THE FLOW OF AQUEOUS HUMOR IN THE HUMAN EYE

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INTRODUCTION

THE CORNEA AND THE CRYSTALLINE LENS, BEING TRANSPARENT AND AVASCULAR, depend for their nutrition on a transport medium, the aqueous humor. Aqueous humor serves not only to inflate the globe but provides substrates, carries away metabolic wastes, sweeps away toxic substances, and, following appropriate stimuli, mediates the inflammatory response for the avascular portions of the eye. Thus, aqueous humor along with the structures that form and drain it, may be regarded as an extravascular extension of the circulatory system.[1,2]

The circulation of aqueous humor has been recognized for decades. Numerous techniques have been employed to study its rate of flow through the anterior chamber.[3-20] Few of these techniques, however, are suitable for frequent use in human studies. A notable exception is tonography, a technique that is easy to perform and can be repeated without harm to the eye. Many studies have been performed using tonographic techniques in which the rate formation of aqueous humor has been inferred from the intraocular pressure (IOP) and the resistance to outflow.[21,22] However, tonography, despite its convenience, is a method of measuring hydraulic resistance and provides only an indirect measure of aqueous humor flow.[23-71]

In studies of aqueous humor flow in the human eye, the method of measurement is critical. The procedure must measure flow as directly as possible. It must permit repeatable measurements of flow to be performed in the undisturbed eye. The method itself must not alter the naturally occurring level of IOP. Fluorescent tracer techniques come the closest to satisfying these criteria and seem to be the most suitable for measurements in human beings.

This thesis will include a discussion of the rationale for choosing a specific protocol for measuring aqueous humor flow, a detailed description of the technique, and a summary of the results of over 1,000 determinations in human eyes under a variety of experimental circumstances.

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HISTORICAL BACKGROUND

At the time this work began, the use of fluorescein as a quantitative tracer for studying physiologic functions of the eye was well established. Ehrlich[72] demonstrated 100 years ago that systemically administered fluorescein would appear in the aqueous humor of the rabbit eye, and his description was followed by many other experiments using fluorescein.[73-76] Lindner[77] made quantitative estimates of the changes in fluorescein concentration in the aqueous humor of human beings after oral ingestion by absorbing the excitatory slit beam with solutions of known concentration and thickness. Amsler and Huber[78] devised a simpler, although nonlinear, method for estimation of the concentration of fluorescein in the eye by reducing the current to the filament of the slit lamp until the green fluorescence had disappeared. Their method was used by many others to study the properties of the blood-aqueous barrier.[75,79]

Goldmann,[80,81] in 1950, made three important contributions to the application of this methodology. First, he developed a more accurate method of quantifying anterior chamber fluorescence by means of a subjective matching technique. Second, he recognized the need to quantify the concentration of the dialyzable portion of circulating fluorescein. Third, he developed a method of quantifying the permeability of the iris and the rate of aqueous humor flow through the anterior chamber in human beings. With rare exceptions,[82-86] Goldmann's method has not been imitated by others, probably because of its complexity. Nevertheless, his studies in normal subjects and in eyes affected with glaucoma represent a milestone in quantitative fluorophotometry of the eye and in our understanding of aqueous humor dynamics.

Langley and MacDonald[87] were the first to introduce fluorescein into the eye by topical administration to measure flow. Their method was extended by Weekers and Delmarcelle,[88] but their measurements related as much to corneal endothelial permeability as to the aqueous humor flow.

Langham and Wybar[89] constructed the first objective slit lamp fluorophotometer. Their instrument measured the intensity of fluorescence in the eye with a photomultiplier tube. Langham and Taylor[82] used this instrument to study the rate of aqueous humor formation in anesthetized rabbits. The method of data analysis was devised by Maurice and was similar to that of Goldmann.

Other investigators working with a variety of substances besides fluorescein devised detailed mathematic models for describing the exchange between the blood, the posterior chamber, the anterior chamber, and the

cornea. Davson and Matchett[90] described the kinetics and measured the factors that controlled the rate of penetration of a wide variety of substances into the blood-ocular barrier. Bárány and Kinsey[3,4] devised a method of measuring aqueous humor flow that exploited the anterior chamber disappearance kinetics of substances that would enter the anterior chamber from the blood and be cleared rapidly by the kidney. Kinsey and Reddy[91] and Kinsey and Palm[92] worked out a systematic mathematic model of the exchange of tracer substances among these compartments. Friedenwald and Becker[93] derived a similar model that was useful for any tracer substance. Becker[7] later devised methods based on this model that permitted aqueous formation to be measured with radioactive iodide in such a way that the eye was undisturbed during the critical period.

Research using fluorescein as a tracer was enhanced significantly by the work of Maurice.[94] In 1963, he described a new fluorophotometer that was suitable for laboratory and clinical work. The signal-to-noise ratio was greatly improved over preexisting instruments by the use of a more intense, modulated light source, better filters, and an operator-observable fluorescence window. His instrument was capable of measuring fluorescent intensity in an accurately defined area small enough to make measurements in the corneal stroma or in an area large enough to include the entire cornea and anterior chamber. This sensitive instrument permitted objective measurements of fluorescein kinetics in the eye well beyond those of any previous instrument. With Maurice's instrument,[95] the range of fluorescence measurements was limited mainly by the properties of fluorescein and by the eye itself.

Later, Jones and Maurice[96] developed a new method of measuring aqueous humor flow. The tracer was introduced via the cornea. Their work clarified the important role of the cornea in affecting the shape of the fluorescence intensity curve of the anterior chamber after topical administration. It also provided a relatively simple method for measuring fluorescein clearance from the anterior chamber and, consequently, for measuring aqueous humor flow through the anterior chamber. Maurice's method or some variation thereof has been used subsequently by several investigators.[97-111] A modification of this technique has been used in the present study.

A photogrammetric technique for measuring aqueous flow, in which fluorescein was used in an entirely different manner, was developed about the same time by Holm and Wiebert and by Kraukau.[112-116] Although simple in concept, this method is difficult to perform and has not been used by others. The work is important, however, because the method can be calibrated by using geometric optics to produce results in

absolute units of flow. Thus, it can be used as a standard of accuracy for other methods.

The use of fluorescent tracer techniques has increased in recent years because of the development of new fluorophotometers,[94,117-120] because of clarification of the kinetics of fluorescein in the cornea and other parts of the eye,[83,100,121-128] and because of new methods of analyzing the kinetics of fluorescein following administration by a number of routes.[83,96,98,105,106,129-131] These techniques are now within the grasp of clinicians who wish to study human subjects under a variety of experimental conditions.

PROPERTIES OF FLUORESCEIN

Many of the properties of fluorescein make it an ideal tracer for use in ophthalmic research.[95] Table I lists some of these properties. Of greatest importance is the fluorescent efficiency of fluorescein, the lack of varia-

TABLE I: PHYSICAL PROPERTIES OF FLUORESCEIN

DESCRIPTION	VALUE	REFERENCE NO.
Formula	$C_{20}H_{12}O_5$ or $C_{20}H_{10}O_5 \cdot Na_2$	135
Molecular weight	332 (Sodium salt 376)	136
Effective cross section (λ = 495, pH = 7.4)	6×10^{-16} cm^2	137, p16
Molecular extinction coefficient (pH 7.4)		
at λ = 495	1.6×10^5 cm^{-1} mol^{-1}	*
over emission band	1.7×10^4 cm^{-1} mol^{-1}	*
Quantum efficiency		
pH 13.0	0.92	. . .
pH 7.0	0.65	138
Fluorescence lifetime	4.6 nsec	139
Rotational relaxation time (37° C)	0.52 nsec	137, p59
Polarization of rigidly bound fluorescein	0.47	140
Polarization of unbound fluorescein (37° C)	0.02	140
Angle between absorption and emission dipoles	12°	137, p57
Excitation peak	490 nm	141
Absorption peak	490 nm	141
Emission peak	515 nm	141
Ether/water coefficient	460/1	*
pK_a	6	95
Diffusion coefficient in water, 37°, pH = 7.0	3.6×10^{-4} cm^2 min^{-1}	142

*Determined in author's laboratory.

tion in this efficiency at physiologic pH, the lack of oxygen quenching at normal atmospheric pressures, and the lack of photodegradation of fluorescence.

Table II lists some of fluorescein's interactions with ocular tissue. The lack of toxicity, the lack of binding except loose reversible binding to albumin, and the apparent lack of local metabolism by the eye are all important properties that make it useful as a quantitative tracer.

Peculiarities of fluorescein distribution, however, have to be kept in mind. Fluorescein is bound loosely to albumin. In human plasma, the unbound concentration of fluorescein is approximately 15% of the total, and this fraction is constant for fluorescein concentrations of less than 10^{-4} g/mL.[132] The binding of fluorescein by albumin partly quenches its

TABLE II: BIOLOGICAL INTERACTIONS OF FLUORESCEIN

DESCRIPTION	VALUE	REFERENCE NO.
Permeability to fluorescein		
Corneal epithelium	2.5×10^{-7} cm^2/min	143, 144
Corneal endothelium	$1.8 - 3.6 \times 10^{-4}$ cm^2/min	96, 100, 106, 125, 128, 145
Distribution ratio cornea/anterior chamber	1.2 – 1.7	96, 123, 125, 128, 145
Diffusion coefficient		
Corneal stroma	$5.3 - 6.6 \times 10^{-5}$ cm^2/min	142, 146
Vitreous	4.0×10^{-4} cm^2/min	121, 122
Transfer coefficient by diffusion		
Anterior chamber to plasma, K_d	3.3×10^{-4} min^{-1}	81
	9.7×10^{-4} min^{-1}	83
	4.5×10^{-4} min^{-1}	130
Sclera to plasma, K_{sp}	1.5×10^{-2} min^{-1}	*
Plasma binding		
Binding site concentration	4×10^{-3} molar	132
Dissociation constant	6×10^{-4} molar	132
Unbound/total ratio	0.15	83, 132
Effect of binding on fluorescence		
Excitation spectrum	Peak reduced 40%, shifted 11 nm	133
Emission spectrum	Peak reduced 68%, shifted 6 nm	133
Molar fluorescent intensity of		
Unbound/bound, I_f/I_b	2.2/1	132, 147
Polarization of plasma-bound form	0.42	132
Active transport sites	Retina Ciliary body	121, 122, 134

*Determined in this study.

fluorescence and shifts its excitation and fluorescence spectra toward longer wavelengths.[133]

Fluorescein penetrates the corneal epithelium poorly, but readily penetrates the endothelium. Its entrance into the vitreous and posterior chamber from the plasma is greatly retarded by an active transport mechanism.[121,122,134] The fluorescent intensity of fluorescein in the corneal stroma is higher than that in the anterior chamber when the two compartments are in equilibrium. The ratio of intensity of the stroma to the anterior chamber in vivo at equilibrium in the human eye has been found to be 1.2 to 1.7.[96,123,125,128] The reason for this intensity distribution is unknown, but it may be caused partly by optical artifacts or by binding of fluorescein to some substance in the stroma. This unequal distribution of fluorescent intensity has also been observed in vitro[123] and in the absence of the limiting layers of the stroma.[146]

It must be kept in mind that the wavelength-dependent extinction coefficient of fluorescein determines the useful upper limit of the concentration and depth of fluorescein that can be measured accurately with a given fluorophotometer. In some instances, a correction for extinction can be applied.[106] In general, for 490-nm excitation, the upper limit of the useful range of concentrations for the cornea is 10^{-4} g/mL, and for the anterior chamber, 10^{-5} g/mL.[95]

MEASUREMENT OF FLUORESCENCE IN THE EYE

There are many factors that affect the ability of scientists to measure fluorescein in the living human eye. Such measurements appear to be simple, since the excitation and emission spectra of fluorescein are within the transmission spectrum of the cornea.[148] However, the environment of such measurements differs substantially from that of the rigorously controlled environment of the fluorescence spectroscopist who makes measurements of isolated solutions in transparent cuvettes. Table III outlines some of the additional problems encountered when such measurements are made in vivo.

Stromal measurements are particularly challenging because of the small size of the target and the motion of the eye. The cardiac pulse and the respiratory cycle can cause axial translations in some patients a distance greater than the corneal thickness. In addition, there are fluorescent materials in the cornea that determine the lower limit of useful fluorescent measurements in the stroma.[95] For corneal measurements, as with all measurements within the eye, one must consider the angle-de-

TABLE III: FACTORS THAT CAN AFFECT MEASUREMENT OF FLUORESCENT INTENSITY AND TOTAL FLUORESCENCE IN THE LIVING EYE

FACTOR	COMMENT
Transmission spectrum of cornea	Excitation wavelength should be longer than 400 nm
Ocular light scattering	
Cornea	Intense fluorescence in cornea interferes with measurement of weak fluorescence in anterior chamber
	Increases fluorescent intensity in cornea
Lens	Little effect on anterior segment measurements
Sclera	Total fluorescence window must not include sclera
Ocular light reflections	
Cornea	Angle dependency of reflection; angles of incidence should be $< 65°$
Lens	Little effect on anterior segment measurements
Iris	Mass measurement can be higher in blue-eyed subject
Optical power of cornea	Distorts target
Conjunctiva at limbus	Hides approximately ⅕ of anterior chamber from view
Autofluorescence	
Cornea	Equivalent to 8×10^{-9} g/mL fluorescein
	Interferes with measurement of extremely low levels of fluorescein in cornea
Lens	Equivalent to 1.2×10^{-7} g/mL fluorescein
Stromal binding of fluorescein	Accounts partly for observed distribution ratio of fluorescent intensity between cornea and anterior chamber
	Probably quenches fluorescence to small extent
Mixing of fluorescein	Regional differences in fluorescence can exist in stroma and anterior chamber
Light sensitivity of eye	Excitation exposure limited by safety considerations
	440 nm most dangerous for blue light hazard

pendency of the reflected and refracted light that is incident upon the tear-air interface. For angles of incidence less than approximately 65°, 90% or more of unpolarized light is refracted, but over 65°, a significant portion can be reflected.[149]

Scattering and reflection from surfaces within the normal eye usually do not interfere with fluorescence measurements in the cornea and the anterior chamber. However, under some conditions, care must be taken. For example, relatively high concentrations of fluorescein in the cornea can interfere with measurements of low concentrations in the anterior chamber.[104,106] Possible mechanisms for this interference include internal reflection and scattering in the cornea and are diagrammed in Fig 1. The apparent fluorescent intensity in the center of the anterior chamber (measured before fluorescein can enter it) is proportional to the fluorescent intensity in the cornea. The proportionality constant increases the nearer the cornea one attempts to measure anterior chamber fluorescence, however, for a given eye and a given location in the anterior

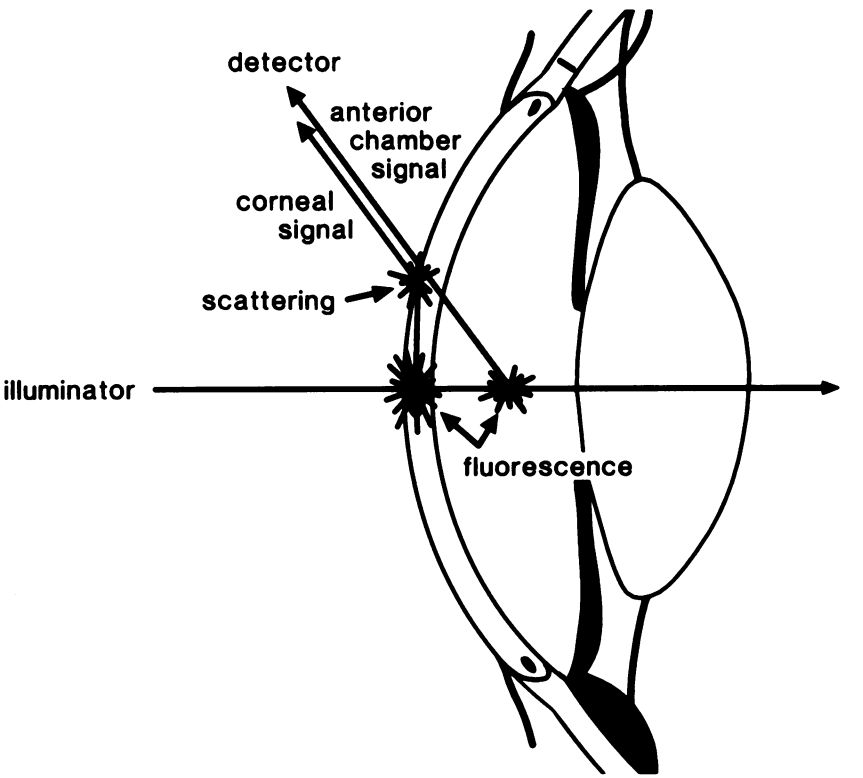

FIGURE 1
Optical arrangement for measurement of fluorescent intensity in anterior chamber illustrating interference from fluorescence in stroma.

chamber, this proportionality constant appears to be stable. In a study of 23 young, normal subjects, the apparent anterior chamber fluorescent intensity was found to rise to 1.2% ± 0.4% (mean ± standard deviation [SD]) of the stromal fluorescent intensity immediately following iontophoresis.[103,106] In a similar study in five monkey eyes, the proportionality constant was found to be 3.3% ± 0.2%.[104]

Other factors can affect measurements of total fluorescence of the combined cornea and anterior chamber, ie, measurements to determine the mass of fluorescein in the cornea and anterior chamber. Corneal autofluorescence will contribute a background signal.[95] Interference from autofluorescence of the crystalline lens will depend on pupillary size. In making mass measurements, scleral scatter must be eliminated by limit-

ing the diameter of the fluorescence window; only the cornea must be included. Otherwise, a large artifact is introduced. As a consequence, these mass measurements must exclude that portion of the anterior chamber that is hidden by the overhanging limbus. It has been estimated from photogrammetric measurements that 10% to 20% of the cornea and anterior chamber is thus hidden from view.[106]

Jones and Maurice[96] observed that iris reflectivity can increase the measured fluorescence from the anterior chamber. They estimated that the blue iris introduces a 10% error into such measurements but that the brown iris causes only a negligible error. A large error would be expected if a large amount of dye were located in the aqueous humor in intimate contact with the iris. Consequently, a method was devised for this study that depends on a single mass measurement, when nearly all of the dye is located in a circumscribed area of the cornea.

Measurement of fluorescence in the cornea can be affected by binding of the dye to substances in the stroma if the quantum efficiency is affected by the binding or if there is a shift of the excitation and the emission spectra. These effects occur when fluorescein is bound to macromolecules.[133,150] The molar fluorescent intensity (excitation $\lambda = 490$, emission $\lambda = 515$) of fluorescein bound to human plasma is less than half that of unbound fluorescein.[132]

Maurice has shown that the concentration of albumin in the rabbit stroma is one-fifth to one-fifteenth that in plasma.[151] Since fluorescein is known to bind to albumin,[147,152] one would expect to find higher fluorescein concentrations in the stroma during a steady-state with the anterior chamber. Measurements of the cornea-to-anterior chamber distribution ratio confirm this expectation.[96,125,128,145] Furthermore, the observed distribution ratio probably does not depend on active metabolism since the same distribution has been found in vitro as in vivo.[123,146] However, no detailed study of the role of albumin in quenching or concentrating fluorescein in the stroma has been made.

In light of the previous discussion, it is appropriate to point out that the fluorescent intensity of fluorescein in the stroma is practically the same as in free solution. Maurice[96] made this observation for his own fluorophotometer by comparing the total fluorescent intensity of fluorescein in an excised corneal button before and after chemical digestion of the cornea. In this study, Maurice's observations have been confirmed by observing the time profile of fluorescent intensity of a known mass of fluorescein as it diffuses into a corneal button in vitro. Thus, the observed intensity of fluorescence is an accurate measure of the total mass of fluorescein in the stroma. It remains to be answered whether this circumstance depends on

the fortuitous coexistence of optical effects that augment fluorescent intensity and quenching effects that reduce fluorescent intensity.

FLUOROPHOTOMETERS

Clinical fluorophotometry cannot be performed satisfactorily without a well-designed and sturdily constructed fluorophotometer. In general, satisfactory instruments have not been commercially available but have been fabricated by individual investigators to meet the requirements of their investigative protocols.[80,89,96,117-120] Such is the case in this study. The rationale for the configuration of the fluorophotometer used in this study is based on principles common to all fluorophotometer design.

Despite the high quantum efficiency of fluorescein, the amount of light available to a clinical fluorescence detector is extremely limited. First, the target is moving, so short exposure times are necessary. Second, the target volume is small, especially in the case of stromal measurements. Third, dilute concentrations of fluorescein must be measured, limited by the autofluorescence of the cornea, to provide an adequate range of concentrations for analysis. Fourth, the light intensity to which the eye can be exposed without danger of damage is limited.[153,154]

One can calculate the total flux of light emitted by a target area containing fluorescein from the following simplified formula[137]:

$$F_e = \Phi \cdot I_i \cdot V \cdot C_f \cdot \varepsilon\ (\lambda)$$

F_e is the flux or total power of fluorescent light emitted by the sample, Φ is the quantum efficiency of fluorescein, I_i is the irradiance of the excitatory light, V is the volume of the selected target containing fluorescein concentration C_f, and $\varepsilon\ (\lambda)$ is the extinction coefficient for fluorescein at the wavelengths present in the excitation beam, I_i. Suppose the target in the eye is 0.25 mm in diameter, is illuminated by a slit beam 0.25 mm thick, and contains 1×10^{-8} g/mL of fluorescein. The emitted fluorescent light for the most efficient wavelength for excitation, 490 nm, and for the maximum permissible intensity of collimated light (American National Standards Institute [ANSI] standard for a 5-min exposure[153]) entering the eye is calculated to be

$$F_e = 0.9 \cdot (1.28 \times 10^{-5}\ \text{W}/6.25 \times 10^{-4}\ \text{cm}^2) \cdot 1.2 \times 10^{-5}\ \text{cm}^3 \cdot 10^{-8}\ \text{g/cm}^3 \cdot 4.6 \times 10^{5}\ \text{cm}^2/\text{g}$$

$$F_e = 1.02 \times 10^{-9}\ \text{W}.$$

This light is emitted in all directions, and only that portion which is collected and focused on a light sensor can be measured.

The efficiency of the aperture E_a of the photoslitlamp camera (Zeiss) at its greatest magnification ($\times 40$) can be calculated from geometric considerations. We have measured the efficiency of the optics E_o, including a 50/50 beam splitter, in transmitting the incident power to the camera plane of the photoadapter (Zeiss) at its maximum f-setting, f14. From these data, the fluorescent power F_d available to any light detector placed in the camera plane can be calculated.

$$F_d = F_e \cdot E_a \cdot E_o$$

$$F_d = 1.02 \times 10^{-9}\ \text{W} \cdot 1.4 \times 10^{-3} \cdot 5 \times 10^{-2}$$

$$F_d = 7.1 \times 10^{-14}\ \text{W} = 1.9 \times 10^{5}\ \text{photons/sec}$$

Under these circumstances the intensity of light available to a detector is small. Clearly, a good fluorophotometer must have a light source of maximum permissible intensity, efficient collection optics, and a sensitive light detector.

Several combinations of light sources, filters, and light detectors offer potential usefulness in a clinical fluorophotometer. Table IV summarizes the spectral characteristics of some commercially available light sources as well as the transmission characteristics of several filters (including the cornea), the excitation and emission spectra of fluorescein, the sensitivity spectrum of a commonly used photomultiplier tube for fluorescein research, and the spectrum of blue light hazard to the retina. From this table, one can estimate from convolution integrals how a given system of filters and a light source will perform in comparison with other systems. Table V compares several of these light sources as they might be used with the Zeiss photoslitlamp camera. The table shows that the 488-nm output of an argon laser is the most efficient source but that others, such as a high-pressure xenon source, can be improved by spectral enhancement near 490 nm. Many of these light sources can be configured and used in such a way that the maximum permissible exposure guidelines are exceeded.

Table VI is a comparison of the features of several fluorophotometers that have been used for the measurement of aqueous flow. Each of these instruments is satisfactory for clinical work, but none has every desirable feature for flow measurement.

Commercially constructed instruments are now beginning to become available to investigators, including instruments from Metricon, Coher-

TABLE IV: SPECTRAL CHARACTERISTICS OF FLUOROPHOTOMETER ELEMENTS

WAVELENGTH nm	POWER SPECTRA* $\mu W/cm^2 \cdot nm$ AT 50 cm: 150 W XENON	200 W MERCURY	1000 W HALOGEN-TUNGSTEN	250 W *Xe Zn* I_2	FLUORESCEIN: EXCITATION SPECTRUM (MAXIMUM = 1.0)	FLUORESCEIN: EMISSION SPECTRUM (MAXIMUM = 1.0)	TRANSMISSION SPECTRA OF FILTERS, %: FLUORESCENCE EXCITATION B-4	MERCURY 436 LINE	CYAN DICROIC	ORIEL IR BLOCKER (5196)	FLUORESCEIN BARRIER B-5	HUMAN CORNEA	BIALKALI PHOTOMULTIPLIER SENSITIVITY (MAXIMUM = 1.0)	BLUE LIGHT HAZARD (MAXIMUM = 1.0)
400	0.9	4.0	2.3	. . .	0.15	. . .	. . .	. . .	91	80	. . .	80	1.00	0.10
410	1.0	20.0	2.7	2.1	0.18	. . .	. . .	. . .	92	79	. . .	81	1.00	0.40
420	1.1	2.4	3.1	2.1	0.23	. . .	. . .	2	92	77	. . .	82	1.00	0.90
430	1.1	3.0	3.6	2.1	0.33	. . .	. . .	27	92	78	. . .	83	0.99	0.98
440	1.2	30.0	4.1	2.1	0.45	. . .	. . .	35	92	79	. . .	84	0.97	1.00
450	1.3	2.6	4.6	2.1	0.57	. . .	6	3	89	79	. . .	85	0.94	0.94
460	1.5	1.7	5.1	2.0	0.73	. . .	78	. . .	89	79	. . .	86	0.90	0.80
470	2.0	1.7	5.7	3.6	0.87	. . .	83	. . .	90	79	. . .	87	0.85	0.62
480	1.5	1.5	6.4	7.2	0.95	. . .	78	. . .	88	80	. . .	87	0.80	0.45
490	1.4	2.0	7.0	5.7	1.00	0.11	11	. . .	86	81	. . .	88	0.76	0.22
500	1.3	1.2	7.7	1.5	0.83	0.44	1	. . .	87	81	3	89	0.71	0.10
510	1.3	1.1	8.3	1.6	0.33	0.78	. . .	. . .	91	80	68	90	0.64	0.06
520	1.3	1.0	9.0	1.4	0.01	0.96	. . .	. . .	89	79	76	90	0.57	0.04
530	1.3	1.1	9.7	1.1	. . .	0.98	. . .	. . .	81	79	81	91	0.51	0.03
540	1.3	15.0	10.4	1.1	. . .	0.89	. . .	. . .	81	80	83	91	0.44	0.02
550	1.3	10.0	11.1	1.0	. . .	0.78	. . .	. . .	92	81	83	92	0.37	0.01
560	1.3	2.2	11.8	1.1	. . .	0.63	. . .	. . .	89	80	79	93	0.31	0.01
570	1.3	2.5	12.5	1.2	. . .	0.52	. . .	. . .	55	80	71	94	0.26	. . .
580	1.3	30.0	13.2	1.1	. . .	0.41	. . .	. . .	25	79	63	94	0.20	. . .
590	1.3	2.8	13.9	1.0	. . .	0.31	. . .	. . .	10	78	65	95	0.14	. . .
600	1.3	1.4	14.7	1.6	. . .	0.22	. . .	. . .	4	76	49	96	0.08	. . .
610	1.3	1.3	15.3	1.6	. . .	0.17	. . .	. . .	2	73	7	96	0.07	. . .
620	1.3	1.2	16.0	1.2	. . .	0.11	. . .	. . .	. . .	71	1	96	0.05	. . .
630	1.4	1.1	16.7	0.6	. . .	0.07	. . .	. . .	. . .	68	. . .	96	0.04	. . .
640	1.4	1.1	17.4	3.9	. . .	0.04	. . .	. . .	. . .	64	. . .	96	0.03	. . .
650	1.4	1.1	18.1	1.1	. . .	0.02	. . .	. . .	. . .	60	. . .	96	0.01	. . .

*References 155 and 156.

TABLE V: COMPARISON OF LIGHT SOURCES FOR FLUOROPHOTOMETRY

LIGHT SOURCE	NOMINAL POWER	FLUORESCEIN EXCITATION B.A. 33-18-50 (B-4)	MERCURY 436 LINE B.A. 10-28-9	CYAN DICROIC	INFRARED (ORIEL 5196)	f2 CONDENSER	BEAM EXPANDER	⅛" × 36" FIBEROPTIC CABLE	ZEISS PHOTOSLITLAMP SYSTEM 0.1 mm × 3.0 mm SLIT	DOMINANT WAVELENGTH	POWER INCIDENT THROUGH 0.1 mm × 3.0 mm SLIT ON: a) CORNEA	b) ANTERIOR CHAMBER	c) RETINA	RELATIVE PHOTOPIC BRIGHTNESS (COMPARED TO 550 nm) (NORMALIZED TO ARGON LASER)	RELATIVE FLUORESCENT EXCITATION (NORMALIZED TO ARGON LASER) a) CORNEA	b) ANTERIOR CHAMBER	RELATIVE "BLUE LIGHT HAZARD" PER W (NORMALIZED TO ARGON LASER)	EXTINCTION COEFFICIENT cm^{-1} mol^{-1} × 10^5	RELATIVE EFFICACY-SAFETY OF SOURCE (FLUORESCENT EXCITATION) / (BLUE HAZARD)
										nm	*μW*	*μW*	*μW*						
Xenon arc*	150 W	X	. . .	X	X	X	. . .	X	X	470	1.81	1.58	1.32	0.06	0.11	0.11	2.26	1.50	0.37
Mercury arc*	200 W	. . .	X	X	X	X	. . .	X	X	440	4.90	4.11	2.66	0.04	0.16	0.15	3.70	0.75	0.12
Tungsten filament, (30 W)* (new bulb)	30 W	X	. . .	X	X	. . .	. . .	. . .	X	475	5.20	4.52	3.76	0.17	0.33	0.32	2.15	1.50	0.40
Xenon-zinc iodide	250 W	X	. . .	X	X	X	. . .	X	X	480	4.64	4.04	3.41	0.18	0.30	0.30	1.98	1.70	0.45
Argon laser (488 nm)	75 mW 35 mW @ 488	. . .	. . .	. . .	. . .	. . .	X	X	X	488	13.9	12.3	10.84	1.00	1.00	1.00	1.00	1.70	1.00
Helium-cadmium laser	18 mW	. . .	. . .	. . .	. . .	. . .	X	X	X	442	3.34	2.81	1.84	0.03	0.11	0.10	3.67	0.80	0.12
Xenon flash (W-Sec)	840† W-Sec	X	. . .	X	X	. . .	. . .	. . .	X	470	12.11 (μW •S)	10.54	8.81	0.39	0.75	0.74	. . .‡	1.50	. . .‡

*Based on measurements with International Light Radiometer, SEE-100 probe. Other power figures are estimates based on power spectra of source, transmission spectra of filters, and measured efficiency of delivery system.

†Flash setting IV (Zeiss slit lamp).

‡Blue light hazard not known for short exposures.

TABLE VI: COMPARISON OF FEATURES OF FLUOROPHOTOMETERS USED FOR MEASURING AQUEOUS HUMOR FLOW

	MAURICE	WALTMAN AND KAUFMAN	BRUBAKER
Light source	Mercury arc (modulated)	Tungsten (continuous)	Xenon arc (flash lamp)
Optics	Custom-designed	Haag-Streit slit lamp	Zeiss photoslitlamp
Light sensor	High-gain photomultiplier	High-gain photomultiplier	High-gain photomultiplier
Amplifier	AC-coupled amplifier	DC-coupled amplifier	Custom-designed timer-integrater
Readout	Meter visible through observation optics	Strip chart recorder	Printing voltmeter
Lower limit of useful sensitivity, g/mL fluorescein	10^{-9}	10^{-9}	10^{-9}
Automatic compensation for background light	Yes	No	Yes
Automatic compensation for lamp intensity fluctuations	No	No	Yes
Duration of measurement	Continuous	Continuous	0.1 msec
Fluorescence window directly visible	Yes	Yes	No
Mass measurement in large diameter field	Yes	No	Yes

ent Radiation, and Topcon (Table VII). Each of these instruments incorporates newer technical features that can facilitate clinical fluorophotometry. An ideal instrument would provide the investigator a quantitative image of fluorescence of the anterior segment in three dimensions. Such an instrument is within the reach of current technology, but no state-of-the-art instrument has yet been described or marketed.

METHODS

METHODS OF ANALYSIS

There are a number of methods that can be used to calculate aqueous humor flow from the measurements of the time course of ocular fluorescent intensity. A good method must be objective and should put the greatest weight on the most accurate data. In addition, the method should be reasonably simple to accomplish. In these studies, we have pursued

TABLE VII: NEW COMMERCIALLY AVAILABLE FLUOROPHOTOMETERS FOR THE EYE POSSIBLY SUITABLE FOR MEASUREMENT OF AQUEOUS HUMOR FLOW

	COHERENT RADIATION*	METRICON*	TOPCON
Inventor	J Gray	A Vassiliadis	S Mishima
Light source	Quartz halogen	Tungsten	Tungsten
Optics	Custom-designed	Haag-Streit slit lamp	Topcon slit lamp
Light sensor	Photon-counting photomultiplier	Linear diode array	Photon-counting photomultiplier
Amplifier	Photomultiplier	Scanner/averager	Photomultiplier
Readout	Computer printout	Computer printout	Computer printout
Lower limit of useful sensitivity, g/mL fluorescein	2×10^{-10}	10^{-9}	. . .
Automatic compensation for background light	No	No	Yes
Automatic compensation for lamp intensity fluctuations	Yes	No	No
Duration of measurement	0.01 - 0.50 sec	1.5 - 5.0 sec	0.4 - 2.0 sec
Fluorescence window directly visible	No	No	Yes
Mass measurement in large-diameter field	No	No	No

*Designed primarily for vitreous fluorophotometry.

methods that depend on two models of the movement of fluorescein in the anterior segment of the eye, the two-compartment model of Jones and Maurice[96] and a multiple-compartment model.

The two-compartment model is simpler and is more generally applicable. This model requires that epithelial losses and limbal losses of fluorescein from the cornea be negligible as compared with losses into the anterior chamber through the endothelium. In addition, it requires that the rate of loss of fluorescein from the cornea to the anterior chamber, discounting its lateral distribution in the cornea, is dependent on the total mass of fluorescein in the cornea. Since the corneal stroma is not a "stirred" compartment, this assumption implies at least two others: (1) that the corneal endothelial surface is uniformly permeable to fluorescein and (2) that the corneal stroma is sufficiently thin that axial concentration gradients of fluorescein in the stroma are negligible. Finally, the model implies passive exchange of fluorescein across the endothelium. Under these conditions, fluorescein enters and leaves the cornea according to a

differential equation. (The system of symbols used in this thesis follows closely the system proposed by Duke-Elder and Maurice.[157] Specific definitions of all symbols are given in the section "Definitions and Symbols.")

$$\frac{dM_c}{dt} = -K_{ca} M_c + K_{ac} M_a \quad (1)$$

In this symbolic representation of fluorescein exchange, the rate has been described in terms of mass rather than concentration because concentration is not necessarily uniform in either compartment. M_c and M_a are the masses of fluorescein in the cornea and the anterior chamber, K_{ca} is the cornea-to-anterior chamber transfer coefficient, and K_{ac} is the anterior chamber-to-cornea transfer coefficient.

In the two-compartment model, the rate of loss of fluorescein from the anterior chamber must be proportional to the total mass of fluorescein in the anterior chamber. This necessity does not imply that the anterior chamber must be a perfectly stirred compartment. The model is valid if imperfections in mixing are offset by the wide distribution of the sites of fluorescein loss. These sites include the surface of the iris, the peripheral cornea, and the circumferentially disposed trabecular meshwork in the iridocorneal angle.

Under the conditions of the two-compartment model, fluorescein exchanges with the anterior chamber according to the equation stated below.

$$\frac{dM_a}{dt} = K_{ca} M_c - K_{ac} M_a - K_o M_a \quad (2)$$

The anterior chamber loss coefficient, K_o, is the sum of two other coefficients, K_f, the loss coefficient caused by aqueous humor outflow, and K_d, the loss coefficient caused by diffusional loss.

In the case where fluorescein is placed by iontophoresis into a stromal depot, the initial conditions of the model are as follows:

$$M_c(0) = M_o$$
$$M_a(0) = 0.$$

Under these conditions, equations 1 and 2 can be solved as simultaneous linear differential equations to yield double exponential equations for the

cornea and for the anterior chamber. The equation for the anterior chamber is

$$X_a = \gamma e^{-\alpha t} - \gamma e^{-\beta t} \tag{3}$$

and the corneal equation is

$$X_c = \delta e^{-\alpha t} + (1-\delta)e^{-\beta t} \tag{4}$$

The symbol X is used to denote the dimensionless fraction of the initial "dose" of fluorescein that remains in the cornea or has entered the anterior chamber after the application of fluorescein to the cornea. The fractional notation simplifies interpretation since both X_c and X_a are confined to the range 0 to 1. The Greek letters represent "hybrid" coefficients, that is, coefficients that have no readily interpretable meaning as regards physiologic processes. Their definitions are given in the section "Definitions and Symbols."

Equations 3 and 4 can be used in a variety of ways to deduce the fluorescein loss coefficient from the anterior chamber, K_o, and hence to estimate the rate of flow of aqueous humor through the anterior chamber. Some of these methods are used in general pharmacokinetic analyses of more complicated systems and include curve stripping (method 1 of Jones and Maurice[96]), terminal clearance (method 2 of Jones and Maurice[96]), the area-under-the-curve (AUC) method of Wagner and Nelson,[158] Coakes and Brubaker,[106] or a number of curve fitting methods.[106,130,159] A comparison of some of the available methods is discussed in detail by Coakes and Brubaker.[106]

In this study, two methods of flow calculation have been used, a nomographic method and a least-squares method. The nomographic method is based on the idea that the coefficients K_{ca} and K_o can be determined uniquely if X_a can be measured accurately at any two times, other than time zero. (The method is analogous to determining the position of a plane by knowing its origin and the location of two other points in the plane.)

The times for the two X_a measurements must be chosen carefully to minimize the effects of errors of measurement or the effects of variations of aqueous humor flow. These measurements must be made at widely spaced times following iontophoresis. They should be made when the fluorescein concentration is high enough to be measured easily and when the rate of change in fluorescein concentration in the anterior chamber is low. These criteria are met near the peak of anterior chamber fluorescent intensity (approximately two hours for the normal eye) and several hours

after the peak. In these studies, all nomographic calculations of K_o have been made from X_a measurements two hours and seven hours after application of fluorescein.

The nomogram is a two-dimensional display of $X_a(2)$, $X_a(7)$ pairs (Fig 2). To construct the nomogram, values of $X_a(2)$ and $X_a(7)$ are calculated from equation 3 for the full range of K_{ca}, K_o pairs likely to be encountered. For convenience, these points are plotted on logarithmic graph paper. Isopters of K_{ca} and K_o are connected with curved lines. Because the relation among the K values and the X values is partly dependent on anterior chamber volume, three nomograms were constructed, one each for anterior chamber volumes of 100 μL, 200 μL, and 300 μL. This range of anterior chamber volumes covers most of those likely to be encountered in human studies.[160]

To use the nomogram to determine the aqueous humor flow in a human subject, the investigator must measure the initial dose, M_o, the volume of the anterior chamber, and the anterior chamber concentrations of fluorescein at two and seven hours. The fraction in the anterior chamber is calculated from the relation

$$X_a = C_a \cdot v_a / M_o$$

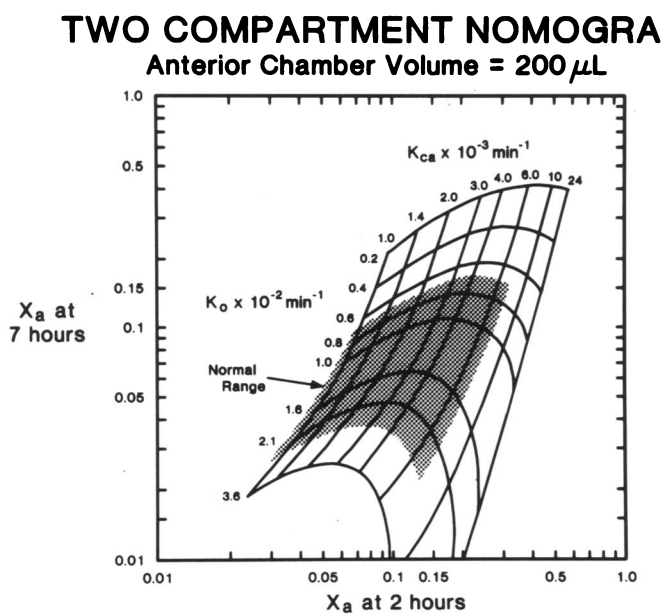

FIGURE 2
Two-compartment nomogram[106] indicating normal range for K_{ca} and K_o.

The X_a pair is plotted on the appropriate nomogram as a single point, and the corresponding values of K_{ca} and K_o, deduced by visual extrapolation from the nearest isopters.

The measurement of X_a at seven hours is the most critical for the determination of K_o and, hence, aqueous humor flow. In the situation where aqueous humor flow is variable rather than steady, the nomographic method calculates a result that is not the simple numerical average of the flow rates during each individual time segment. Rather, the flow rate immediately prior to the seven-hour measurement has the greatest effect on the calculated result.

The relative weight given to flow occurring during each hourly time period was calculated for an eye with normal endothelial permeability and a normal average rate of flow, but with fluctuations in the rate of flow between 50% and 200% of the average rate. These relative weighting factors are listed in Table VIII. One can see from this table that the nomographically calculated rate of flow depends almost entirely on the rate of flow that actually occurs during hours four through seven. If the rate of flow is steady, the calculated rate is the same as the steady rate for the seven-hour period.

The method of successive approximation can be employed to determine K_{ca} and K_o, eliminating the need for a nomogram and permitting the use of a specific anterior chamber volume. In these studies, a modified Newton-Raphson method has been used to fit equation 3 to the data.[106] The nomographic method and the method of successive approximation should produce identical results except that the latter is more accurate

TABLE VIII: NOMINAL WEIGHTING FACTORS INHERENT IN THE NOMOGRAPHIC METHOD OF CALCULATING AQUEOUS HUMOR FLOW UNDER CONDITIONS OF VARIABLE FLOW RATE

TIME WINDOW (HRS)	RELATIVE WEIGHT GIVEN TO AQUEOUS FLOW TAKING PLACE DURING TIME WINDOW (TOTAL WEIGHT = 1.00)
0–1	0.01
1–2	0.04
2–3	0.05
3–4	0.07
4–5	0.13
5–6	0.24
6–7	0.46
Total	1.00

because of its use of the measured anterior chamber volume in the calculation and its lack of dependency on visual extrapolation.

In many of these studies, measurements of X_a were made every hour for eight hours, permitting observation of a more complete and reliable picture of the dye transit through the anterior chamber. In these studies, the best fitting parameters were determined by statistical methods. Initial estimates of K_{ca} and K_o were made. The hybrid coefficients γ, α, and β of equation 3 were calculated from these initial values. The difference between the measured value $X_a(t_i)$ and the calculated value $\gamma_e{}^{-\alpha t_i}$ $\gamma_e{}^{-\beta t_i}$ was determiined for each measurement of anterior chamber concentration.

$$\text{Difference } (i) = X_a(t_i) - \gamma_e{}^{-\alpha t_i} - (\gamma_e{}^{-\beta t_i}) \tag{5}$$

The root-mean-square (RMS) difference between the data set and the initial estimates of the parameters of the two-compartment model were calculated.

$$RMS = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (\text{Difference } (i))^2} \tag{6}$$

This process was repeated for a new pair of K_{ca}, K_o estimates. Ultimately, the paired estimate of K_{ca}, K_o that produced the smallest RMS difference was accepted as being correct. The minimum RMS was used as a measure of satisfactory fit for an individual determination of flow.

Several methods were tried for finding the minimum RMS difference. Errors in measurement of fluorescence and probable variations of the living eye from the two-compartment model impose practical limits on the accuracy with which the best K_{ca} and K_o values must be calculated. Thus, a two-dimensional search of K values in the region of the expected minimum has proved to be a sufficient and rapid method. More elaborate methods of determining the best K values have been described,[86,130,159] but these other methods, because of lengthy computations, are more costly in computer time.

A multiple-compartment model was used to evaluate certain theoretic limitations of the two-compartment model. The multiple-compartment model divides the cornea into layers and concentric rings so that the diffusional movement of fluorescein in the cornea, the exchange of fluorescein at the limbus, and the exchange of fluorescein with the anterior chamber can be simulated. A diagram illustrating this model and scaled

drawings of the human eye from which the dimensions were taken for this model are shown in Figs 3 through 5.

Diffusion between adjacent compartments within the cornea or between the cornea and the sclera is modeled according to Fick's law.

$$\frac{\Delta \text{Mass}}{\Delta t} = \frac{D_c \cdot A_{12} \cdot (C_2 - C_1)}{\Delta x} \tag{7}$$

In this equation, D_c is the diffusion coefficient of fluorescein in the stroma, A_{12} is the area of the interface between the two compartments, C is the concentration of unbound fluorescein in each compartment, Δt is the time interval, and Δx is the distance between the centers of the compartments. The corneal endothelium is modeled as a thin membrane of uniform permeability P_{ca} and surface area A_{ca}. The rate of exchange across this surface is dependent on the unbound concentrations of fluorescein in the inner layers of the cornea and the anterior chamber.

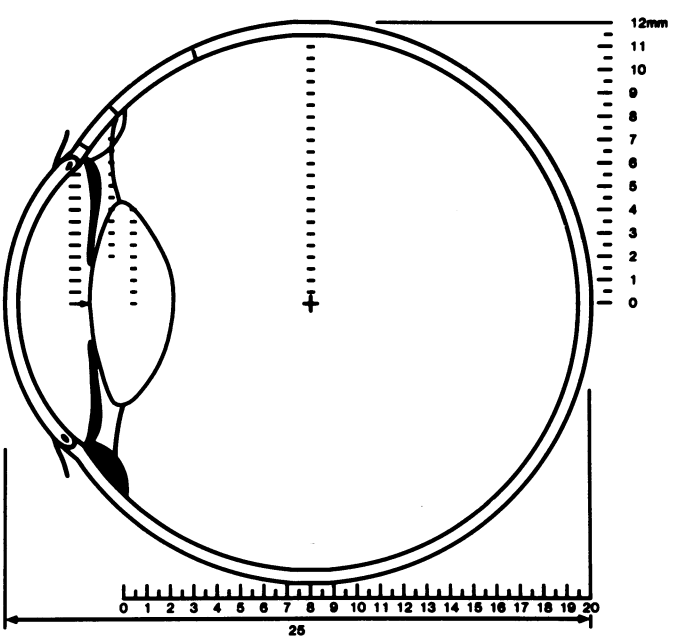

FIGURE 3
Scaled cross section of human eye from which dimensions were obtained for multiple-compartment model.

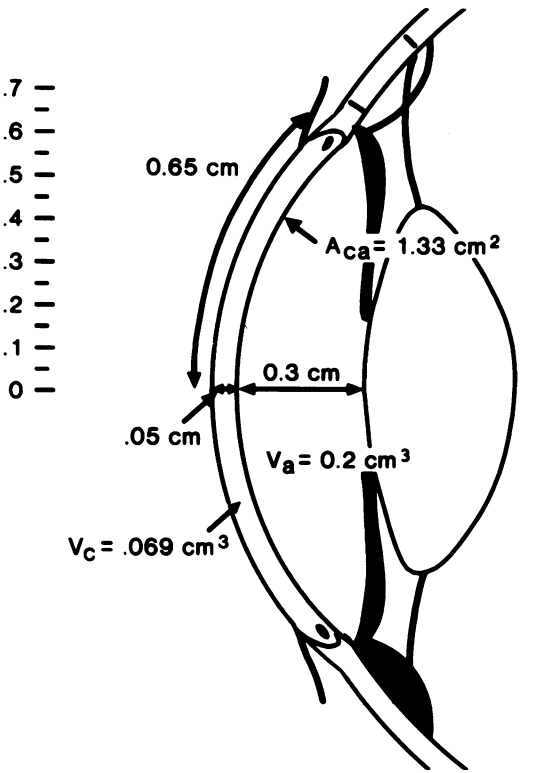

FIGURE 4
Scaled cross section of anterior segment of human eye indicating normal dimensions used for multiple-compartment model.

$$\frac{\Delta \text{Mass}}{\Delta t} = P_{ca} \cdot A_{ca} \cdot (C_a - C_c) \tag{8}$$

The loss of fluorescein from the anterior chamber into vessels of the iris is simulated as a first-order process.

$$\frac{\Delta \text{Mass}}{\Delta t} = K_d \cdot M_a \tag{9}$$

Also, the loss of fluorescein from the anterior chamber at the iridocorneal angle because of flow is simulated as a first-order process.

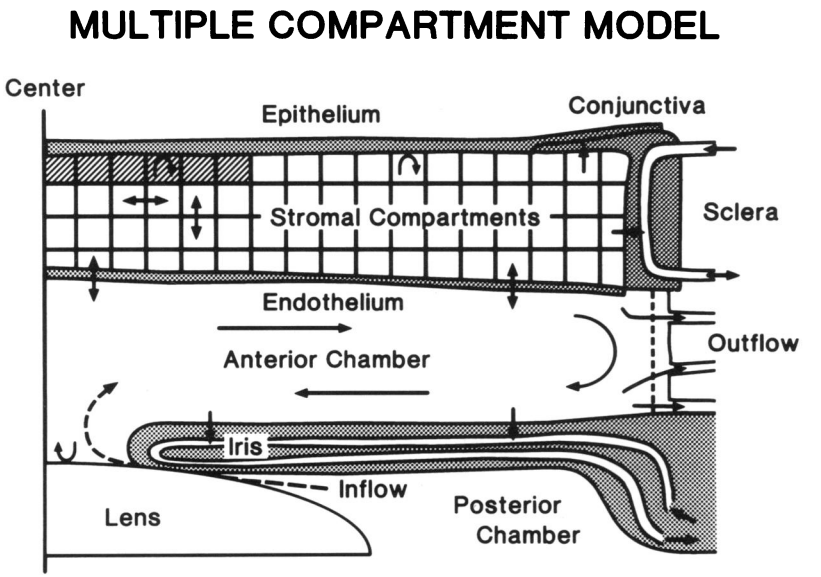

FIGURE 5
Diagram of cross section of multiple-compartment model illustrating compartments and boundaries. See Table IX for values of parameters.

$$\frac{\Delta\text{Mass}}{\Delta t} = K_f \cdot M_a \tag{10}$$

The loss of fluorescein from the scleral compartments into limbal vessels is simulated by first-order kinetics. Here $M_s(i)$ is the mass of fluorescein in each scleral compartment, and K_{sp} is the sclera-to-plasma transfer coefficient.

$$\frac{\Delta\text{Mass}}{\Delta t} = K_{sp} \cdot M_s \tag{11}$$

The same equation is used to simulate loss of fluorescein from the most superficial layers of the peripheral stroma covered by overlying conjunctiva (Fig 5).

A summary of the parameters of the model and the values used for purposes of simulation of fluorescein movement in the eye are given in Table IX.

TABLE IX: PARAMETERS OF THE MULTIPLE-COMPARTMENT MODEL OF PHARMACOKINETICS OF FLUORESCEIN IN THE HUMAN EYE

PARAMETER	VALUE	SYMBOL	UNITS
Cornea			
Thickness of stroma	0.05	X_c	cm
Diameter of cornea	1.30	. . .	cm
Geometric volume of stroma	0.069	v_c	cm^3
(Apparent volume of distribution of fluorescein in stroma)	0.108	V_c	cm^3
Number of stromal rings	48	. . .	. . .
Number of stromal layers	4	. . .	. . .
Cornea/anterior chamber distribution ratio	1.60	R_c	. . .
Diffusion coefficient of fluorescein in stroma	6.6×10^{-5}	D_c	cm^2/min
(Area of endothelium)	1.33	A_{ca}	cm^2
Permeability of epithelium	0.00	. . .	. . .
Cornea-to-anterior chamber transfer coefficient	3.0×10^{-3}	K_{ca}	min^{-1}*
(Endothelial permeability)	2.3×10^{-4}	P_{ca}	cm/min
Scleral diffusional loss coefficient	1.52×10^{-2}	K_{sp}	min^{-1}
Diameter of initial depot of fluorescein	0.50	. . .	cm
Anterior chamber			
Volume of anterior chamber	0.200	v_a	cm^3
Anterior chamber loss coefficient due to diffusion	1.5×10^{-3}	K_d	min^{-1}
Anterior chamber loss coefficient due to flow	1.35×10^{-2}	K_f	min^{-1}*
(Aqueous humor flow rate)	2.7×10^{-3}	. . .	cm^3/min

*These values varied to produce multiple-compartment nomogram.

At time zero, fluorescein is placed into one or more compartments of the cornea. Thereafter, the concentrations and masses of fluorescein and the fluorescent intensity of each compartment as viewed externally are calculated for small increments of time. This model was used to test certain theoretic limitations of the two-compartment model and to generate multiple-compartment nomograms for determining aqueous humor flow.

LABORATORY PROCEDURES

The relation between fluorescent intensity and fluorescein mass in the cornea was determined by agar-to-cornea diffusion experiments in vitro. The corneal epithelium of a freshly killed rabbit was scraped away, and a 5-mm button of the central cornea removed with a trephine. The endothelium was wiped away. The button was weighed and stored temporarily in a moist chamber. A solution of 10^{-6} g/mL sodium fluorescein in phosphate buffer, pH 7.4, was prepared and heated with 2% agar. The heated fluorescent agar solution was poured onto glass plates and allowed to harden at room temperature. Circular buttons of the fluorescent agar were cut with a 5-mm trephine and weighed. The total fluorescent intensity of the agar button alone and the corneal button alone were determined with a fluorophotometer. The agar button and the corneal button were then placed together. The fluorescent intensity was measured at frequent intervals in the center of the specimen for one to two hours as fluorescein entered the corneal button by diffusion from the agar (Fig 6).

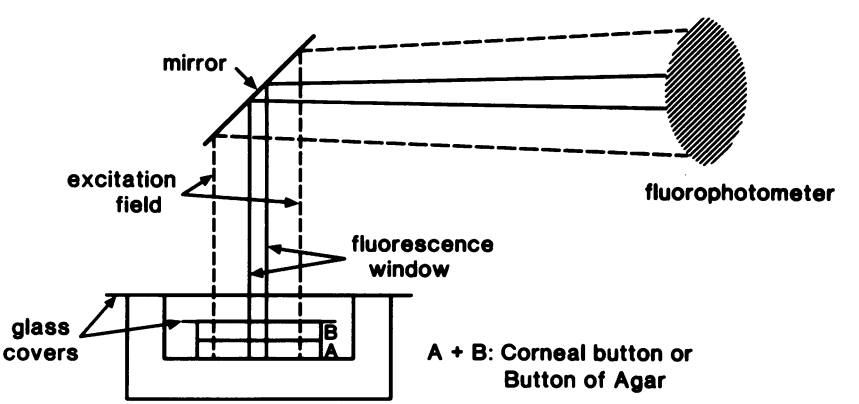

FIGURE 6

Experimental arrangement to measure apparent fluorescent intensity of fluorescein in corneal stroma. In some instances, button of agar occupied position A and button of stroma position B. In other instances, these positions were reversed.

The effect of ocular reflectance on the measurement of fluorescent intensity of fluorescein in the rabbit cornea was measured in vitro. Reflectance was measured in arbitrary units with a fluorophotometer from which the barrier filter had been removed. The test material was illuminated with a blue 10-mm diameter excitation field and observed at a nominal angle of 10° with an 8-mm diameter light collection window. The reflectance of the human eye was measured in subjects having a wide range of iridal color. Reflectance was measured also in an albino rabbit eye. An artificial anterior chamber was constructed from black plastic, and a transparent acrylic contact lens served as the "cornea." The contact lens was 0.5 mm thick and 10 mm in diameter. Its anterior radius of curvature was 7.8 mm, and its posterior radius of curvature was 7.3 mm. Rough-textured paper was cut into circular discs and placed into the plane of the iris of the artificial chamber, as shown in Fig 7. These paper discs varied in reflectivity from black to white. The reflectivity of the artificial chamber was measured with each of the discs in place. The barrier filter was replaced into the fluorophotometer. A depot of fluorescein was placed by iontophoresis into the central 5 mm of the cornea of an anesthetized rabbit. The central 10 mm of the cornea was excised and laid over the contact lens of the artificial chamber. The fluorescent intensity of this depot was measured with each of the reflective discs in place. The corneal button was placed over the cornea of the fellow eye of the anesthetized

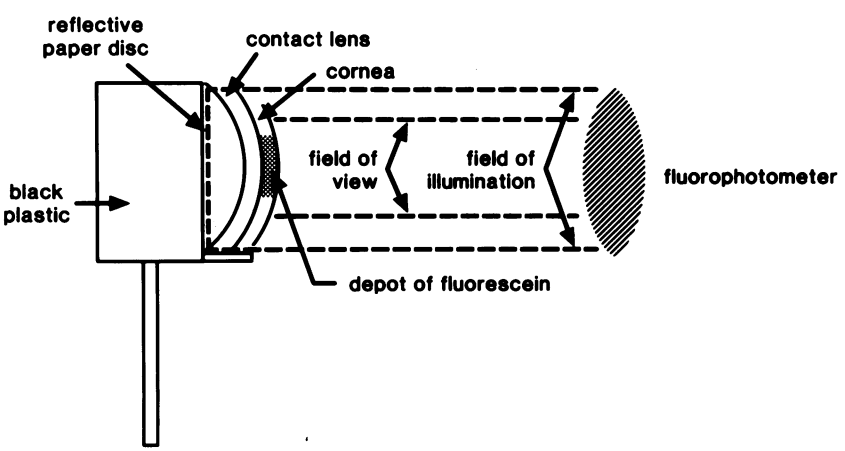

FIGURE 7
Experimental arrangement to determine effect of iridal reflectance on measurement of fluorescein mass in stroma. Reflective paper discs were used to simulate range of reflectivity to blue light encountered in human eyes.

rabbit, and the fluorescent intensity measured with the same fluorophotometer settings as used with the artificial chamber.

The effect of corneal fluorescence on the apparent fluorescent intensity in the anterior chamber was studied in vivo. A rhesus monkey was anesthetized with pentobarbital and placed in a head-holding apparatus, permitting measurement of the fluorescent intensity of the cornea and the anterior chamber. The anterior chamber was cannulated with two 23-gauge disposable needles and perfused with a tissue culture medium (TC 199) at a rate of 150 μL/min. The end of the drainage tubing was immersed in a beaker that was placed at a height sufficient to maintain the IOP at 12 mm Hg. A 5-mm diameter depot of fluorescein was placed into the central corneal stroma by iontophoresis. Excess fluorescein was flushed out of the tear film with water. The apparent fluorescent intensity in the corneal stroma and in the anterior chamber was measured at 15-minute intervals for nearly three hours. The experimental arrangement is illustrated in Fig 8.

The polarization of fluorescence in the corneal stroma of the rabbit eye was measured in vitro. The corneal epithelium of a freshly killed rabbit was scraped away with a wooden spatula. A solution of 10^{-4} g/mL sodium fluorescein in phosphate buffer, pH 7.4, was placed on the corneal surface

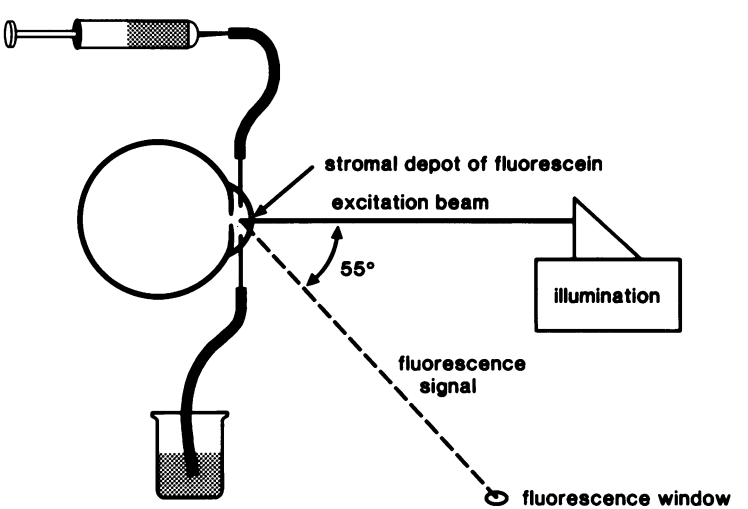

FIGURE 8
Experimental arrangement to determine effect of stromal fluorescence on measurement of fluorescent intensity in anterior chamber. Rate of perfusion of anterior chamber with a non-fluorescent medium was sufficiently rapid to wash out any fluorescein which entered from corneal depot.

for one minute and then blotted. The cornea was excised, and the endothelium wiped away with a cotton-tipped applicator. A strip of cornea 3 mm × 8 mm was cut from the stroma and placed between two strips of clear plastic. The plastic strips and the cornea were slid into a 3 × 3-mm glass cuvette containing phosphate buffer. Once inside the cuvette, the gap available to accommodate the thickness of the stroma was 0.3 mm. Fluorescence measurements were made in a photon-counting spectrofluorometer (SLM 8000). The instrument was used in a symmetric "T" arrangement with two emission monochromators and a dual excitation monochromator. Nichol prisms were used on all three sides to permit passage of linearly polarized light. The excitation monochromator was set at 495 nm, and the emission monochromators were set at 515 nm; the band width of all monochromators was 0.5 nm. Polarization measurements were made with the cuvette in each of its four possible orientations. Polarization was calculated from the formula

$$p = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

$I_{\parallel}$ and $I_{\perp}$ are the observed intensities of fluorescent light, the "e" vectors of which are parallel and perpendicular to the "e" vector of the excitation light.

The autofluorescence of the central cornea, the peripheral cornea, and the center of the crystalline lens was measured in six normal subjects. A 0.1-mm slit width was used for excitation along the visual axis, and fluorescence was measured at an angle of 55°.

The fluorescein concentrations of the cornea and anterior chamber were measured in two glaucoma patients who received hourly tonometry with instillation of a solution of 0.25% fluorescein and 0.4% benoxinate. Fluorescence was measured 30 minutes after the last instillation of fluorescein. These concentrations were compared to the concentrations reached in the normal eye after application of 350 ng of fluorescein by iontophoresis, the average dose used in the clinical studies described later.

The rate of exchange of fluorescein between blood and the tissues adjacent to the limbus was determined in five normal human subjects after oral ingestion of fluorescein and in four albino rabbit eyes after intravenous (IV) administration of fluorescein. An area of the conjunctival surface was selected in each instance to avoid including large vessels in the field of view. The axes of illumination and detection were separated by 10°. Total fluorescent intensity was measured in an 8-mm circular field

in the human subjects and in a 3-mm field in the rabbit experiments. The fluorescent intensity, I_s, of this target and the unbound concentration of fluorescein in the plasma were measured every 15 to 30 minutes for several hours.

The exchange between plasma and the "scleral" tissues was assumed to be a first-order process.

$$\frac{dC_s/R_s}{dt} = K_{sp}\,(C_p/R_p - C_s/R_s) \tag{12}$$

C_s/R_s is the unbound concentration of fluorescein in the scleral tissue and C_p/R_p is the unbound concentration in the plasma. C_s/R_s was assumed to bear a fixed but unknown relationship to the observed fluorescent intensity, I_s.

$$C_s/R_s = gI_s$$

For every experiment, a two-dimensional search was made to obtain the K_{sp}, g pair that produced the least squares deviation between the I_s data set and the theoretic C_s/R_s curve obtained by the integration of equation 12. The half-life of exchange between the scleral tissue and the plasma was calculated as shown below.

$$T\frac{1}{2} = \frac{ln\ 2}{K_{sp}}$$

CLINICAL PROCEDURES

Over the past five years, the author's laboratory has made over 1,000 determinations of aqueous humor flow through the anterior chamber using topically applied fluorescein (Table X). In all cases, the volume of

TABLE X: FLOW STUDIES IN HUMAN EYES CARRIED OUT IN AUTHOR'S LABORATORY

CATEGORY	NO. OF DETERMINATIONS
Normal eyes	
No drugs used	407
Drug studies	333
Abnormal eyes	264
Total	1004

the anterior chamber was determined by the photogrammetric method described by Johnson et al.[161] All measurements of fluorescent intensity were made with a stroboscopic fluorophotometer described previously.[120] This instrument has undergone three modifications. The current model requires no modification of the Zeiss photoslitlamp camera system and is connected directly to a desk top computer (Hewlett-Packard 9815A). Photographs of the components of the system, except for the slit lamp, are shown in Fig 9.

This instrument was calibrated with standards of sodium fluorescein in phosphate buffer, pH 7.4, beginning with a commercially available 10% solution. The range of concentration was 10^{-8} to 10^{-5} g/mL. Measurements were made in the artificial anterior chamber described previously. The geometric arrangement of the test chamber simulated that of the anterior chamber of the human eye.

Measurements of concentration of fluorescent intensity were made with an excitation beam 0.1 mm wide and 3 mm high. This beam was centered in the pupil and directed along the visual axis of the eye. The angle between the axis of the excitation beam and the axis of the light-sensing aperture was 55°. The aperture was a thin, curved slit. The curvature of the aperture coincided with the curvature of the image of the normal corneal stroma as it appears in the film plane of the Zeiss photoslitlamp camera system at a magnification setting of $\times 40$. The width of the aperture was approximately half the width of the image of the cross section of the cornea.

Measurements of total fluorescent intensity were made by illuminating a 10-mm diameter field along the visual axis and observing a field 8 mm or 12 mm in diameter at a nominal angle of 10°. The fluorophotometer was calibrated for mass measurements with a cuvette having a path length of 1 mm and a circular window 4.6 mm in diameter. Solutions of 10^{-5} and 10^{-6} g/mL fluorescein in buffer, pH 7.4, were used as standards.

The fluorophotometer was recalibrated daily with fluorescent uranium glass (Corning No. 3750). The glass standard had been ground and polished into a plano convex lens, front radius of curvature 7.8 mm. This glass standard was glued onto a black plastic support. A steel rod that snapped into an opening on the chin rest of the Zeiss photoslitlamp was used to support the standard at a convenient height for measurement (Fig 9). The fluorescent intensity of the glass standard was equivalent to a fluorescein solution of approximately 2×10^{-5} g/mL.

In many cases, background fluorescence of the cornea, lens, and anterior chamber was measured, but under most circumstances these fluorescence intensities were so low that background correction was not neces-

FIGURE 9
Ocular fluorophotometer currently used in author's laboratory. Four circuit boards, shutter/filter assembly, excitation filter attachment, and uranium glass standard are shown at top. Front panel with built-in voltmeter is seen at bottom. Desk top computer is connected directly to voltmeter and provides number of user programmable options as well as hard copy of results.

sary. In nearly all cases, fluorescein was applied iontophoretically to the central 5 mm of the cornea; in a few cases, fluorescein was applied to the upper portion of the cornea to study the effect of peripheral deposition on the clearance of fluorescein from the eye. The method of Jones and Maurice[96] was used for the iontophoretic application.

A 45-V battery was used to apply the current through a 220-kΩ resistor. The subject was instructed to hold the positive electrode in one hand, and the negative electrode, consisting of a disposable applicator of 10% fluorescein in 2% agar, was applied to the cornea. The apparatus permitted the battery voltage to be tested before each application. The current was monitored during application; it was usually 200 μA. The current was permitted to flow for five to seven seconds. An application of this duration would suffice to produce a bright 5-mm diameter circle of fluorescence in the stroma. This depot was usually found upon measurement to range from 100 to 600 ng of fluorescein. The apparatus that was used for iontophoresis is shown in Fig 10.

Care was taken to apply the electrode to the cornea before the circuit was closed (with a foot switch) and to break the circuit before removing the agar electrode from the eye. This precaution was taken to minimize the chance of producing a focal area of high current density. The agar electrode itself contained no conducting salt other than sodium fluorescein and thus had limited conductivity.

The method appeared to be quite safe and simple. Out of 1000 applications, only 1 significant corneal abrasion was produced. It was thought this abrasion was caused by contact between the eye and the edge of the polyethylene container, due to insufficient protrusion of the agar, resulting in direct trauma to the epithelium. This abrasion healed completely within 24 hours. In most eyes, the application produced a fine epithelial stippling that disappeared by the time topical anesthesia from ½% proparacaine hydrochloride had worn off. By the time the mass of the depot was measured (30 minutes), corneal luster had returned to normal.

Disposable agar electrodes were made in batches using 0.25 mL polyethylene microcentrifuge tubes (Fig 10). These electrodes were prepared under sterile conditions and stored at refrigerator temperatures until needed. The opening of the microcentrifuge tube conveniently fitted into a standard Luer taper. The agar electrode was held with a 1-cc tuberculin syringe fitted with a stainless steel insert. Alternatively, the electrode was held with a special applicator containing a capacitor that could be charged sufficiently to carry out iontophoresis without any external wires. In this case, the examiner's body served as the return pathway for the current.

The polyethylene container was placed into a cutting jig, and its tip cut

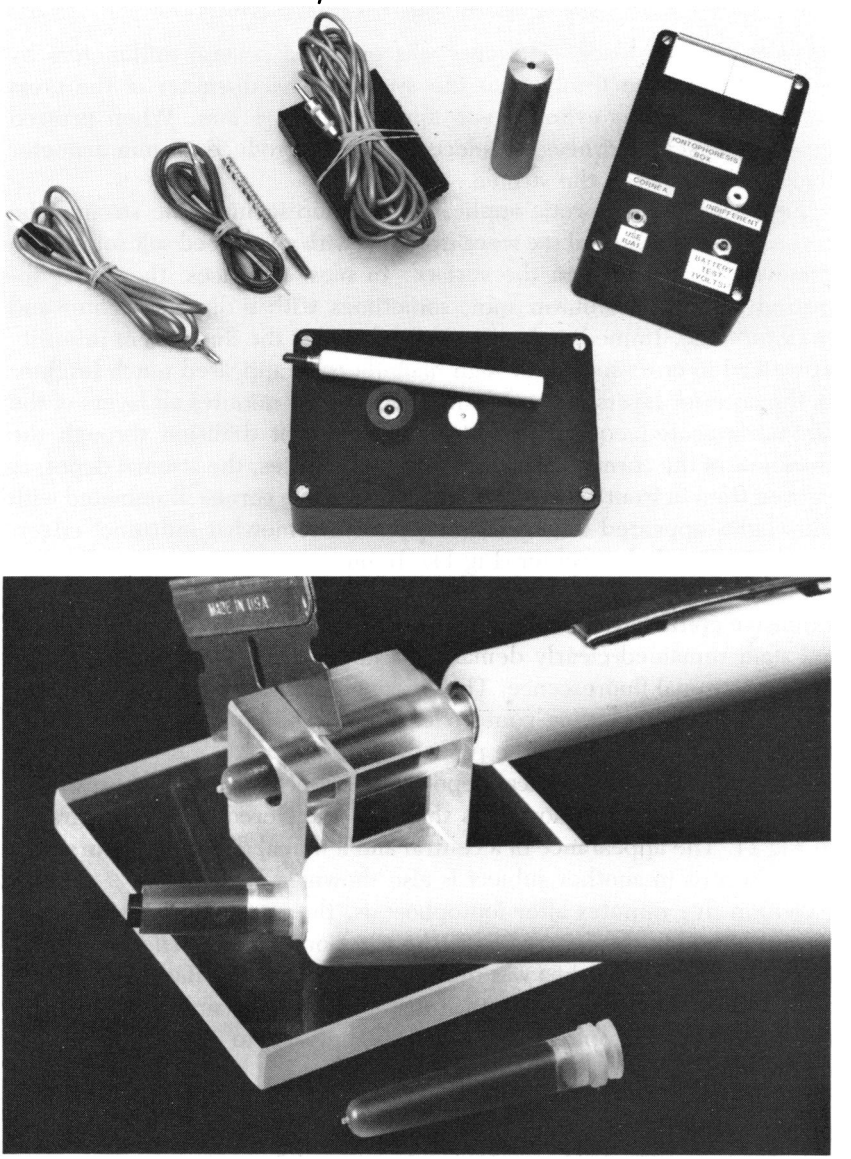

FIGURE 10

Equipment for performing iontophoresis. Top, upper portion, shows from right to left: battery-operated apparatus to monitor current, positive electrode for subject's hand, foot-switch and wire, electrode, and ground wire. Top, lower portion, shows charger and capacitor-operated corneal electrode that requires no external wires (examiner's hands serve as return pathway). Bottom, note disposable agar electrode, cutting jig, and assembled electrode ready for use.

off with a razor blade. The agar was extruded several millimeters by inserting the tube firmly onto the syringe. The diameter of the cross section of the agar cylinder was approximately 4 mm. When pressed gently against the cornea, this electrode would produce a 5-mm diameter fluorescent depot in the stroma.

Following iontophoretic application of fluorescein to the stroma, the cornea and conjunctival sac were irrigated with a balanced salt solution to remove fluorescein from the surface. In most instances, the depot appeared as a fairly uniform spot, sometimes with a dimmer center and brighter edge. Immediately after iontophoresis, the fluorescent intensity as viewed in cross section at high magnification appeared much brighter in the anterior layers of the stroma, but after 15 minutes all layers of the stroma appeared equally bright, indicating that diffusion through the thickness of the cornea was rapid. After 30 minutes, the stromal depot, as viewed from in front of the cornea with the whole cornea illuminated with blue light, appeared as a uniform spot with somewhat indistinct edges, having lost its darker center (Fig 11). In one instance, it was noted that a contact lens wearer who removed the lens just prior to iontophoresis had extensive epithelial staining in the depot area. In this subject, the epithelial stain remained clearly demarcated for over an hour before it faded into the stromal fluorescence. This phenomenon was not observed in any other subjects, including contact lens wearers who had removed their lenses the evening before the procedure.

The appearance of a typical depot immediately after iontophoresis and 30 minutes after iontophoresis is shown in the stereoscopic photographs in Fig 11. The appearance of a central and a limbal depot two hours after iontophoresis in another subject is also shown.

Within five minutes after iontophoresis, the intensity of fluorescence was measured in the cornea and in the anterior chamber. The intensity of fluorescence in the cornea was quite high, as expected. Rather unexpectedly, the intensity of fluorescence in the anterior chamber jumped immediately after iontophoresis to approximately 1% to 3% of the intensity measured in the cornea as long as the excitation slit beam passed through the site where corneal fluorescence had been measured. This apparent fluorescent intensity in the anterior chamber in a given eye was always found to bear a fixed relationship to the corneal fluorescent intensity and was presumed to be caused by stray fluorescent light originating in the excitation beam as it traversed the cornea. Figure 1 suggests one possible pathway the light might take in reaching the detector.

It was desirable to place the depot of fluorescein in the center of the cornea to simplify mass measurements (described below). Also, it was

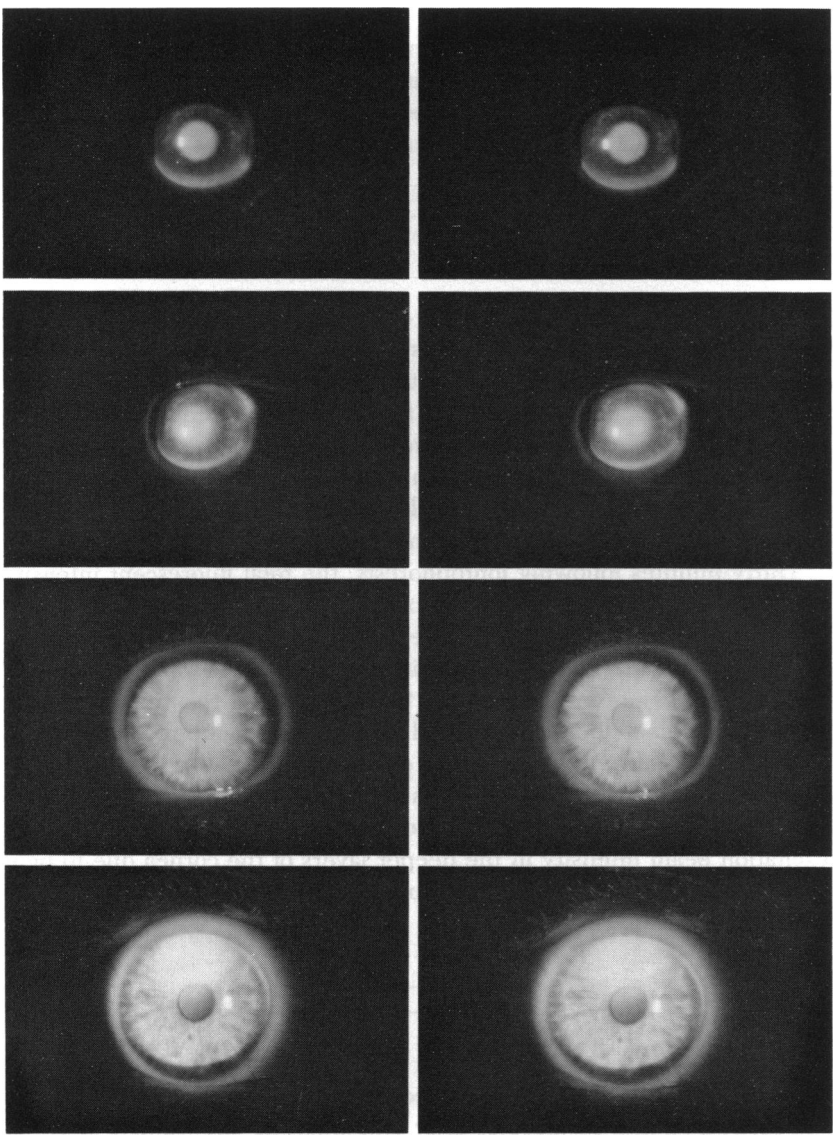

FIGURE 11

Stereoscopic photographs of appearance of eye after application of fluorescein to corneal stroma by iontophoresis. Top to bottom, (1) Brown-eyed subject immediately after iontophoresis and irrigation of cornea and conjunctival sac. (2) Same subject 30 minutes later. (3) Blue-eyed subject (subject 2, Table XVII) two hours after central application. (4) Fellow eye of subject two hours after limbal application.

most convenient to measure fluorescent intensity as diagrammed in Fig 1. Hence, to make accurate measurements of anterior chamber fluorescence, it was necessary to introduce a correction factor to eliminate the unwanted signal originating from the cornea. This correction was applied to all anterior chamber measurements as follows:

$$\begin{aligned} I_a(t) &= I'_a(t) - I_c(t) \cdot \frac{I'_a(0)}{I_c(0)} \\ &= I'_a(t) - I_c(t) \cdot I_{ac} \end{aligned} \tag{13}$$

Here $I_a(t)$ is the corrected anterior chamber intensity at time t, I'_a the uncorrected anterior chamber intensity, and I_c the corneal intensity. The ratio of intensity of fluorescence of the anterior chamber-cornea at time zero, I_{ac}, was found to be related to the distance along the optic axis between the cornea and the location of the fluorescence window during the anterior chamber measurement. In this study, anterior chamber concentration measurements were always made in the center of the optical cross section of the anterior chamber.

Thirty minutes following iontophoresis, the total fluorescent intensity was measured in the central 8 mm of the cornea and the anterior chamber. The 30-minute time was chosen to allow the fluorescein depot to become more uniformly distributed in the central cornea and to allow any residual fluorescein in the tear film to disappear. At this time, less than 5% of the original depot has entered the anterior chamber of the normal eye and even less will have left the eye completely.

Because of the high concentrations of fluorescein in the depot, a correction was applied to account for the inner filter effect, ie, the loss of excitation beam intensity in the deeper layers of the cornea due to light absorption by fluorescein in more superficial layers.

The form of the correction is

$$M = \frac{-ln\ (1 - a \cdot M')}{a} \tag{14}$$

In this equation, M' is the uncorrected mass of fluorescein measured in the corneal stroma 30 minutes after iontophoretic application. M is the corrected mass. The value of "a" depends on the extinction coefficient of fluorescein and on the lateral distribution of fluorescein in the cornea. The lateral distribution was determined from the multiple-compartment model; the profile is illustrated in Fig 12. The value of "a" for the fluorophotometer currently in use is 8.9×10^{-4} ng^{-1}. The rationale for the

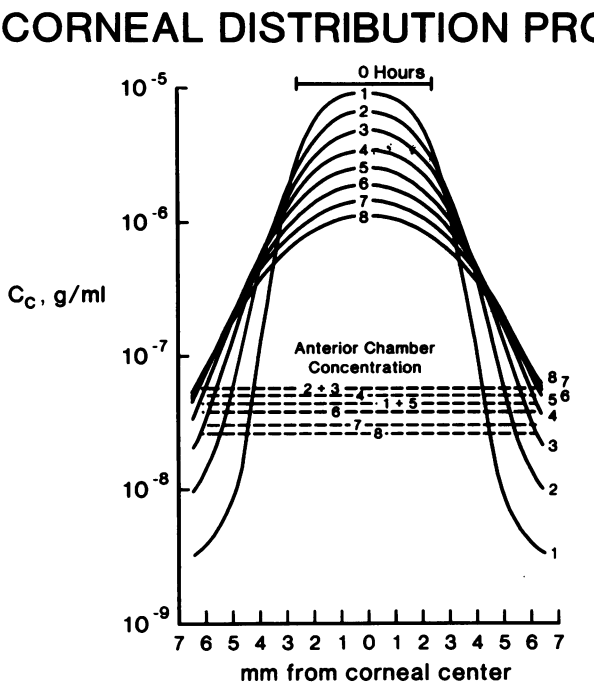

FIGURE 12

Simulation of lateral distribution of fluorescein in corneal stroma with multiple-compartment model following 5 mm diameter into central cornea. Normal values of K_{ca}, K_o, and v_a were used (Table IX). Note corneal concentration (solid curves) and concentration in anterior chamber (dotted lines).

form of the correction is given by Coakes and Brubaker.[106] The usual uncorrected dose of fluorescein in these studies was approximately 310 ng. The correction for extinction for such a dose is approximately 10%. It is noteworthy that extremely large doses cannot be measured accurately since the effect of extinction increases exponentially with dosage in a fixed area of the cornea. To measure higher doses, the fluorescein must be deposited in a larger area of the cornea or a shorter excitatory wavelength must be used.

In some subjects, intensity of fluorescence was measured in the cornea and anterior chamber every hour after iontophoresis for seven to nine hours. In most of these same subjects, total fluorescent intensity was measured every hour, also. In other subjects, these measurements were made only at two hours and seven hours.

The rate of aqueous humor flow through the anterior chamber was

calculated from the clearance of fluorescein on the assumption that 90% of the clearance was due to flow and 10% due to diffusional loss.

$$\text{Flow} = K_o \cdot v_a \cdot 0.9 \tag{15}$$

In five normal subjects, the depot of fluorescein was placed in the central cornea in one eye and tangent to the superior limbus in the fellow eye. The calculated rate of aqueous humor flow in the two eyes was compared.

In all topical drug studies, one eye of a subject was treated with the drug and the fellow eye was treated with a placebo. All assignments were masked and determined by random. In the case of acetazolamide, each eye served as its own control. Each eye was tested on two occasions, once after administration of the drug and once after administration of an identically appearing placebo.

In patients with a unilateral disease of the eye, the fellow eye served as the control. In patients with a bilateral ocular disease, comparisons were made with groups of normal subjects. In the longitudinal study with timolol, each eye was tested on several occasions, and each served as its own control.

In all groups of subjects, a test of the integrity of the blood-ocular barrier was performed in a portion of the group with orally administered fluorescein to determine if equation 15 was valid for the calculation of flow. (No subjects in the carotid occlusion group have yet been tested with systemic fluorescein.)

RESULTS

ACCURACY OF FLUORESCENCE MEASUREMENTS

In six normal subjects, the autofluorescence of the peripheral cornea, the central cornea, and the crystalline lens was measured before administration of fluorescein. Table XI summarizes the results of these fluorescence measurements. The mean autofluorescence of the central cornea, expressed as the equivalent concentration of fluorescein, was $7.15 \times 10^{-9} \pm 1.10 \times 10^{-9}$ g/mL. Slightly higher autofluorescence was seen in the peripheral cornea where the mean was $8.13 \times 10^{-9} \pm 1.21 \times 10^{-9}$ g/mL. This difference, however, was not statistically significant (two-sided t test, $P > 0.05$). The autofluorescence of the crystalline lens was found to be many times higher than that of the cornea. The mean value in these subjects was $120 \times 10^{-9} \pm 25 \times 10^{-9}$ g/mL. The autofluorescence

TABLE XI: AUTOFLUORESCENCE OF CORNEA AND CRYSTALLINE LENS (FLUORESCENCE EXPRESSED AS EQUIVALENT CONCENTRATION OF FLUORESCEIN, g/mL)

SUBJECT NO.	PERIPHERAL CORNEA, g/mL × 10^{-9}	CENTRAL CORNEA, g/mL × 10^{-9}	LENS, g/mL × 10^{-9}
1	7.88	6.07	117
2	7.49	6.60	96
3	7.15	8.16	113
4	7.05	5.95	153
5	10.00	7.61	147
6	9.21	8.53	96
Mean	8.13	7.15	120
SD	1.21	1.10	25

	COMBINED CORNEA AND LENS (12-mm FIELD), ng	
	NORMAL PUPIL	PUPIL DILATED
1	4.3	16.2
2	5.0	20.2
3	7.2	20.3
4	4.3	17.4
5	10.4	24.0
6	5.5	13.3
Mean	6.1	18.6
SD	2.4	3.7

of the lens and the cornea did not appear to interfere with the determination of fluorescence in the anterior chamber. No fluorescence greater than the background of the fluorophotometer could be detected in the anterior chamber of any eye that was tested before the application of fluorescein.

After application of fluorescein to the cornea, a fluorescent signal that seemed to originate from the anterior chamber was detectable. The apparent fluorescence had a constant relation to the observed fluorescence intensity of the corneal stroma. Table XII summarizes measurements made in a rhesus monkey eye before and after application of fluorescein to the cornea by iontophoresis. The experimental arrangement for the results in this table is shown in Fig 8. The ratio of fluorescent intensity in the anterior chamber to that in the cornea remained relatively fixed at 1.5% during the entire experiment.

Fluorescent intensity was measured immediately after iontophoresis in the central corneal stroma and in the central anterior chamber along the visual axis in 46 eyes of young normal subjects. The mean apparent intensity in the anterior chamber following iontophoresis was 1.2% ± 0.3% of the fluorescent intensity in the stroma in this group.[106] In another group of normal human subjects ranging in age from 20 to 83, the mean

TABLE XII: FLUORESCENT INTENSITY IN CORNEA AND ANTERIOR CHAMBER IN MONKEY EYE DURING CONTINUOUS ANTERIOR CHAMBER WASHOUT FOLLOWING FLUORESCEIN IONTOPHORESIS INTO CENTRAL CORNEA

TIME, HR:MIN	FLUOROPHOTOMETER READING FROM ANTERIOR CHAMBER, V	FLUOROPHOTOMETER READING FROM CORNEA, V	RATIO OF INTENSITY, AC/CORNEA
Baseline	0.000008	0.00042	0.019
0:05	0.037	3.17	0.012
0:30	0.033	3.00	0.011
0:45	0.033	2.20	0.015
1:05	0.026	1.56	0.017
1:15	0.020	1.30	0.015
1:30	0.020	1.55	0.013
1:45	0.018	1.34	0.013
2:00	0.017	1.25	0.014
2:10	0.015	0.96	0.016
2:40	0.013	0.87	0.015

ratio I_{ac} was found to be higher, 2.6% ± 0.8%. The ratio did not correlate with the age of the subject per se or the color of the iris stroma but did correlate with the depth in the anterior chamber at which the measurement was made. The ratio was higher the closer to the cornea one measured anterior chamber fluorescence. In abnormal subjects with hazy epithelium or thickened corneal stroma, the apparent fluorescent intensity in the anterior chamber was a much greater percentage of the fluorescent intensity in the cornea. These subjects were unsuitable for flow determination by this method. In all of these subjects, the lack of normal transparency of the cornea was obvious.

The effect of autofluorescence of the cornea and crystalline lens on the measurement of the mass of fluorescein in the corneal stroma was measured in six subjects. In one eye of these subjects the pupil was dilated, and in the other eye the pupil was normal. The total fluorescent intensity in the central 12 mm of these eyes was greater on the dilated side because of the greater contribution of the crystalline lens. On the undilated side, the intensity was equivalent to 6.1 ± 2.4 ng of fluorescein and on the dilated side, equivalent to 18.6 ± 3.7 ng (Table XI). For the undilated pupil, this intensity of fluorescence was too low to require subtraction from the fluorescent intensity after iontophoresis. In almost all cases studied, the initial dose of fluorescein was greater than 100 ng, and the average dose was 350 ng. Thus, in most of the subjects studied, autofluorescence of the cornea and lens was less than 2% of the total fluorescence immediately following iontophoresis.

A study was carried out in excised rabbit corneas to determine the accuracy with which the mass of fluorescein in the cornea could be

measured. The experimental arrangement is illustrated in Fig 6. No consistent difference in fluorescent intensity was found as fluorescein diffused from the button of agar into the button of corneal stroma. In some instances fluorescent intensity drifted slowly for a short period of time but one or two hours later had returned to an intensity that was within 2% of the original value. These experiments were interpreted as demonstrating that the apparent molar fluorescent intensity of fluorescein in the stroma was the same as that in the standard solutions.

The effect of iridal reflectance on the measurement of fluorescein mass in the cornea was measured in vitro. The experimental arrangement is diagrammed in Fig 7. Table XIII summarizes the range of reflectance encountered in human subjects having dark brown to light blue irides. The reflectance in the subjects with light irides was twice that in the subjects with dark irides. The most reflective human eye exhibited less than half the reflectance of that of an albino rabbit eye. After iontophoresis in the central cornea of the rabbit eye, a measurement of fluorescence was made. The cornea was excised, and the measurement of fluorescence was determined in an artificial chamber. Different surfaces were used in the artificial chamber to recreate different ocular reflectivity. Only a slight difference, about 5%, in the intensity of the fluorescent depot of the corneal button was observed over the range of reflectivity of human eyes. The fluorescent signal, however, was much larger with reflective surfaces equivalent to that of the albino rabbit eye. This experiment was inter-

TABLE XIII: OCULAR REFLECTIVITY: EFFECT ON MEASUREMENT OF FLUORESCEIN MASS IN CORNEA

SUBJECT	REFLECTED LIGHT SIGNAL FOR 10-mm FIELD, V	FLUORESCENT SIGNAL FROM SINGLE RABBIT CORNEA WITH CENTRAL FLUORESCEIN DEPOT IN VIVO AND AFTER EXCISION, V
Human subjects in vivo		
1. Dark-brown iris	0.126	
2. Brown iris	0.146	
3. Light-brown iris	0.186	
4. Blue iris	0.218	
5. Light-blue iris	0.240	
Animal		
6. Albino rabbit in vivo	0.544	8.0 (in vivo)
Artificial anterior chamber		
7. Black "iris"	0.145	6.0 (excised, black "iris" background)
8. Gray "iris"	0.330	6.2 (excised, gray "iris" background)
9. Tan "iris"	0.552	8.5 (excised, tan "iris" background)

preted as demonstrating that brown-eyed and blue-eyed human subjects could be studied by the technique described in this thesis without regard to iridal reflectance. As will be pointed out later, no difference in flow was found between two groups of subjects stratified for iris color.

ACCURACY OF FLOW CALCULATION

In a series of 113 normal subjects[109] ranging in age from 20 to 83 years, the mean (± SD) value of the anterior chamber loss coefficient of fluorescein was $1.5 \times 10^{-2} \pm 0.43 \times 10^{-2}\ min^{-1}$. The volume of the anterior chamber in these eyes was 186 ± 37 μL. The calculated rate of clearance of fluorescein from the anterior chamber was 2.7 ± 0.6 $\mu L/min^{-1}$. The rate of aqueous humor flow through the anterior chamber was calculated to be 2.4 ± 0.6 $\mu L/min^{-1}$.

Table XIV lists the rates of aqueous humor flow measured by a number of investigators with fluorescent tracer techniques in human eyes. The results of flow calculation in this study is in general agreement with those of other investigators. Most investigators who have administered fluorescein by systemic routes have calculated flows that are lower than the flows determined by investigators who have administered fluorescein via the cornea. The reasons for these differences cannot be explained satisfactorily at the present time.

The method of Holm[113] and Holm and Wiebert,[114,115] although tedious, permits the most direct calculation of flow with the fewest assumptions. The mean results by this method are in closer agreement with the absolute flow figures determined by procedures in which fluorescein has been applied topically. As will be shown later, the higher flow figures of Holm may have been caused by the use of miotics. The mean flow rate determined in this study in pilocarpine-treated eyes is in close agreement with the flow rates measured by dynamic photogrammetry.

The rate of loss of radioactive albumin from the anterior chamber has been determined in ten human eyes by O'Rourke.[18] The mean loss coefficient, K_o, was $1.5 \times 10^{-2} \pm 0.26 \times 10^{-2}\ min^{-1}$. This coefficient is identical to the mean loss coefficient that was found in our group of normal subjects. The agreement between experiments with albumin and fluorescein is reassuring since albumin is unlikely to be lost in any significant quantity from the normal anterior chamber except by the process of bulk outflow. The agreement between these two techniques is additional evidence that diffusional loss of fluorescein from the anterior chamber is small compared with the loss by flow.

The question of the accuracy of the topical fluorescein method in measuring flow has been addressed by Pederson et al.[104] However, in

TABLE XIV: AQUEOUS FLOW DETERMINED IN NORMAL HUMAN EYE USING FLUORESCEIN

INVESTIGATOR	YR	METHOD OF ADMINISTRATION	NO. OF SUBJECTS	AQUEOUS FLOW μL/min MEAN ± SD
Goldmann	1950	IV	10	1.9 ± 0.4
Goldmann	1951	IV	36	2.2 ± 0.4
Jones and Maurice	1966	Iontophoresis	10	2.4 ± 0.5
Starr	1966	Iontophoresis	. . .	1.9 ± ?
Holm	1968	Iontophoresis (photogrammetric)	17	3.1 ± 1.6
Holm and Wiebert	1968	Iontophoresis (photogrammetric)	11	3.4 ± 1.7
Nagataki	1975	IV	14	1.8 ± 0.8*
Nagataki and Mishima	1976	IV (fellow eye abnormal)	7	1.8 ± 0.2*
Bloom et al	1976	Iontophoresis	19	2.8 ± 0.6
Yablonski et al	1978	Topical	15	2.3 ± 0.7*
Coakes and Brubaker	1978	Iontophoresis	20	2.9 ± 0.4
Coakes and Brubaker	1978	Iontophoresis (timolol, fellow eye)	23	2.6 ± 0.4
Townsend and Brubaker	1980	Iontophoresis (epinephrine, fellow eye)	24	2.5 ± 0.4
Araie et al	1980	Oral	10	1.8 ± 0.4*
Araie and Takase	1981	Oral (various drugs, fellow eye)	52	1.5 ± 0.4*
Nagataki and Brubaker	1981	Iontophoresis (epinephrine, fellow eye)	8	2.6 ± 0.4
Brubaker et al	1981	Iontophoresis	113	2.4 ± 0.6
Wentworth and Brubaker	1981	Iontophoresis (Horner's fellow eye)	21	2.1 ± 0.6
Lee et al	1982	Iontophoresis (thymoxamine, fellow eye)	25	2.4 ± 0.6

*Anterior chamber volume assumed to be 200 μL to calculate flow from published value of K_o.

human subjects, the accuracy of this technique or other techniques cannot be determined. The best one can do is to estimate its precision from repeated measurements.

The calculated flows of each of the two eyes of normal subjects, determined on the same day, or the calculated flow of normal eyes, determined twice on different days, were compared. Observed differences included errors of measurement, right-to-left differences, and day-to-day variations in flow. Table XV summarizes several such comparisons and gives the SD of the differences between paired flow measurements, expressed as the percentage of the mean flow.

TABLE XV: PRECISION OF PAIRED FLOW MEASUREMENTS IN NORMAL SUBJECTS

COMPARISON	NO. OF PAIRS OF MEASUREMENTS	METHOD OF FLOW CALCULATION	DIFFERENCES DUE TO			SD OF DIFFERENCES OF PAIRED MEASUREMENTS (% OF MEAN FLOW)
			MEASUREMENT ERRORS	OD-OS DIFFERENCES	DAY-TO-DAY DIFFERENCES	
A. OD to OS, flows measured same day	30	Nomogram	X	X	. . .	20%
B. OD to OS, flows measured same day	10	Least squares	X	X	. . .	16%
C. OD to OD, or OS to OS, flows measured different days	12	Least squares	X	. . .	X	15%
D. OD to OS, flows measured different days	15	Least squares	X	X	X	18%

The SD of the differences, resulting from measurement errors and other factors such as right-to-left differences, is slightly lower with the least squares method. The SD due to experimental error alone must be somewhat less than 15% of the mean flow in eyes undergoing a technically satisfactory determination. In a given subject, a single determination of flow is likely to give a result that is within 25% of the mean of several determinations. The variance in flows of right-left pairs of normal subjects can be used to estimate the sample size required to carry out studies of the effects of topical drugs with this technique.

A comparison was made in four groups of subjects between the results of flow calculated by the nomographic method and flow calculated by the least squares method. Table XVI summarizes the results of this comparison. In a group of 15 subjects treated in one eye with pilocarpine and in the other eye with a placebo, the nomographic calculation yielded a higher value of flow than did the method of least squares. In these two groups, the method of least squares was based on hourly data points beginning one hour after iontophoresis and continuing until nine hours. The difference of the means of the two methods was approximately 12%. The variability of test points from the fitted curve was much greater for the pilocarpine-treated group than for the placebo-treated group. This finding was interpreted as resulting from poorer mixing in the presence of miosis, an advantage for the method of Holm[113] but a disadvantage for a dye dilution method.[98]

In two other groups, one a group of eyes treated with 2.5% phenylephrine and the fellow eye treated with a placebo drop, a similar comparison was made.[162] In these groups the least squares procedure was based upon hourly measurements beginning two hours after iontophoresis and ending eight hours after iontophoresis. A small difference between the means of the two methods is seen in Table XVI. The two methods produce similar, but seldom identical, results in a given eye. The SD of the differences between the results of the two methods is about 10% of the mean of the two methods. As outlined previously and as can be determined by inspection of the two-compartment nomogram, the nomographic result is critically dependent upon the measurement of anterior chamber concentration seven hours after iontophoresis. Thus, the least squares method, which depends on a number of data points, is inherently more reliable although more tedious, than the nomographic method.

A small number of subjects were tested to determine if the position of the placement of the fluorescein depot was critical in determining the rate of aqueous humor flow. In five subjects, fluorescein was placed in the central cornea of one eye and at the superior limbus of the cornea in the

TABLE XVI: COMPARISON OF NOMOGRAPHIC (TWO DATA POINTS) AND LEAST SQUARES (SEVEN TO NINE DATA POINTS) DETERMINATIONS OF FLOW (μL/min)

GROUP	n	NOMOGRAM	LEAST SQUARES	MEAN DIFFERENCE	SD OF DIFFERENCES (%)	NO. OF DATA POINTS USED FOR LEAST SQUARES METHOD
Pilocarpine control	15	2.67	2.37	0.30	0.12 (5)	9
Pilocarpine-treated	15	3.09	2.72	0.37	0.44 (16)	9
Phenylephrine control	22	2.60	2.64	0.04	0.32 (12)	7
Phenylephrine-treated	22	2.73	2.64	0.09	0.26 (10)	7

fellow eye. In the eye with the central depot, the measurement of initial mass was simpler since the depot was easy to center in the 8-mm field of observation. Also, there was no concern about light scattering in the sclera. On the side with the limbal depot, the alignment was less simple. The examiner had to exercise caution to ensure that the entire depot was included in the field but that the sclera was excluded. On the other hand, the determination of anterior chamber fluorescent intensity was simpler in the eye with the eccentric depot. In this eye, there was little corneal fluorescence to interfere with anterior chamber measurement. In the eye with the central depot, careful measurements of corneal intensity and correction with equation 13 for corneal interference, as outlined under "Methods," had to be made.

The calculated rate of aqueous humor flow was almost identical in the two groups of eyes (Table XVII). The cases were too few to detect small differences due to the site of fluorescein deposition, but a large difference should have been detectable. The appearance of the depot of fluorescein in the two eyes of one subject is depicted in Fig 11; the data on this subject are shown graphically in Fig 13.

It was concluded from this experiment that the position of placement of the corneal depot is not critical to the calculated flow. However, small differences in flow because of systematic differences in placement of the depot cannot be excluded with such a small number of subjects.

ADEQUACY OF TWO-COMPARTMENT MODEL

The multiple-compartment model described under "Methods" was used to test certain theoretic aspects of the adequacy of the two-compartment model as representing the movement of fluorescein in the cornea and to

TABLE XVII: LIMBAL V CENTRAL APPLICATION OF FLUORESCEIN

	CORNEA-TO-ANTERIOR CHAMBER TRANSFER COEFFICIENT, $min^{-1} \times 10^{-3}$		AQUEOUS HUMOR FLOW, $\mu L\ min^{-1}$		
SUBJECT	LIMBAL (OD)	CENTRAL (OS)	LIMBAL (OD)	CENTRAL (OS)	DIFFERENCE (OD-OS)
1	2.8	2.8	4.04	3.44	0.60
2	2.4	3.0	2.28	2.29	−0.01
3	3.8	4.0	1.44	1.76	−0.32
4	3.6	3.6	2.84	2.72	0.12
5	2.6	3.2	2.51	2.77	−0.26
Mean	3.0	3.3	2.62	2.60	0.03
SD	0.6	0.5	0.94	0.62	0.37

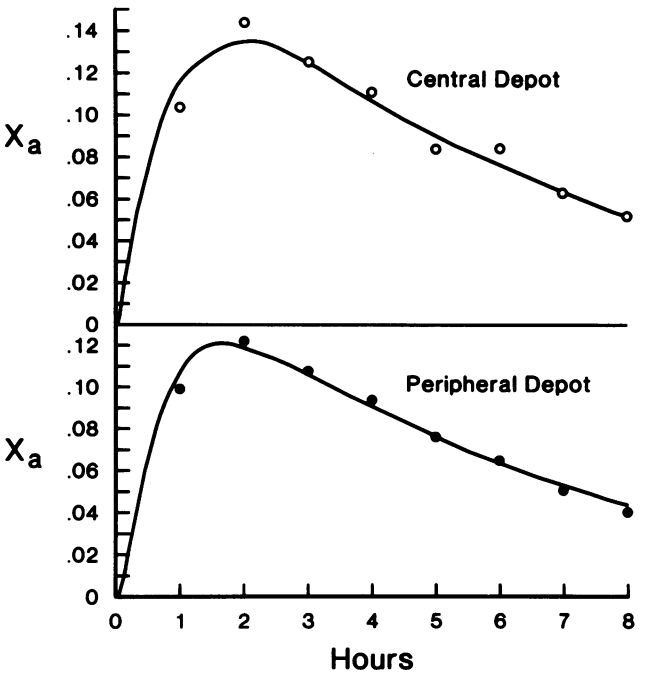

FIGURE 13
Fraction of fluorescein in anterior chamber (X_a) in normal subject (subject 2, Table XVII) after application of fluorescein by iontophoresis in central cornea of one eye and tangent to superior limbus of fellow eye. Curves were fitted by method of least squares (two-compartment model).

determine the importance of fluorescein loss at the limbus. To determine the importance of limbal loss, it was necessary to calculate the rate of exchange of fluorescein between plasma and the tissues adjacent to the peripheral cornea.

This rate of exchange was determined in four rabbit eyes and in five human eyes. In the experiments with the rabbit eye, fluorescein was administered IV, and in the human experiments, fluorescein was given orally after being mixed with a carbonated beverage to ensure rapid absorption. In the rabbit, the rate of exchange between the scleral tissues and the plasma, K_{sp}, was found to be $0.74 \times 10^{-2} \pm 0.16 \times 10^{-2}$ min^{-1}. The half-time of exchange between the plasma and the sclera in these animals was determined to be 96 ± 19 minutes. The results are summarized in Table XVIII. A more rapid rate of exchange was observed in the five human eyes that were studied. The mean value for the scleral

TABLE XVIII: SCLERAL EXCHANGE COEFFICIENT

SUBJECTS	$K_{sp} \times 10^{-2}$ min^{-1}	T½ min
Rabbit no.		
1	0.61	113
2	0.99	70
3	0.67	104
4	0.72	96
Mean	0.74	96
SD	0.16	19
Human no.		
1	1.1	62
2	1.5	46
3	0.91	76
4	2.7	26
5	1.4	48
Mean	1.52	52
SD	0.70	19

exchange coefficient was $1.52 \times 10^{-2} \pm 0.70 \times 10^{-2}$ min^{-1}. The half-time of exchange of fluorescein between the plasma and the sclera was calculated to be 52 ± 19 minutes in the human eye. For the purpose of simulating fluorescein exchange at the limbus with the multiple-compartment model, a value of the scleral exchange coefficient of 1.52×10^{-2} min^{-1} was used. (See Table IX for values of all parameters of the multiple-compartment model.)

A graph of the results of a simulation with the multiple-compartment model is shown in Fig 14. Most of the fluorescein that is lost from the model eye is lost because of the process of outflow of aqueous humor. In addition to this loss and to the loss by direct diffusion into the vessels of the iris, some fluorescein is lost from the model eye by way of the limbus. This loss is insignificant for several hours but by the eighth hour is similar magnitude to the loss through the normal iris.

The corneal distribution profile of fluorescein is illustrated in Fig 12. The initial rise of fluorescence in the peripheral cornea is caused by reentry of fluorescein into the peripheral cornea from the anterior chamber. Many hours after iontophoresis some fluorescein begins to reach the limbus as a result of lateral diffusion within the corneal stroma. The average concentration of fluorescein in the stroma always remains higher than the anterior chamber concentration.

The mass of fluorescein in the anterior chamber as a fraction of the initial dose is depicted in Fig 15. Three of these curves are generated by the multiple-compartment model and one of them, by the two-compart-

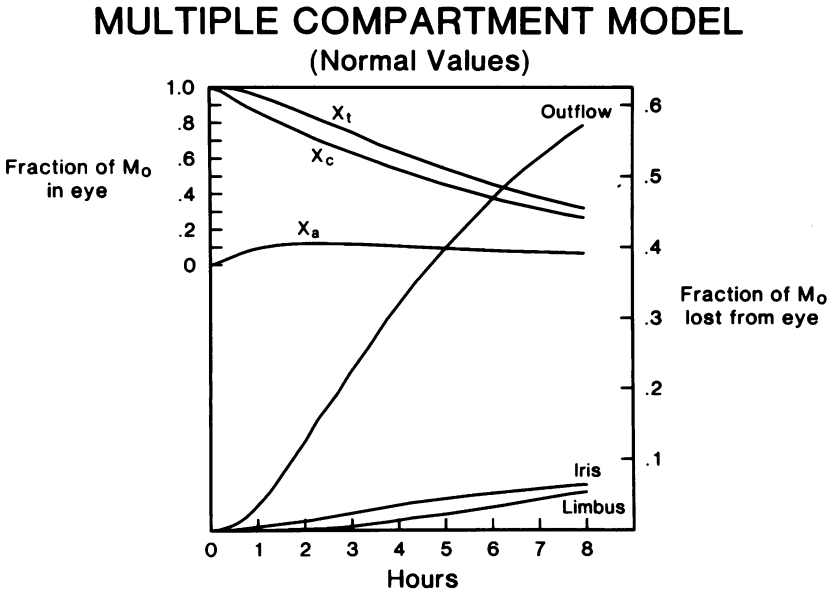

FIGURE 14
Simulation of fluorescein movement in eye with multiple-compartment model following 5 mm diameter central depot of fluorescein. Normal values of K_{ca}, K_o, and v_a were used (Table IX).

ment model. For all curves, the values of K_{ca}, K_o, and v_a are the normal values. One curve results from fluorescein placement in the central stroma of the multiple-compartment model at time zero; no limbal exchange is permitted. Another curve depicts the same conditions except that limbal exchange is permitted to occur at a rate dependent on the concentration of fluorescein in the peripheral cornea and sclera; the rate of exchange between limbal tissues and plasma is the mean rate measured in human eyes (Table XVIII). Another curve shows the situation where the depot of fluorescein is placed adjacent to the limbus.

The two curves in the middle are almost identical, implying that limbal loss of fluorescein following application in the center of the model cornea is negligible. The greater loss from the limbus with a peripheral depot accounts for the downward displacement of the lowest curve. As compared with the multiple-compartment model, the two-compartment model predicts a more rapid accumulation of fluorescein in the anterior chamber, a higher peak, and a slightly more rapid fall. These differences are due to the thickness of the stroma, which is not entirely negligible. The stroma of the multiple-compartment model retards, to some extent,

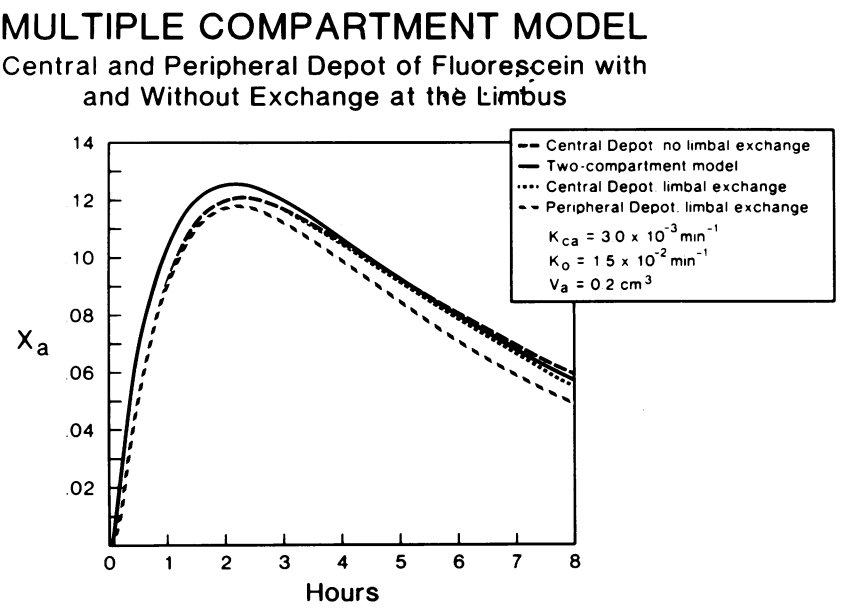

FIGURE 15

Comparison of simulations of kinetics of fluorescein in normal eye with two-compartment model and multiple-compartment model. K values for all four curves are same: $K_{ca} = 3 \times 10^{-3}$ min^{-1}, $K_o = 1.5 \times 10^{-2}$ min^{-1}, $v_a = 0.2$ cm^3.

the penetration of fluorescein from the subepithelial depot into the anterior chamber.

A nomogram was constructed from the multiple-compartment model for central application of fluorescein in an eye with an anterior chamber volume of 200 μL. The nomogram is depicted in Fig 16. Critical comparison of this nomogram with that based on the two-compartment model (Fig 4) shows that the two nomograms will produce almost identical results for normal or rapid aqueous humor flow but might show somewhat different results for extremely low rates of aqueous humor flow. The calculated cornea-to-anterior chamber transfer coefficient, K_{ca}, is higher with the two-compartment nomogram, especially for abnormally high values for K_{ca}. In this circumstance, stromal resistance to fluorescein, accounted for in the multiple-compartment, but not the two-compartment model, becomes increasingly important in determining the overall rate of fluorescein loss from the cornea.

The differences between the two models are illustrated further in Table XIX. In this table, two- and seven-hour anterior chamber fraction values

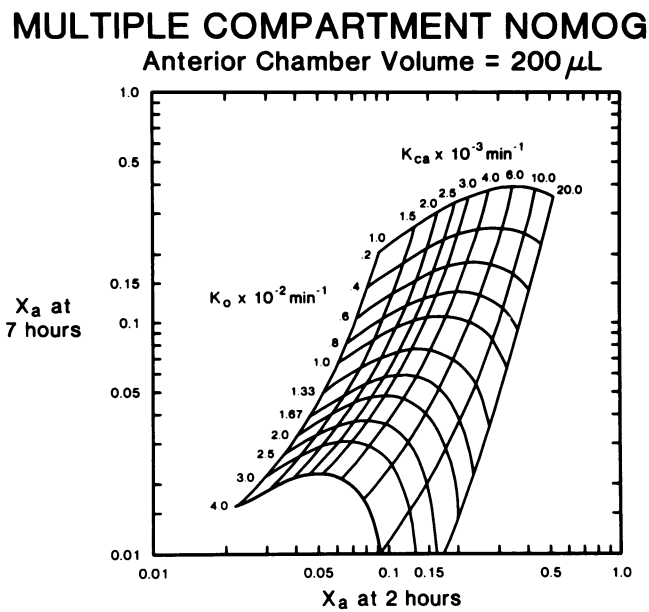

FIGURE 16
Nomogram to determine K_{ca} and K_o from experimentally measured values of X_a at two hours and seven hours following iontophoresis into central cornea. Nomogram is generated from multiple-compartment model. (See Table IX for values of parameters.)

were determined for the normal eye with the multiple-compartment model. These values were then plotted as a single point on the two-compartment nomogram to determine the K values. The result demonstrates the small systematic error inherent in the two-compartment model. Two circumstances are depicted, one with the central depot method and the other with the peripheral depot method. The systematic errors are small and clinically insignificant. The two-compartment model appears to be adequate for clinical research and justified by its relative simplicity.

Figure 17 illustrates the average anterior chamber fractions, X_a, of 22 normal eyes. For comparison, the average fractions, determined by the two-compartment model using the method of least squares, are shown. (The one-hour data point was not used in determining the best fit.) The continuous curve was calculated from the multiple-compartment model, the K values of which were determined with the multiple-compartment nomogram (Fig 16) from the data points X_a (2) and X_a (7). The shapes of the curves fitted by the two techniques are quite similar.

The systematic deviation of the data from the models at the first hour in

TABLE XIX: SIMULATED DATA WITH MULTIPLE-COMPARTMENT MODEL: CORRESPONDENCE WITH TWO-COMPARTMENT NOMOGRAM

PARAMETER VALUES OF SIMULATION	RESULTS OF MULTIPLE-COMPARTMENT SIMULATION		TWO-COMPARTMENT NOMOGRAPHIC DETERMINATION OF K VALUES		SYSTEMATIC ERROR BETWEEN MULTIPLE-COMPARTMENT K VALUE ("TRUE" VALUE) AND TWO-COMPARTMENT NOMOGRAPHICALLY DETERMINED K VALUE	
$K_{ca} = 3.0 \times 10^{-3}\ min^{-1}$ $K_o = 1.5 \times 10^{-2}\ min^{-1}$ $v_a = 0.200$ mL						
	X_a (2)	X_a (7)	K_{ca}	K_o	K_{ca}	K_o
Simulation no. 1 (central depot)	0.119	0.066	2.6×10^{-3}	1.55×10^{-2}	13% low	3% high
Simulation no. 2 (peripheral depot)	0.116	0.058	2.8×10^{-3}	1.75×10^{-2}	7% low	17% high

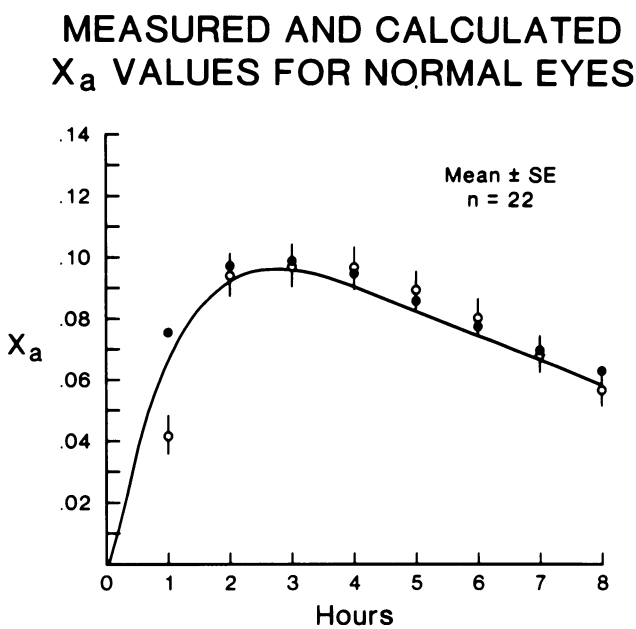

FIGURE 17

Mean experimental values (open circles) and standard errors (bars) of X_a for 22 eyes (control eyes for phenylephrine experiment). Values at hours two to eight were used as data. Two-compartment model was fitted to these data by method of least squares. Best fit is depicted (closed circles). X_a data at hours two and seven were also used to determine K values using multiple compartment (Fig 16). Smooth curve is simulated multiple-compartment model using nomographically determined K values. Both models fit shape of average data of 22 eyes well except for first hour's data point.

this group of eyes is typical and has been noted in almost all of our other studies. This systematic deviation may be due to several factors, one of which is the tendency of fluorescein to circulate in the anterior chamber near the cornea and near the iris during the time of rapid rise of anterior chamber fluorescence. The central anterior chamber appears somewhat darker during the first hour. Measurements taken from the center of the anterior chamber at this time may underestimate the average concentration of fluorescein in the anterior chamber. It is likely that more accurate calculations of flow are made when the first hour's measurement is omitted.

Measurements of polarization of fluorescence were made in excised strips of rabbit corneal stroma. These measurements were used to look for evidence of binding of fluorescein in the stroma. Three specimens were studied. In all three specimens, polarization of fluorescence was ob-

served. These data raise the suspicion that fluorescein is bound in the stroma. If binding occurs and is due exclusively to albumin, the ratio of bound-to-free fluorescein can be determined. The average value of the bound-free ratio for these corneal strips is 0.54. (See Table XX for method of calculation.) The bound-free ratio in the central stroma caused by

TABLE XX: POLARIZATION OF FLUORESCENCE IN CORNEAL STROMA OF THE RABBIT

SPECIMEN NO.	DETERMINATION NO.	POLARIZATION	BOUND-FREE RATIO*
1	1	0.09	0.31
	2	0.09	0.31
	3	0.10	0.37
	4	0.09	0.31
2	1	0.16	0.77
	2	0.14	0.62
	3	0.16	0.77
	4	0.14	0.62
3	1	0.17	0.85
	2	0.11	0.43
	3	0.14	0.62
Mean	. . .	0.13	0.54

*Calculated from formula below using values of I_f/I_b and P_b measured in this study for rabbit plasma. See reference 132 for methods and basis of equation.

$$\text{Bound-Free Ratio} = I_f/I_b \frac{(P\text{-}P_f)}{(P_b\text{-}P)} = 1.7 \frac{(P\text{-}0.02)}{(0.47\text{-}P)}$$

albumin binding can also be calculated from the law of mass action and the following: (1) the concentration of albumin in the stroma,[151] (2) the dissociation constant of the albumin-fluorescein complex,[122,132] and (3) the average number of binding sites for fluorescein per unit concentration of albumin.[122,132] Such a calculation yields a result of 0.5 for the bound-free ratio, similar to that calculated from polarization of fluorescence. If the bound-free ratio calculated from the polarization data is used to calculate the steady-state intensity ratio between the cornea and the anterior chamber, R_{ca}, a value of 1.3 is calculated, somewhat less than the value of 1.6 measured in the living eye by Ota et al[125] and Ota[145] and somewhat greater than the value of 1.2 measured by Jones and Maurice.[96] The finding of polarization of fluorescence of fluorescein in the corneal stroma in vitro raises the suspicion that the physiologic basis for unequal distribution of fluorescent intensity between the stroma and the anterior chamber is due mainly to albumin binding in the stroma.

TABLE XXI: STUDY OF NORMAL EYES (n = 113)

	MEAN	SD	RANGE	
Age, yr	41	18.50	20	83
Anterior chamber volume, v_a, μL	186	36.90	113	272
K_o, $min^{-1} \times 10^{-2}$	1.51	0.43	0.68	2.57
Flow, μL/min	2.44	0.57	1.01	4.24
Initial "dose" of fluorescein, ng	350	255	107	1620

FLOW STUDIES IN NORMAL EYES

A group of 113 normal subjects was identified among our study population.[109] These subjects ranged in age from 20 to 83 years. Data from flow studies in each of these subjects were coded and a statistical analysis carried out with the Statistical Package for the Social Sciences (SPSS) version H, release 7.2, June 1978, as documented by Nie and co-workers.[163] The results of this analysis are described in the following paragraphs and tabulated in Tables XXI through XXIV.

As described by previous investigators,[160,164,165] the volume of the anterior chamber was found to diminish with age. The mean anterior chamber volume of the whole group was 186 ± 37 μL (Table XXI). A statistically significant decrease of volume of 14 μL per decade of age was found (Table XXII). The anterior chamber loss coefficient of fluorescein, K_o, was found to increase with age. The mean K_o for the group was $1.51 \times 10^{-2} \pm 0.43 \times 10^{-2}$ min^{-1}. An increase of K_o of 0.08×10^{-2} min^{-1} per

TABLE XXII: RELATION OF AGE TO AQUEOUS HUMOR DYNAMICS*

AGE	NO.	$K_o \times 10^{-2}$ min^{-1}	v_a, μL	FLOW, μL/min
20–29	49	1.33 ± 0.39	212 ± 28	2.58 ± 0.48
30–39	13	1.41 ± 0.33	193 ± 32	2.39 ± 0.52
40–49	11	1.32 ± 0.23	172 ± 18	2.06 ± 0.38
50–59	15	1.78 ± 0.41	158 ± 26	2.49 ± 0.47
60–69	14	1.65 ± 0.54	163 ± 29	2.38 ± 0.75
70–79	9	1.88 ± 0.58	145 ± 24	2.40 ± 0.80
80–89	2	1.56 ± 1.05	132 ± 11	1.82 ± 1.10
Mean	113	1.51 ± 0.43	186 ± 37	2.44 ± 0.57
Regression/decade		0.08×10^{-2}/min/10 yr	−14 μL/10 yr	−0.06 μL/min/10 yr
Probability		$P < 0.001$	$P < 0.001$	$P = 0.014$

*From Brubaker et al.[109]

decade of age was found. The rate of aqueous humor flow was surprisingly stable with age. The mean flow rate for the group was 2.44 ± 0.57 μL per minute. A statistically significant decrease of flow with age of 0.06 μL per minute per decade was found. However, this decrease represents a fall of aqueous humor flow of only 2% per decade of age.

The relation between anterior chamber volume and the loss coefficient K_o and the relation between anterior chamber volume and flow were studied. A statistically significant negative correlation between K_o and the volume of the anterior chamber was found, indicating that the smaller the anterior chamber the more rapid the turnover in the chamber.

Figure 18 depicts this correlation ($P < 0.001$), which is obvious from the pattern of the individual measurements. A correlation is also found between flow and anterior chamber volume; the larger the anterior chamber volume the greater the rate of aqueous humor flow. However, this correlation is not as striking ($P = 0.024$), and Fig 19 not as convincing. Taken together, these two plots suggest that the rate of aqueous humor flow is relatively independent of anterior chamber volume whereas the rate of turnover in the anterior chamber of a dye or drug is more rapid in eyes with smaller anterior chambers. If either flow or

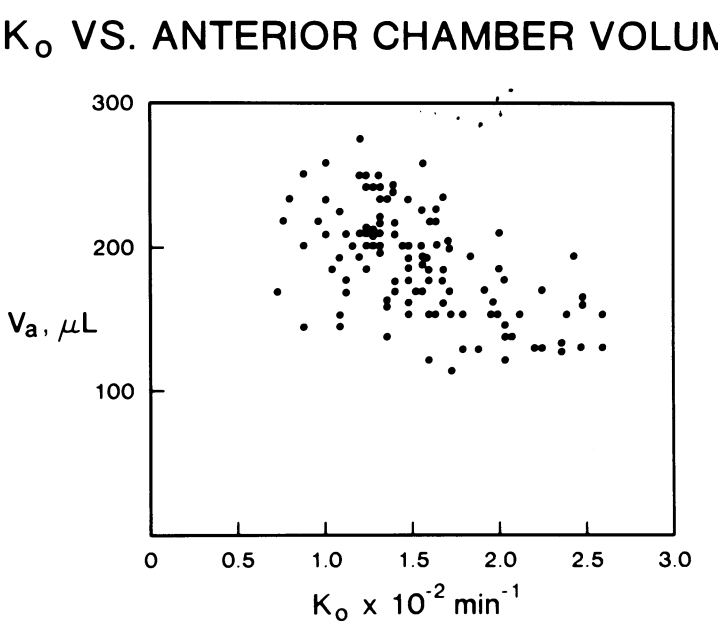

FIGURE 18
Scatterplot of v_a vs K_o in 113 normal eyes showing obvious decrease of K_o with increasing v_a.

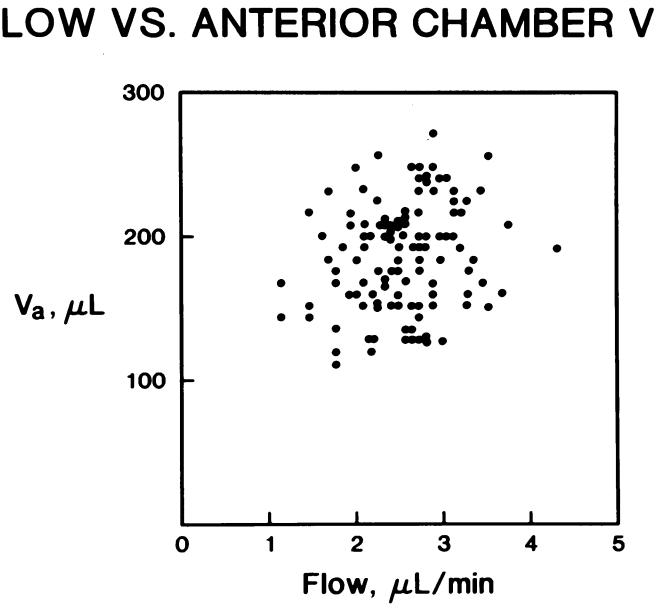

FIGURE 19
Scatterplot of v_a vs flow in 113 normal eyes showing lack of obvious correlation.

turnover rate is a biologically controlled variable, the former rather than the latter must be controlled.

The study group was stratified by sex and iris color. No statistically significant difference in the rate of aqueous humor flow through the anterior chamber was observed between men and women (Table XXIII). Ninety of these subjects had light irides, and 23 had dark. The flow in the group with light irides was 3% higher than in the group with dark irides. The difference was in the proper direction to have been explained by systematically higher initial mass measurements having been made in the eyes with more reflective irides. Such an explanation was credible, but

TABLE XXIII: RELATION OF FLOW OF AQUEOUS HUMOR TO SEX AND EYE COLOR IN NORMAL EYES

SEX	NO.	FLOW, μL/min	% DIFFERENCE	STATISTICAL SIGNIFICANCE
Male	51 (8 D/43 L)	2.40 ± 0.55	4	0
Female	62 (15 D/47 L)	2.49 ± 0.56		
Iris color				
Light	90 (43 M/47 F)	2.46 ± 0.54	3	0
Dark	23 (8 M/15 F)	2.39 ± 0.62		

the magnitude of the differences was too small to be of concern and was not statistically significant.

In all subjects, iontophoresis was carried out for five to seven seconds. The mean dose of fluorescein delivered in this way was 350 ng with a range from 107 ng to 1620 ng. Only four subjects had initial doses greater than 1000 ng. No correlation was found between the applied dose, the flow, the anterior chamber loss coefficient, the volume of the anterior chamber, or the age of the subject (Table XXIV). The lack of correlation between the applied dose and the calculated rate of flow is indirect evidence that the correction for extinction, which was small for low doses and large for high doses, was appropriate.

ACUTE DRUG STUDIES IN NORMAL EYES

A number of acute drug studies were carried out in normal subjects, and the results are tabulated in Table XXV. The adrenergic agonists, phenylephrine and epinephrine, were tested. Phenylephrine, an α agonist, was not found to have any statistically significant effect on the rate of aqueous flow through the anterior chamber. The mean rate of flow in the treated eye was 2.64 ± 0.67 μL/min and in the control eye, 2.64 ± 0.58 μL/min. Epinephrine, a mixed α and β agonist, was found to increase the flow in the normal, previously untreated eye by 15% after an acute dose.[107,108] This effect was observed in two series of subjects. In a third series, designed to determine if this effect was time-dependent, the effect was barely discernible, and time-dependency could be neither proved nor disproved.[110] The finding that epinephrine increases flow is counter to the results of a number of other studies of the effect of this drug on flow.[16,21,85,131,166-173]

The adrenergic antagonists thymoxamine, an α blocker, and timolol, a β blocker, were studied. Thymoxamine-treated eyes had a 6% higher flow than fellow eyes, but the difference was not statistically significant.[162] The combination of thymoxamine and epinephrine raised the rate of aqueous humor flow 17%.[175]

TABLE XXIV: STUDY OF NORMAL EYES (n = 113): CORRELATIONS (PEARSON, KENDALL, SPEARMAN*)

	AGE	v_a	K_o	FLOW
V_a	−, $P < 0.001$	. . .	. . .	. . .
K_o	+, $P < 0.001$	−, $P < 0.001$	. . .	. . .
Flow	−, $P = 0.014$	+, $P = 0.024$	+, $P < 0.001$	. . .
Dose	NS	NS	NS	NS

*Highest probability of three tests displayed: NS = $P > 0.05$.

TABLE XXV: SUMMARY OF ACUTE DRUG STUDIES IN NORMAL EYES

DRUG	NO. OF SUBJECTS	DOSE	FLOW		% DIFFERENCE	REFERENCE
			CONTROL EYE MEAN ± SD μL/min	TEST EYE MEAN ± SD μL/min		
Acetazolamide	21	250 mg × 2	2.08 ± 0.50	1.51 ± 0.34	−27*	174
Epinephrine	32	1% × 2	2.48 ± 0.43	2.86 ± 0.65	+15*	108, 110
Phenylephrine	22	2.5% × 2	2.64 ± 0.67	2.64 ± 0.58	0	. . .
Pilocarpine	15	2% × 2	2.37 ± 0.38	2.79 ± 0.59	+18*	159
Thymoxamine	25	1% × 2	2.35 ± 0.61	2.48 ± 0.62	+6	162
Timolol	44	0.5% × 2	2.34 ± 0.44	1.54 ± 0.31	−34*	103
Timolol + acetazolamide	21	0.5% × 2	2.08 ± 0.50	1.23 ± 0.24	−41*	174
Timolol + epinephrine	25	0.5% × 2	2.53 ± 0.50	1.36 ± 0.31	−46*	107
Thymoxamine + epinephrine	25	0.5% × 2	2.35 ± 0.61	2.74 ± 0.45	+17*	175

*$P < 0.01$.

Timolol, on the other hand, was observed to lower the rate of aqueous flow 34% after an acute dose.[103] This effect was noted in every subject tested. An even greater lowering effect, 46%, was observed in eyes treated with a combination of timolol plus epinephrine as compared with the untreated control eye.[107] These results were in agreement with the results of other investigators.[105]

The carbonic anhydrase inhibitor, acetazolamide, was observed to lower the rate of flow by 27%.[174] The combination of acetazolamide and timolol was found to lower the rate of flow by 41%, greater in this group of 21 subjects than either timolol or acetazolamide alone, suggesting that the effects of the two drugs were partly additive; this had been determined in clinical studies in which IOP had been measured.[176]

The parasympathomimetic agent, pilocarpine, was found to increase the rate of aqueous humor flow by 18%. An even greater increase in K_o was observed with this drug because of its anterior chamber shallowing effect. The rate of flow with pilocarpine was extremely close to that determined by Holm, and the stimulatory effect of parasympathomimetics has been demonstrated by other investigators.[131,177-179]

CHRONIC DRUG STUDIES

The effect of dexamethasone 0.1% was studied after instillation in one eye of normal subjects four times a day for one week. The rate of aqueous humor flow was unchanged in the first 11 subjects tested. The results of flow calculations are shown in Table XXVI.

A long-term study was carried out in a small group of glaucoma patients. In these patients, timolol was found to reduce flow by 44% after a week's treatment. The lowering effect was observed in all eyes tested. After one year's treatment, the effect had dropped to one half that observed at the one-week test period. This lessening of the effect of timolol was observed in every subject tested. The reduction of the effect of the

TABLE XXVI: SUMMARY OF CHRONIC DRUG STUDIES

DRUG	DURATION OF APPLICATION	NO. OF SUBJECTS	FLOW, μL/min	% DIFFERENCE
Dexamethasone	Control	11	2.61 ± 0.38	. . .
	1 wk	11	2.51 ± 0.79	. . .
Timolol	Control	13	2.42 ± 0.60	. . .
	1 wk	13	1.35 ± 0.53	−44*
	1 yr	13	1.88 ± 0.57	−22†

*Significantly different from control period.
†Significantly different from control period and one-week period.

drug was too great to have been explained by a worsening of the disease process and must have been caused by some form of ocular adaptation to the drug.

STUDIES IN ABNORMAL EYES

A group of 21 patients was identified with unilateral third neuron Horner's syndrome.[111] This group was studied to determine if any abnormality of aqueous dynamics existed under conditions of chronic adrenergic denervation in the human eye. Intraocular pressure was 1 mm Hg lower in the affected eye, but no other abnormality was found. The rate of flow was 2.21 ± 0.54 μL/min in the affected eye and 2.14 ± 0.57 μL/min in the unaffected eye. The difference was not statistically significant (Table XXVII).

Some of these patients underwent drug testing with adrenergic agents. Those tested were found to have a normal response to timolol in both eyes. All patients tested with epinephrine in this group responded with a decrease in flow in the affected eye and an increase in flow in the unaffected eye.[111]

A group of 26 patients with myotonic dystrophy, all of whom had extremely low IOP (mean = 7 mm Hg), were studied with fluorophotometry.[180] The calculated rate of flow in these subjects was normal, 2.51 ± 0.62 μL/min. However, it was found after systemic administration of fluorescein that these patients had a profound defect in the permeability of the blood-ocular barrier to fluorescein, sufficient to interfere with the determination of aqueous humor flow. The interference could cause the calculated flow to be higher than the actual flow, and thus these patients probably have a lower than normal rate of aqueous humor formation, but exactly how low remains unknown.

Nine patients with the clinical signs in one eye of the exfoliation syndrome with glaucoma were studied. The rate of flow was 2.27 ± 0.77 μL/min in the affected eye and 2.85 ± 0.88 μL/min in the unaffected eye. This difference was statistically significant. The IOP was much higher in the affected eye, 32 ± 11, than in the unaffected eye, 18 ± 5. Thus, the difference of flow may have been due to the difference of IOP per se. Like the eyes of patients with myotonic dystrophy, the affected eyes of these patients demonstrated a leaky blood-ocular barrier to fluorescein. Thus, the true rate of flow in the affected eye was probably lower than the rate calculated from fluorescein clearance by equation 15.

A group of patients with cornea guttata without epithelial edema were studied, primarily in regard to corneal endothelial permeability, but the flow of aqueous humor was also measured.[181] The rate of flow was 3.00 ±

TABLE XXVII: SUMMARY OF STUDIES IN ABNORMAL EYES

DISEASE	NO. OF SUBJECTS	CALCULATED FLOW, μL/min		COMMENT	REFERENCE
		AFFECTED EYES	NORMAL EYES		
Horner's syndrome	21	2.21 ± 0.54*	2.14 ± 0.57	Difference insignificant	111
Myotonic dystrophy	26	2.51 ± 0.62	. . .	Blood-ocular barrier too leaky for accurate flow determination	180
Unilateral exfoliation syndrome with glaucoma	9	2.27 ± 0.77	2.85 ± 0.88	Flow lower in affected eye; blood-ocular barrier leaky in affected eye	. . .
Cornea guttata	18	3.00 ± 1.03	. . .	No different from age-matched controls	181
Controls	15	. . .	2.69 ± 0.65		
Iridocorneal endothelial syndrome	5	3.18 ± 1.82	2.47 ± 1.13	Difference insignificant	. . .
Unilateral carotid artery occlusion	5	2.61 ± 0.32	2.64 ± 0.21	Difference insignificant†	. . .
Bilateral carotid artery occlusion	1	1.40 OD 1.45 OS	. . .	Flow low-normal in one case	. . .

*Affected eyes responded normally to timolol, responded abnormally to epinephrine.
†No subject in this group has yet been tested with systemically administered fluorescein.

1.03 μL/min in this group. This rate was not significantly different from a group of age-matched controls without guttata in whom a flow rate of 2.69 ± 0.65 μL/min was measured.

A small group of patients with the iridocorneal endothelial syndrome (ICE syndrome: essential iris atrophy, Chandler's syndrome, Cogan-Reese syndrome) was studied. These patients had a dramatically lower K_{ca} (and lower endothelial permeability to fluorescein), but no difference was found in the rate of aqueous humor formation. In two patients, polycoria and corectopia were sufficient that fluorescein may have been able to leak into the posterior chamber, causing the flow to appear abnormally high.

Five patients with unilateral carotid occlusion and asymmetric ophthalmodynamometric readings and one patient with bilateral carotid occlusion were studied. The rate of aqueous humor flow was calculated as being normal in all these subjects, but none of them has yet undergone testing with systemically administered fluorescein to demonstrate integrity of the blood-ocular barrier.

DISCUSSION

The use of fluorescent tracers provides a method to study the flow of aqueous humor through the anterior chamber in human subjects and offers certain advantages over other methods. Fluorescent tracer techniques do not alter the naturally existing state of the IOP, do not require invasion of the anterior chamber with a needle, and appear to be harmless to the eye. Human subjects are the best suited for studies with the technique described in this thesis since they can cooperate fully with the procedure and can pursue their normal visual activities. These activities permit maintenance of normal corneal transparency, pupillary responses, and mixing of the aqueous humor in the anterior chamber by thermal and mechanical means.

These methods can be used for animal studies. However, general anesthesia is usually required, maintenance of normal corneal luster may be difficult, reproducible alignment with the fluorescent target is more difficult, and mixing in the anterior chamber may be marginally adequate. These problems have been circumvented in one way or another for animal studies, and successful studies using fluorescein have been reported.[82,86,104,182,183] The need for general anesthesia and the lack of subject cooperation detract significantly from the advantages inherent in topical fluorescein tracer techniques as used in human subjects.

The convenience and safety of this means of measuring aqueous humor flow must be considered. In regard to convenience, many of our subjects have been persons with occupations near the test area and have carried out their normal daily duties throughout the day of the test. The interference with visual function produced by the iontophoresis of fluorescein is no greater than that caused by applanation tonometry with a fluorescein-anesthetic combination.

In regard to safety, several facts need to be considered. First, there is a risk of corneal abrasion in performing iontophoresis, but the risk is low if the procedures outlined in the "Methods" section are followed. In using this procedure in 1000 eyes, only one eye suffered a significant corneal abrasion. On slit-lamp examination, the abrasion apparently was caused by contact between the edge of the polyethylene container of the disposable agar electrode, a procedural error. The abrasion healed completely within 24 hours. Bloom et al[101] reported a higher incidence of abrasion, but applied the current for a much longer time.

It is possible to produce a corneal abrasion if one attempts to perform iontophoresis in a small area of the cornea with a highly conductive electrode. For this reason, we have used agar electrodes containing no conductive ions except sodium fluorescein itself. The agar is applied to the surface of the cornea before the current is activated, and the current is deactivated before the agar is removed from the corneal surface. This sequence assures a low current density as well as a more uniform depot.

The slit-lamp appearance of the corneal epithelium immediately following iontophoresis is a fine stippling, which disappears in approximately 15 minutes. Most of our subjects have been questioned directly about foreign body sensation, and few of them have noted any such sensation whatever.

Fluorescein is commonly instilled into the conjunctival cul-de-sac in order to demarcate areas of corneal abrasions or to demarcate the flattened area during applanation tonometry. My co-workers and I measured the concentration in the cornea and in the anterior chamber of both eyes of two subjects who underwent hourly measurement of IOP with hourly instillation of 0.25% fluorescein with 0.4% benoxinate. The concentrations of fluorescein in these two subjects are recorded in Table XXVIII. The concentrations reached in the anterior chamber were higher in these two patients than the peak concentration expected in the anterior chamber of a normal eye following iontophoresis of 350 ng of fluorescein. However, the corneal concentration in the center of a depot is over ten times (Fig 12) that found in the two subjects after hourly tonometry. We have not measured the corneal concentrations in persons with corneal

TABLE XXVIII: FLUORESCEIN CONCENTRATION REACHED IN TWO GLAUCOMA PATIENTS UNDERGOING HOURLY TONOMETRY WITH 0.25% FLUORESCEIN AND 0.4% BENOXINATE

		CONCENTRATIONS, g/mL	
		CORNEA	ANTERIOR CHAMBER
Patient 1 (seven instillations)	OD	2.0×10^{-6}	2.6×10^{-7}
	OS	3.2×10^{-6}	3.0×10^{-7}
Patient 2 (nine instillations)	OD	2.7×10^{-6}	5.4×10^{-7}
	OS	1.4×10^{-6}	3.1×10^{-7}
Peak concentrations reached after iontophoresis of 350 ng fluorescein into central cornea in normal eye		3.6×10^{-5}	2.1×10^{-7}

abrasions following instillation of fluorescein. The clinical appearance of corneas stained in this way suggests that the fluorescein concentration in this situation is at least as high as that achieved after iontophoresis. It is doubtful that fluorescein in these concentrations has any toxic effect on the cornea.

Several investigators have demonstrated that commonly used ophthalmic diagnostic instruments may expose the eye to light levels that are dangerously near the threshold for retinal damage.[154,184,185] Long exposures to blue light are particularly dangerous in this regard.[186] For this reason, measurements were carried out to determine the light levels to which subjects are exposed while undergoing fluorophotometry for the measurement of aqueous humor flow with the instrument used in these studies. The light output of the fluorophotometer was measured with a radiometer (International Light, model 700) with an SEE-100 light sensor. The measurements were made with the settings of the fluorophotometer as it is currently used. A summary of the measurements is outlined in Table XXIX. The exposure to the eye is within the ANSI[153] and ACGIH[154] guidelines. These exposures are lower than those produced by the unfiltered lamp output of the Zeiss photoslitlamp system or other slit lamps. It is easy to exceed the recommended safe levels of illumination with existing light sources, but it is not necessary to exceed them to obtain accurate measurements of fluorescence in the cornea and the anterior chamber. The most suitable light source is the 488-nm output of the argon laser, which has the highest ratio of efficiency-to-safety of any light source that we have tested (Table V).

The cost of carrying out fluorophotometry for the measurement of aqueous flow is high enough so that it may never be used widely except for investigative purposes. The nomographic technique is the easiest and

TABLE XXIX: SUMMARY OF TYPICAL EXPOSURE PARAMETERS FOR CONTINUOUS TUNGSTEN AND XENON FLASH WITH B-4 EXCITATION (BLUE) AND IR FILTERS

	3-mm PUPIL		7-mm PUPIL	
	10-mm DIAMETER SPOT	0.1 mm × 3.0-mm SLIT	10-mm DIAMETER SPOT	0.1 mm × 3.0-mm SLIT
Continous tungsten				
Power measured (μW)	72.0	3.60	72	3.60
Total power through pupil (μW)	6.50	3.60	35	3.60
Retinal irradiance* (μW/cm^2)	325	180	1768	180
Time to MPE†	3.8 hr	6.9 hr	42 min	6.9 hr
Flash exposure				
Energy measured per flash (μJoules)	205	2.10	205	2.10
Total energy through pupil (μJoules)	18.50	2.10	100	2.10
Retinal irradiance* (μJoules/cm^2)	926	105	5008	105
Number of flashes to MPE†	4800	4.2×10^4	890	4.2×10^4

*Retinal irradiance defined by: $E_r = \frac{\tau n^2 A_p L_s}{f^2} = \frac{\tau n^2 P_p}{f^2 \Omega}$

†E_r maximum = 4.45 Joules/cm^2.

the least expensive method we have used. A single technician can perform all of the testing procedures and paperwork for approximately three to four subjects per day if this method is followed. Such a procedure would cost approximately $35 per subject considering the costs of equipment, space, and supplies. A maximum of two per day can be performed if the least squares method, which requires hourly measurements, is used. Techniques requiring measurement of plasma concentration of fluorescein as well as anterior chamber concentrations cost more per subject than do topical methods. Also, subject inconvenience is greater because of the necessity for frequently withdrawing blood samples. On the other hand, methods based on analysis of systemically administered fluorescein are more precise, as judged from right-to-left differences in normal subjects. Moreover, these methods provide direct measurements of diffusional exchange.[130,131] For these reasons, studies of topical drugs require fewer subjects as long as the study is properly designed and the study population is fairly homogeneous.

Despite its many advantages, measurement of aqueous humor flow with the corneal depot method also has a number of disadvantages and limitations. The use of a depot in the center of the cornea can interfere significantly with the measurement of anterior chamber concentration at times when the anterior chamber concentration is low. In cases where the investigator is primarily interested in endothelial permeability, this interference can introduce significant errors into the procedure. Application of fluorescein away from the center of the cornea or the use of systemic administration of fluorescein is likely to be more accurate for studies of endothelial permeability.[128]

Problems are also encountered with measurements of total mass in the cornea and anterior chamber many hours after application of fluorescein. An ideal instrument for such measurement would illuminate and gather light from a field exactly equal to the size and shape of the cornea of each subject. Furthermore, the intensity of illumination and the sensitivity of the light detector must be uniform throughout this field. Even an ideal instrument, however, cannot visualize the area of the cornea and the anterior chamber obscured by the overhanging conjunctiva. For these reasons, procedures for calculating aqueous humor flow that depend on late mass measurements are not used in the author's laboratory.

The dynamics of fluorescein movement in the stroma are not fully understood. There are reasons to believe that fluorescein binds to albumin in the stroma. If so, this binding must effect the exchange of fluorescein across the endothelium and the fluorescent intensity of fluorescein in the cornea. It is possible that albumin binding may be greater in the limbal portions of the cornea than in the central portions.[151] Also, little is known about the exchange of fluorescein at the limbus. Evidence is presented in this study that limbal exchange may be of little importance after topically applied fluorescein. However, unaccounted limbal loss after topical administration of fluorescein and unaccounted limbal gain after systemic administration may be the basis of the systematic differences in flows reported from laboratories using one or the other of these methods.

It is possible that the limbus is a major source of entry of fluorescein into the anterior chamber following systemic administration. Preliminary measurements of the rate of exchange of the sclera, episclera, and conjunctiva in this study suggest that the rate of exchange with plasma is fairly slow in the normal eye. Thus, the scleral tissue near the limbus could act as a reservoir of fluorescein from which the dye gradually enters the anterior chamber via the peripheral cornea. It is known that fluorescein administered in the subconjunctival space readily enters the anterior

chamber.[187,188] If unaccounted for, limbal entry of fluorescein would depress the calculated rate of flow by methods that depend on systemically administered fluorescein.[130] It is likely, therefore, that proper correction for limbal exchange would bring the results of topical and systemic methods closer to a common result.

A major disadvantage of all techniques using topically applied fluorescein is that they cannot be applied to eyes exhibiting inflammation or abnormalities of the blood-ocular barrier. The loss of fluorescein through the normal iris is almost negligible, but iridal loss may be substantial in abnormal eyes. In all of our studies, it has been assumed that iridal loss is 10% of the observed rate of fluorescein from the anterior chamber. Diminution in the rate of loss of fluorescein through the iris would have an insignificant effect on the results, but a twofold or threefold increase in permeability of the iris to fluorescein could affect the results.

A large increase in the permeability of the iris, however, is easy to detect with systemically administered fluorescein. In all of the studies reported here (except for the group with carotid occlusion), the status of the blood-ocular barrier has been measured in a subset of the test group. In the subjects with myotonic dystrophy and in those with the syndrome of exfoliation with glaucoma, the blood-ocular barrier was found to be quite leaky to fluorescein. In such subjects, the relation between fluorescein clearance from the anterior chamber and aqueous flow is unclear.

It must be remembered that topically applied fluorescein cannot be used to measure flow unless the normal iris-lens barrier is intact to prevent posterior losses of fluorescein. Thus, persons with large iridectomies, aphakia, aniridia, or other abnormalities of the lens-iris diaphragm pose problems for the experimental fluorophotometrist.

All noninvasive fluorescent tracer studies in suitable subjects measure only that portion of newly formed aqueous humor that enters and leaves the anterior chamber. That portion of aqueous humor that leaves the eye through its posterior portion is not included. Experimental procedures that satisfactorily quantify the loss of aqueous humor from the posterior portions of the eye have not been devised. Estimates of such losses made from measurements of hydraulic conductivity of ocular tissues to water suggest that they may not be insignificant in the normal eye[189-191] and may take on added significance in the diseased eye.[192]

The use of topical fluorescein as a tracer is rather ideally suited to the study of the effect of topically applied drugs on the rate of aqueous humor flow through the anterior chamber. One eye conveniently serves as a control, and the flows of the two eyes can be measured simultaneously. Variations in systemic metabolism that are likely to affect the flow through

the anterior chamber affect both eyes simultaneously. Table XV can be used to assist in calculating the sample size (number of subjects) needed to test the effect of a topical drug on flow. In determining sample size, the newcomer to fluorophotometry of the anterior segment needs to determine for himself the reproducibility of his measurements. However, persons familiar with slit-lamp biomicroscopy but unfamiliar with fluorophotometry seem able to learn the latter skill quite rapidly and can make fairly reproducible measurements after some practice.

The adrenergic agents studied by this technique demonstrate a pattern of effect on aqueous humor flow. α-Adrenergic agonists and antagonists appear to have only a minor effect on the rate of aqueous flow. No statistically significant effects of α-active drugs have been found in these studies. Possible conclusions are that these drugs have not reached the ciliary body, that these drugs have no effect on aqueous humor flow, or that the α system plays a less important role in regulating the rate of aqueous humor formation. The findings in Horner's syndrome are consistent with the last hypothesis. These eyes, all selected because of their α-adrenergic defect, exhibit a normal rate of aqueous humor flow.

Statistically significant changes were seen in aqueous humor flow with β-adrenergic agents. Epinephrine is a weak stimulator of aqueous humor flow as measured by this technique, and timolol is a potent suppressor of aqueous humor flow. The suppression by timolol has been confirmed by other investigators, but the effect of epinephrine remains a puzzle. It has been shown by fluorophotometry that the selective β agonists, salbutamol[193] and metaproterenol,[131] increase the rate of aqueous humor flow. It seems reasonable from these studies to generalize that the rate is stimulated by β-adrenergic agonists and inhibited by β-adrenergic antagonists in the normal human eye when acute doses are given topically. However, it is not known from studies of topically applied drugs whether the drug produces its observed effect because it is acting as a specific agonist or antagonist at a high-affinity receptor site or whether the drug produces its observed effect by some nonspecific mechanism. Such conclusions can be drawn only from studies in which receptor sites themselves are studied and the physiologic process of aqueous formation is linked directly to the binding of the specific drug to the receptor. Such studies are difficult for practical reasons to carry out in human eyes, but have been carried out extensively in animal eyes. Exemplary in this regard are the studies of Gregory and co-workers[194] who have identified and studied a β receptor site that is localized in the ciliary process of the rabbit eye. Gregory et al[195] also have shown that irreversible stimulation of intracellular cAMP production with cholera toxin produces a dramatic

lowering of the rate of aqueous humor flow through the anterior chamber. These workers have concluded that the β receptor of the ciliary epithelium is linked in some rather direct way to the physiologic process of aqueous humor formation, that such a receptor may regulate the rate of aqueous humor formation, and that natural or artificial stimulation of the receptor decreases the rate of formation.[196]

The observation that β-adrenergic drugs alter the rate of aqueous humor flow through the anterior chamber is consistent with the idea of a β receptor for aqueous humor formation, but the direction of the change in these studies is unlike what would have been expected from the studies cited. This apparent disparity need not be of major concern, since a number of reasonable explanations can be invoked to account for it. In addition, one cannot draw conclusions about specific cellular mechanisms from topical drug studies such as this one because of the complexity of the system of the living eye, as pointed out by Neufeld.[197] Clinically, extremely high concentrations are applied to the cornea, the concentration of the drug at the receptor is not known, and the action of the drug is superimposed on whatever endogenous mechanisms are currently taking place in the eye. In spite of such limitations, fluorophotometric studies in the living human eye can assist in establishing patterns of drug effect that can help in formulating a clinically rational approach to their use. It is to this end that the data of these studies can most usefully be applied.

There are many areas of research that would benefit by future improvements in the technology of ocular fluorophotometry. Fluorescent materials other than fluorescein could be used for studying oxygen tension or protein binding. The polarization of fluorescence of fluorescein can be used to quantify the concentration of albumin in the anterior chamber and in the corneal stroma. This technique can be applied to the living eye but awaits the invention of an accurate polarizing fluorophotometer for its clinical application. Such a technique would be particularly valuable in the study of inflammatory diseases.

Other advances in fluorophotometric instrumentation could help answer questions about the mixing of fluorescein in the anterior chamber, the concentration of fluorescein in the pupillary aqueous, and the exchange of fluorescein at the limbus. These questions need more detailed study and would be assisted by a fluorophotometer that could recreate the three-dimensional image of fluorescent intensity in the anterior segment of the eye. Such a fluorophotometer is well within the grasp of currently available technology. It is likely that continual advances in spectrofluorophotometry will give the ocular physiologist new and important tools with which to study the highly specialized circulatory system of the eye.

APPENDIX

DEFINITIONS AND SYMBOLS

I. AQUEOUS HUMOR DYNAMICS

The system of symbols used here follows closely that proposed by Duke-Elder and Maurice,[157] with some minor exceptions, including the fact that the letter *K*, either uppercase or lowercase, always denotes a transfer coefficient and never a permeability.

A. Summary of symbols

1. Subscripts referring to tissue or fluid compartments
   - *a*, Anterior chamber
   - *c*, Corneal stroma
   - *p*, Plasma
   - *s*, Limbal sclera, episclera, and subconjunctival tissue
2. Volumes
   - *v*, Geometric volume (anatomic volume)
   - *V*, Apparent volume of distribution to fluorescein
3. Masses
   - *M*, Mass of fluorescein in a compartment
4. Areas
   - *A*, Interfacial area between two compartments
5. Transfer coefficients
   - *K* or *k*, Transfer coefficient of fluorescein into or out of a compartment
6. Permeabilities
   - *P*, Permeability to fluorescein of interfacial tissue between two compartments
7. Distribution ratios
   - *R*, Steady-state concentration ratio of fluorescein between a compartment and a reference solution containing no bound fluorescein
8. Linear dimensions
   - *x*, Thickness, depth, diameter, etc
9. Dimensionless fractions
   - *X*, Ratio of mass of fluorescein in a compartment to initial mass (dose of fluorescein administered to the cornea)
10. Hybrid coefficients
    - α, β, γ, δ, λ Indicate combinations of parameters collected together for convenience but having no readily interpretable meaning
11. Diffusion coefficients
    - *D*, Diffusion coefficient of fluorescein in ocular tissues

B. Specific definitions

1. Constants

v_c, Geometric volume of the corneal stroma

V_c, Apparent volume of distribution of fluorescein in the corneal stroma

$$V_c = v_c \cdot R_c$$

v_a, Geometric volume of the anterior chamber

V_a, Apparent volume of distribution of fluorescein in the anterior chamber

$V_a = v_a$ in the normal eye[198]

A_{ca}, Surface area between the stroma and the anterior chamber

A_{12}, Surface area between two compartments in the stroma in the multiple-compartment model

x_c, Thickness of the stroma

x, Distance between compartments of the stroma in the multiple-compartment model

R_c, Steady-state concentration ratio between the cornea and a reference solution containing unbound fluorescein

R_s, Steady-state concentration ratio between the limbal sclera and episcleral tissue and a reference solution containing unbound fluorescein

R_p, Steady-state concentration ratio between plasma and a reference solution containing unbound fluorescein

e, Base of the natural logarithm

D_c, Diffusion coefficient for fluorescein in the stroma

M_o, Dose of fluorescein administered at time zero into the corneal stroma by iontophoresis

g, Proportionality constant relating fluorescein concentration in the sclera to observed fluorescent intensity of the sclera

2. Time-dependent variables

t, Time

M_c, Mass of fluorescein in the corneal stroma

M_a, Mass of fluorescein in the anterior chamber

M_s, Mass of fluorescein in the limbal sclera

$M_c(i)$, Mass of fluorescein in individual stromal compartment in the multiple-compartment model

$M_s(i)$, Mass of fluorescein in individual scleral compartment in the multiple-compartment model

M_T, Total mass of fluorescein in the cornea and anterior chamber, $M_c + M_a$

C_c, Concentration of fluorescein in the stroma. Note: Concentration of unbound fluorescein in the stroma is C_c/R_c

C_a, Concentration of fluorescein in the anterior chamber

C_p, Concentration of fluorescein in the plasma

C_s, Concentration of fluorescein in the limbal sclera

X_c, M_c/M_o, fraction of initial mass of fluorescein contained at any time in the corneal stroma

X_a, M_a/M_o, fraction of initial mass of fluorescein contained at any time in the anterior chamber

X_T, $X_c + X_a$, fraction of initial mass of fluorescein contained in the eye at any time

3. Transfer coefficients and permeabilities

K_{ca} or $K_{c.ca}$*, Transfer coefficient for fluorescein from the cornea to the anterior chamber, as defined by

$$\frac{dM_{ca}}{dt} = K_{ca} \cdot M_c$$

K_{ac} or $K_{a.ac}$†, Transfer coefficient for fluorescein from the anterior chamber to the cornea, as defined by

$$\frac{dM_{ac}}{dt} = K_{ac} \cdot M_a$$

Note: $K_{ac} = K_{ca} \cdot V_c/V_a$

*Equivalent to K_a of Coakes and Brubaker[106] and k_{ac}/R_c of Sawa et al.[128]

†Equivalent to K'_a of Coakes and Brubaker.[106]

K_{sp} or $K_{s.sp}$, Transfer coefficient for fluorescein from the limbal sclera and episclera into the plasma, as defined by

$$\frac{dM_s}{dt} = K_{sp} \cdot M_s$$

K_d, Transfer coefficient for fluorescein loss from chamber into plasma by diffusion

K_f, Transfer coefficient for fluorescein loss from the anterior chamber by outflow of aqueous humor

K_o*, Transfer coefficient for fluorescein loss from the anterior chamber by diffusion and flow (excluding exchange with cornea), as defined by

$$\frac{dM_a}{dt} = K_o \cdot M_a = (K_d + K_f) \cdot M_a$$

P_{ca}, Permeability of the corneal endothelium

Note: $P_{ca} = K_{ca} \cdot R_c \cdot x_c$

*Equivalent to K_e of Coakes and Brubaker.[106]

4. Hybrid coefficients

Let $h = (1 + V_c/V_a) \cdot K_{ca} + K_o$

$\alpha = (h - h^2 - 4 \cdot K_{ca} \cdot K_o)/2$

$\beta = (h + h^2 - 4 \cdot K_{ca} \cdot K_o)/2$

$\gamma = K_{ca}/(\beta - \alpha)$

$\delta = (\beta - K_{ca})/(\beta - \alpha)$

5. Miscellaneous

a, A correction factor used to eliminate the effects of extinction from stromal mass measurements. It is determined from the multiple-compartment model and the extinction coefficient of fluorescein for the fluorophotometer used in these studies. Has units mass^{-1}.

II. LIGHT AND FLUORESCENCE

F_e, Flux or total power of light emitted by sampled volume of fluorescein (λ = 520 nm)

F_i, Flux or total power of illumination (λ = 490 nm)

I_i, Irradiance incident on the fluorescent target

Φ, Quantum efficiency of fluorescein

ϵ, Extinction coefficient of fluorescein

E_a, Collection efficiency of objective, due to aperture

E_o, Efficiency of optics of biomicroscope in bringing collected light to light sensor

I, Intensity of fluorescent light detected by fluorophotometer per unit flux of excitation power incident on the target per unit volume of target

I_f, Molar fluorescent intensity of free (unbound) fluorescein

I_b, Molar fluorescent intensity of fluorescein bound to plasma

p, Polarization of fluorescence

$$p = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

, where $I_{\parallel}$ is the intensity measured parallel to the excitation "e" vector and $I_{\perp}$ is the intensity measured perpendicular to the excitation "e" vector

P_f, Polarization of free fluorescein

P_b, Polarization of fluorescein bound to plasma

λ, Wavelength

I_{ac}, Ratio of fluorescent intensity in anterior chamber to cornea immediately after iontophoresis

$I_{ac} = I_a(0)/I_c(0)$

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