant during the course of the experiment. The test substance is initially absent from the aqueous, but its concentration increases over time until it reaches a steady-state concentration, \( C_{\text{ss}} \), which is determined by the concentration in the plasma, the rate of aqueous flow, and the ability of the tracer to diffuse from plasma into aqueous. By measuring \( C_{\text{ss}} \) and the rate at which the concentration of tracer approaches this steady-state level, the rate of aqueous flow can be determined.

In a study of the steady-state distribution of various solutes between the blood and aqueous, Kinsey and Grant [1] presented the mathematical derivation of the equations upon which these methods are based. This derivation as adapted by Becker [2] argues that, if the tracer enters the aqueous strictly by diffusion and mixes completely with the aqueous, the rate of change of concentration of tracer in the aqueous can be expressed:

\[
\frac{dC_a}{dt} = k_d (C_p - C_a) - k_f C_a \tag{1}
\]

where

- \( k_d \) = the coefficient of diffusion exchange of tracer between aqueous and plasma
- \( k_f \) = the coefficient of escape of tracer from anterior chamber by bulk flow of aqueous
- \( C_p \) = the concentration of tracer in the plasma, which remains constant
- \( C_a \) = the concentration of tracer in the aqueous humor

If

\[
k_o = k_d + k_f
\]

then equation 1 can be written:

\[
\frac{dC_a}{dt} = k_d C_p - k_o C_a \tag{2}
\]

At steady state, i.e., when the rate of entrance of tracer by diffusion and the rate of exit of tracer by diffusion and bulk flow are equal,

\[
\frac{dC_a}{dt} = 0, \quad C_a = C_{\text{ss}}
\]

and

\[
k_d = \frac{k_o C_{\text{ss}}}{C_p} \tag{3}
\]

Substituting into equation 2:

\[
\frac{dC_a}{dt} = k_o (C_{\text{ss}} - C_a) \tag{4}
\]

and, by integration:

\[
C_{\text{ss}} - C_a = C_{\text{ss}} e^{-k_o t} \tag{5}
\]

\[
\ln (C_{\text{ss}} - C_a) = \ln (C_{\text{ss}}) - k_o t \tag{6}
\]

Experimental determination of \( C_a \) at at least one point in time, and \( C_{\text{ss}} \) after reaching
the steady state, permits the calculation of $k_s$. By substitution into equation 3 and from the measured value of $C_p$, the value of $k_f$ can be determined.

If the tracer enters the aqueous solely by secretion, and therefore can leave the eye only by bulk flow, where $k_f$ is the coefficient of secretion of tracer into aqueous from the blood, equation 1 simplifies to:

$$\frac{dC_a}{dt} = k_s C_p - k_f C_a$$  \[7\]

and an analogous derivation leads to:

$$\ln (C_{am} - C_a) = \ln (C_{am}) - k_f t$$  \[8\]

In this case, $k_f$ can be determined directly from values for $C_{am}$ and $C_a$.

The relationship between the first-order rate constant for exit of tracer by bulk flow $k_f$ and the rate of aqueous flow can be shown by the following simple derivation. Assuming that the tracer can exit only by bulk flow, the rate of change of mass of tracer in the anterior chamber can be expressed:

$$\frac{dm}{dt} = -C_a \cdot f$$  \[9\]

where

- $m$ = the mass of tracer in the anterior chamber
- $C_a$ = the concentration of tracer in the aqueous
- $f$ = the rate of aqueous flow

But since

$$m = C_a \cdot V_a$$

where $V_a$ is the volume of the anterior chamber, substituting and rearranging, equation 9 becomes:

$$\frac{dC_a}{C_a} = -f/V_a dt$$  \[10\]

Integrating equation 10 we get:

$$C_a = C_o e^{-k_f t}$$  \[11\]

where $C_o$ is the initial concentration of tracer in the aqueous, and

$$k_f = \frac{f}{V_a}$$  \[12\]

demonstrating that $k_f$ represents the fractional rate of turnover of aqueous in the anterior chamber per unit of time.

This technique has been employed, using several different tracer substances, by various investigators. For example, Goldmann [3] and Nagataki [4] each used intravenous fluorescein to measure aqueous flow in humans relative to the volume of the anterior chamber and obtained mean rates of 1.1 percent ± 0.2 percent and 0.974 percent ± 0.094 percent per minute, respectively. This technique had the advantage of allowing the measurement to be made without removing aqueous from the eye,
but still required several blood samples. Becker [2] administered radioactive isotopes of iodine to rabbits by a combination of intraperitoneal and intravenous injection and continuous intravenous infusion. Using two different isotopes administered at different times before sampling the aqueous permitted a single aqueous sample to provide both data points required for the measurement. With this technique he found a relative rate of aqueous flow per minute of 1.5 percent ± 0.17 percent of the volume of the anterior chamber.

In an attempt to alleviate the difficulties associated with maintaining constant plasma tracer levels, Bárány and Kinsey [5,6] used intravenously administered para-aminohippuric acid, radioactive Rayopake®®, and radioactive Diodrast®® to measure the rate of aqueous flow in rabbits. These tracers were chosen because they are quickly excreted by the kidney, providing a relatively constant, near-zero level in the plasma. It was hoped that a detectable quantity of the test substance would remain in the eye until the plasma level was negligible and that diffusion of tracer out of the aqueous would be slow enough to ignore. If these conditions were met, measurement of the rate of disappearance of the tracer would accurately reflect the rate of aqueous flow. The first term of equation 1 would approach zero, allowing the calculation of flow rate from equations 11 and 12.

After measuring the rate of disappearance of tracer in groups of normal rabbits by taking aqueous samples two and three hours after intravenous infusion of the test substance, the authors found that the plasma tracer concentration and diffusion of tracer out of the aqueous were not negligible. A correction factor was therefore introduced which required measurement in a separate group of animals of the steady-state aqueous concentration at a constant plasma tracer level. This value was determined in rabbits with ligated renal arteries to prevent excretion of the tracer, thus maintaining a constant plasma concentration. With this technique, the rate of aqueous flow in normal rabbits was estimated to be in the range of 2.6 to 3.6 μl/minute.

**WASHOUT OF INTRACAMERALLY INJECTED TRACERS**

In order to simplify the mathematical treatment, techniques involving systemic administration of tracers generally require that the plasma level of tracer remain essentially constant. To avoid the difficulties associated with this requirement, methods involving direct injection of a tracer substance into the anterior chamber have been developed by several investigators. A bolus of tracer is injected into the anterior chamber and is assumed to be completely mixed with the aqueous. An equivalent volume of aqueous is removed in order to prevent elevation of intraocular pressure (IOP). As the tracer is washed out of the eye by bulk flow and diffusion, measurements of aqueous tracer concentration are made. Calculation of the rate of aqueous flow under these conditions is analogous to that in the case of systemic administration of tracer, but with an essentially constant plasma tracer level of zero. Furthermore, if the tracer is a relatively large molecule which cannot diffuse out of the anterior chamber but leaves only by bulk flow, the calculation of flow is further simplified, since the concentration follows a simple first-order exponential decay given by equations 11 and 12 above.

This method more effectively overcomes the complexities of systemic tracers which Bárány and Kinsey [5,6] tried to overcome by using systemic tracers that are rapidly eliminated from the blood. It does so, however, at the expense of a directly invasive
procedure which may affect the normal physiology of the eye during the course of the experiment. Tracers used by investigators employing this method have included radioactively iodinated albumin [7,8], iodoantipyrene [9], iodohippurate [9], blue dextran [10], and fluorescein isothiocyanate-(FITC-) dextran [11].

DILUTION METHODS

Another group of methods to measure aqueous flow is based upon the dilution of a tracer during continuous perfusion with a buffer solution containing tracer [12,13]. Through a cannula placed in the anterior or posterior chamber, the buffer is infused at a constant rate, where it is assumed to mix completely with newly forming aqueous. The degree of dilution of the tracer substance is related to the rate of aqueous humor formation. Aqueous leaves the eye through the normal outflow channels and through another cannula in the anterior chamber from which aqueous tracer concentration measurements can be made. The height of the fluid column attached to this cannula is adjusted to maintain IOP at its initial level. Since after reaching a steady state the rate of entry and exit of tracer must be equal,

$$ r_p \cdot C_p = (r_p + f)C_a $$  [13]

where

- $r_p$ = the rate of perfusion with tracer-containing buffer
- $f$ = the rate of aqueous humor formation
- $C_p$ = the concentration of tracer in the perfusate
- $C_a$ = the concentration of tracer in the aqueous

Rearranging equation 13 gives:

$$ f = r_p \cdot \frac{(C_p - C_a)}{C_a} $$  [14]

As the rate of perfusion and concentration of tracer in the buffer are known, measurement of $C_a$ permits calculation of the rate of aqueous flow.

This method allows observation of changes in the rate of aqueous flow in an individual eye. For example, measurement of the concentration of tracer in the aqueous before and after administration of a drug is possible, allowing an eye to be used as its own control. Tracers used in this technique include $^{14}$C-labeled inulin [12], which requires discrete sampling of the aqueous for detection of $^{14}$C in a liquid scintillation counter, and $^{131}$I-labeled albumin [13,14], which permits constant monitoring of the concentration in the aqueous with a $\gamma$ detector.

Animals must be under general anesthesia during the course of the measurements since continuous cannulation of the eye is required. In addition, inflammation induced by the required invasive procedures limits the duration of experimentation to four to six hours for each eye studied.

TOPICAL APPLICATION OF FLUORESCEIN (CORNEAL DEPOT)

Langley and MacDonald [15] suggested that relative changes in aqueous flow could be determined by staining the cornea with fluorescein and measuring changes in its peak concentration in the aqueous. The first thorough quantitative discussion of how this method can be used to determine the absolute rate of aqueous flow was presented by Jones and Maurice [16]. In this report, they asserted that the rate of
aqueous flow can be calculated by measuring the concentration of fluorescein in the aqueous over time. In addition, the determination of either the ratio of corneal fluorescein concentration to that in the aqueous or the total quantity of fluorescein in the eye is required. Their derivation, as adapted by Coakes and Brubaker [17], requires the simultaneous solution of the linear differential equations which describe the rate of change of quantity of fluorescein in the anterior chamber:

$$\frac{dm_a}{dt} = k_a m_a - (k'_a + k_o)m_a$$ [15]

and in the corneal stroma:

$$\frac{dm_c}{dt} = k_c m_c + k'_cm_c$$ [16]

where

- \(m_a\) = the mass of fluorescein in the anterior chamber
- \(m_c\) = the mass of fluorescein in the cornea
- \(k_a\) = the mass transfer coefficient from cornea to anterior chamber
- \(k'_a\) = the mass transfer coefficient from anterior chamber to cornea
- \(k_o\) = the mass transfer coefficient from aqueous to blood by bulk flow and diffusion

The solutions take the form:

$$m_a = \gamma e^{-\alpha t} - \gamma e^{-\beta t}$$ [17]

$$m_c = \delta e^{-\alpha t} + (1 - \delta)e^{-\beta t}$$ [18]

where \(\alpha\), \(\beta\), \(\gamma\), and \(\delta\) are complex expressions consisting of combinations of the mass transfer coefficients and have no readily interpretable physical meaning. The solutions are exponential functions in which the second term decays more quickly than the first. In other words, while both terms contribute to changes in concentration of fluorescein early, only the \(\alpha\) exponential influences the concentration at later times. The semilog plots of the concentration of fluorescein in the aqueous and the cornea, therefore, eventually become linear with slope \(-\alpha\) (Fig. 1). The semilog plot of the total mass of fluorescein in the eye also decreases with the same slope.

Jones and Maurice [16] proposed two methods to calculate the rate of aqueous flow based on the above equations. The first requires the measurement of anterior chamber fluorescence from the time the corneal fluorescein is instilled until some time later when the \(\beta\) term has decayed. Plotting the values on semilog paper, the slope of the linear portion of the curve is, as mentioned above, \(-\alpha\). The difference between the original curve and the extrapolated line with slope \(-\alpha\) represents the \(\beta\) exponential term, and has slope \(-\beta\) (Fig. 1). The values of the mass transfer coefficients can be calculated from the values of \(\alpha\), \(\beta\), and the ratio of the concentration of fluorescein in the cornea to that in the aqueous on the linear portion of the curves. This ratio is constant once the \(\beta\) exponential has decayed and needs to be measured only once during the experiment.

Their second method requires several measurements of anterior chamber fluorescence a few hours after application of fluorescein once the \(\beta\) exponential has become small. The total mass of fluorescein in the eye, \(m_n\), is also measured at the same time
as one of the aqueous fluorescence measurements. Since it is assumed that the dye is
lost from the cornea only by way of the aqueous, it follows that:

\[ \frac{dm_i}{dt} = -C_a V_a k_o \]  

[19]

where \( C_a \) is the anterior chamber fluorescence and \( V_a \) is the volume of the anterior
chamber. Dividing by \( m_i \) leads to:

\[ \left( \frac{1}{m_i} \right) \frac{dm_i}{dt} = \frac{d[\ln(m_i)]}{dt} = -\frac{C_a V_a k_o}{m_i} \]  

[20]

Since, after decay of the \( \beta \) exponential:

\[ -\alpha = \frac{d[\ln(m_i)]}{dt} \]

it follows that:

\[ V_a k_o = \frac{\alpha m_i}{C_a} \]  

[21]

and

\[ f = \frac{\alpha m_i}{C_a} \cdot r \]  

[22]

where \( f \) is the rate of aqueous flow and \( r \) is the fraction of fluorescein which leaves the
eye by bulk flow, as opposed to diffusion. This value has been found to be
approximately 0.80 following intracameral injection [10,18] and approximately 0.86
following intravenous administration of fluorescein [18].

These methods require a corneal depot of known quantity and distribution in
order to obtain consistent results. This purpose can be accomplished either by
instillation of a fluorescein solution into the conjunctival sac or by iontophoresis of a
bolus of fluorescein into the corneal stroma. Topper et al. [19] showed that topical instillation of a fluorescein solution must be done several hours before any meaningful measurement of aqueous fluorescence can be made as it takes this length of time for uniform staining of the corneal stroma to occur. No delay was required for effective results after iontophoresis, as the procedure quickly provides a depot of fluorescein with sharply defined boundaries. Since the first method of Jones and Maurice requires early measurements of aqueous fluorescence, iontophoresis is required for this technique.

Diffusional loss of topically applied fluorescein from the anterior chamber cannot be determined by these techniques. The diffusional and flow components of the anterior chamber loss coefficient, $k_a$, cannot be separately evaluated. Thus, in investigating the effect of a drug or disease on the rate of aqueous flow, separate determination of a possible effect on the rate of diffusional loss of fluorescein must be undertaken.

An estimate of aqueous flow obtained by one of the corneal depot techniques is based on differences in fluorescence observed between at least two points in time. It represents, therefore, an average flow value between the end points rather than a measurement of flow at a discrete point in time. This fact becomes an important consideration in interpreting the results of experiments designed to detect changes in the rate of aqueous flow or to define maximum and minimum flow rates.

With the exception of the iontophoretic placement of the corneal fluorescein depot, these methods are completely noninvasive. As a result, they are useful not only in animal experimentation, but are ideal for clinical studies in humans.

**VITREOUS DEPOT TECHNIQUE**

A method of measuring aqueous humor flow in laboratory animals was devised by Johnson and Maurice [20], using an intravitreal injection of FITC-dextran, which can presumably leave the eye only by diffusing across the interface between the vitreous and posterior chamber, and then exiting with the aqueous by bulk flow into the blood. After allowing several days for the tracer to become uniformly distributed in the vitreous and for the concentration of tracer in the aqueous to reach a maximum, the concentration of fluorescein in the anterior chamber reflects the rate of aqueous flow. As the dye diffuses into the posterior chamber, it is diluted to varying degrees, depending upon the aqueous flow rate. At high rates of flow the tracer is relatively dilute, while at low rates of flow it is more concentrated.

It is assumed that complete mixing occurs, that a steady state exists, i.e., that the rate of diffusion of FITC-dextran from the vitreous into the aqueous is equal to the rate at which it is leaving the anterior chamber by bulk flow, that the rate of aqueous flow is constant, and that the mass of FITC-dextran in the vitreous does not change appreciably during each measurement. Under these conditions, the rate of aqueous flow can be determined by the relationship:

$$ k_v m_v = f \cdot C_a $$

$$ f = \frac{k_v m_v}{C_a} $$

where $k_v$ is the coefficient of diffusion of FITC-dextran from the vitreous into the aqueous and $m_v$ is the mass of FITC-dextran in the vitreous. The authors demon-
strated that once the tracer is uniformly distributed and $C_a$ has reached a maximum, the concentrations of FITC-dextran in the vitreous and aqueous decrease exponentially with the same rate constant, $k_r$. The value of $k_r$ for a given species can be determined, therefore, by following the change of concentration of FITC-dextran in the aqueous or vitreous of a group of animals for several days. A semilog plot of this data is linear, with slope $-k_r$. The rate of aqueous flow in an eye is determined by measuring $C_a$ at some point in time, enucleating the eye, and measuring the quantity of FITC-dextran in the vitreous at the time the animal was sacrificed. Using the value of $k_r$, $m$, at the time of the measurement can be determined, permitting calculation of the absolute rate of aqueous flow.

This technique has the advantage of allowing measurement of aqueous flow over extended periods of time since the tracer remains at readily detectable levels for up to two weeks, depending upon the sensitivity of the detector used. The steady-state assumption required for the flow calculation, however, can lead to errors if aqueous fluorescence measurements are made shortly after changes in flow rate. This problem, which will be discussed in detail below, is not unique to the vitreous depot technique; however, Gaul and Brubaker [21] found that this problem seems to be magnified in this technique, for reasons which were not clear. They compared the vitreous depot method with the second method of Jones and Maurice [16] in the detection of changes in aqueous flow rate after administration of acetazolamide and mannitol. During the hour after administration of either agent, the corneal depot technique showed a 50 percent decrease in aqueous flow rate, while no statistically significant difference was demonstrated by the vitreous depot method.

ENDOGENOUS TRACER SUBSTANCES

Quantification of endogenously produced compounds which diffuse or are secreted from the blood into the aqueous can be used to calculate the rate of aqueous flow. Substances which enter the aqueous at constant or nearly constant rates behave as if there were a systemic "depot" and permit measurement of relative changes in aqueous flow rate by applying the same theory as that underlying the vitreous depot technique. Measurement of aqueous flare, which correlates with aqueous protein concentration, and determinations of aqueous ascorbate concentrations have been used in this way [22,23,24,25,26,27]. It is not generally possible to make absolute flow measurements by these methods, however, as the rate of entrance of the tracer into the aqueous is difficult to determine.

MEASUREMENT OF PUPILLARY FLOW OF AQUEOUS HUMOR

Holm and Krakau [28] proposed a method to measure the flow of aqueous humor as it passes from the posterior to the anterior chamber through the pupil. This method uses fluorescein not as a tracer, but as a marker to delineate bubbles of aqueous humor as they emerge from the posterior chamber. By staining the aqueous in the anterior chamber with topically administered fluorescein, new aqueous passing through the pupil was found to form an unstained volume for up to 30 seconds before mixing began to occur. A photographic method of determination of the volume of the newly formed unstained aqueous was used to estimate the aqueous flow rate. By thoroughly mixing the aqueous through eye movements, the process could be repeated many times in a short period. Values obtained for rabbits were in the range of 1.5 to 3.8 µl/minute.
Krakau later suggested a photoelectric method of quantifying the volume of unstained aqueous by measuring the decrease in fluorescence in a "cuvette" enclosing the pupil created by a suitably masked slit lamp fluorophotometer [29]. Since the intensity of fluorescence is directly proportional to the concentration of fluorescein,

\[ I(t) = I_o - (k \cdot C \cdot f \cdot t) \]  \[25\]

where

- \( I(t) \) = the intensity of fluorescence at time \( t \)
- \( I_o \) = the intensity of fluorescence at time \( t = 0 \) (before any new aqueous is formed)
- \( f \) = the rate of aqueous flow, assumed to be constant
- \( C \) = the concentration of fluorescein in the thoroughly mixed aqueous
- \( k \) = a proportionality constant relating \( C \) and \( I \)

After determining \((k \cdot C)\) by measuring the intensity of fluorescence in a known volume of well-mixed aqueous, flow can be determined by the equation.

\[ f = \frac{I_o - I(t)}{k \cdot C \cdot t} \]  \[26\]

Although this method allows rapid measurements which can be quickly repeated, it is relatively sensitive to eye movements during the period of measurement, which cause mixing of newly formed aqueous with stained aqueous. In addition, the presence of a small pupil is required, necessitating topical treatment with pilocarpine before the period of measurement.

**EFFECT OF VARIATION OF FLOW RATE ON THE RESULTS OF TRACER METHODS**

As noted above, methods of estimating the rate of aqueous flow assume this rate to be constant. Previous studies have demonstrated, however, that diurnal variations in aqueous flow occur in both rabbits [30] and humans [31]. To study the error introduced by this invalid assumption, I have developed the following compartmental model of a circadian system based on the vitreous depot technique. This model demonstrates the degree to which a continuously changing rate of aqueous flow can be expected to limit the ability of this particular method to determine flow rate accurately.

FITC-dextran was assumed to diffuse from the vitreous compartment into the aqueous compartment, then to leave the aqueous only by bulk flow. Back diffusion from the aqueous into the vitreous was assumed to be negligible, as Johnson and Maurice [20] have demonstrated that vitreous fluorescence levels average approximately 26 times greater than aqueous levels, using dextrans of high molecular weight. With these assumptions, the rate of diffusion of tracer into the aqueous is given by:

\[ \frac{dM}{dt}_{in} = k_o M_o e^{-k_o t} \]  \[27\]

and the rate of exit of tracer from the aqueous is given by:

\[ \frac{dM}{dt}_{out} = -C_a \cdot f(t) \]  \[28\]
where

\[ M = \text{the mass of FITC-dextran in the aqueous humor} \]
\[ M_o = \text{the mass of FITC-dextran in the vitreous at } t = 0 \]
\[ f(t) = \text{the function describing the rate of aqueous flow over time} \]

Since

\[ C_a = \frac{M}{V_a} \]

where \( V_a \) is the volume of the aqueous compartment, combining equations 27 and 28 and dividing by \( V_a \) gives:

\[
\frac{dC_a}{dt} = \left( \frac{k_rM_o}{V_a} \right) e^{-k_r t} - \frac{C_a f(t)}{V_a}
\]

Assuming values of \( k_r = 0.09 \text{ day}^{-1} \) [30], \( M_o = 1 \text{ mg} \), and \( V_a = 0.25 \text{ ml} \), and letting the flow function \( f(t) \) be the sinusoidal function with a range of 1.2 to 2.4 \( \mu l/\text{minute} \) and a period of 24 hours, this differential equation was solved numerically for \( C_a \) using the Runge-Kutta fourth-order method [32], programmed in Pascal on an Apple Macintosh computer, using a step size of 0.05 day. The function describing the concentration of FITC-dextran in the aqueous compartment under these conditions is shown in Fig. 2. Substituting values of \( C_a \) given by this curve into equation 24, the equation used to calculate flow from aqueous fluorescence data by the intravitreal depot technique, calculated flow rates were compared with the “true” flow rates defined by the sinusoidal flow function (Fig. 3).

The presence of a continuously changing rate of aqueous flow results in underestimation of the range of the circadian rhythm of aqueous flow and a shift in the phase of the rhythm. This result occurs because the changing concentration of FITC-dextran lags significantly behind the changing rate of aqueous flow, due to the low rate of turnover of aqueous of about 1 percent per minute. Before the concentration of FITC-dextran reaches the level it would attain if the maximum flow rate were sustained, the flow rate begins to decrease.

![Figure 2](image-url)
Although the above analysis is derived from the vitreous depot technique, these principles apply to all tracer methods and can affect their results in a similar fashion.

CONCLUSION

Each of the methods previously discussed has differences in precision of measurement, technical simplicity, invasiveness, and ease of mathematical treatment of data, making a particular technique more suitable for a given research protocol. There are several factors, however, which prevent any of these techniques from measuring with complete accuracy the total quantity of aqueous secreted by the ciliary epithelium. First, it must be remembered that not all of the aqueous leaves the eye by one particular route. While the majority of the fluid passes into the anterior chamber through the pupil and leaves the eye by bulk flow through the canal of Schlemm, the uveoscleral pathway acts as a second site of bulk outflow through which aqueous passes from the anterior chamber into the suprachoroidal space and from there through the sclera into periorbital tissues [33]. In addition, a small portion of aqueous never enters the anterior chamber, but leaves the eye posteriorly through the vitreous and retina. Tracer techniques are measurements only of anterior chamber turnover. The portion which exits the eye posteriorly cannot be measured by any currently available technique.

In addition, the mathematical treatment of data in the above methods assumes the rate of aqueous flow to be constant. Studies have shown, however, that a diurnal rhythm of aqueous flow exists both in rabbits [30] and in man [31]. As demonstrated by the model presented above, which assumes a continuously changing flow rate, the low rate of turnover of aqueous—approximately 1 percent per minute—causes the tracer concentration to lag behind changes in flow and results in some degree of inaccuracy, particularly in predicting maximum and minimum values.

Although such considerations limit the ability of these methods to quantify the rate of aqueous secretion precisely, relative changes in the rate of flow can still be readily detected. Alterations in aqueous flow induced by pharmacologic intervention or interruption of the eye's innervation can be clearly determined. Such measurements enable investigators to define the physiologic regulators of aqueous secretion.
and to help discover the mechanism of action of drugs of potential usefulness in the
treatment of glaucoma.

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