

of patients in each study group, to diurnal fluctuation of IOP, and to subject variability of response to pilocarpine. One would anticipate a smoother time course and more predictable dose-response relationship for a larger study.

The insert is, in effect, a device for prolonging the contact time of the dispersed drug with the corneal tear film. It is therefore analogous to the use of agents which increase tear film viscosity, such as methylcellulose and polyvinyl alcohol,⁸ without the disadvantage inherent in the removal of a drug-soaked carrier such as the cotton pledget.⁹ Since there is a limit to the effectiveness of increasing viscosity on drug penetration,¹⁰ the duration of effect seen with these inserts can be attributed to prolonged release with the slow dissolution of the device.

This study satisfactorily demonstrates the effectiveness of soluble inserts as a drug delivery system. Ultimately, delivery of pilocarpine by this method may be accomplished by the insertion of a device immediately prior to sleep, resulting in the minimization of symptoms while retaining the desired pressure-lowering effect.

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A randomized technique of constant-pressure infusion. M. GARY WICKHAM,* DAVID M. WORTHEN,* AND DARRYL DOWNING.**

A population study of measured inflow values of eyes of a group of adult male rhesus monkeys was done with a randomized technique of constant-pressure infusion. Each eye was presented with a sequence of 18 4-minute runs, consisting of three infusion runs for each of six pressures, in which the order of appearance of a given pressure had been randomized. A statistical analysis of the results indicates that only the independent variables of infusion pressure, type of anesthetic, and position in the sequence of the 18 infusion runs had a significant effect upon measured inflow.

The technique of measuring the facility of aqueous humor outflow in an in vivo preparation dates from the work of Becker and Constant.¹ Bárány's² two-level method estimated a Δ -facility value ($\mu\text{l}/\text{min.}/\text{mm. Hg}$) obtained by subtracting a flow value measured over 4 min. at 2.5 mm. Hg above baseline from one at 11.8 mm. Hg above baseline and then dividing by the pressure difference. Bárány and associates³ have now adopted the use of a four-level technique with seven infusion periods repeated twice and have included the use of higher infusion pressures to 40 mm. Hg. Brubaker⁴ and Brubaker and Worthen⁵ have presented data describing low-pressure infusion, isolation of the aqueous venous barrier, the use of more physiological infusion media, and mathematical modeling of outflow dynamics.

If experimental error (i.e., maximum pressurization of the eye, fluid volume injected prior to facility measurement, total time needle is in eye, fluctuating baseline intraocular pressure) is minimized and kept at equivalent levels from one time to the next, if work is confined to near-physiological pressures, and if all experimental variables except the one being studied are controlled, then the two-level technique will provide statistically sound results in comparison tests. Even under these ideal conditions, the results will still be an expression of the response of the measured eye

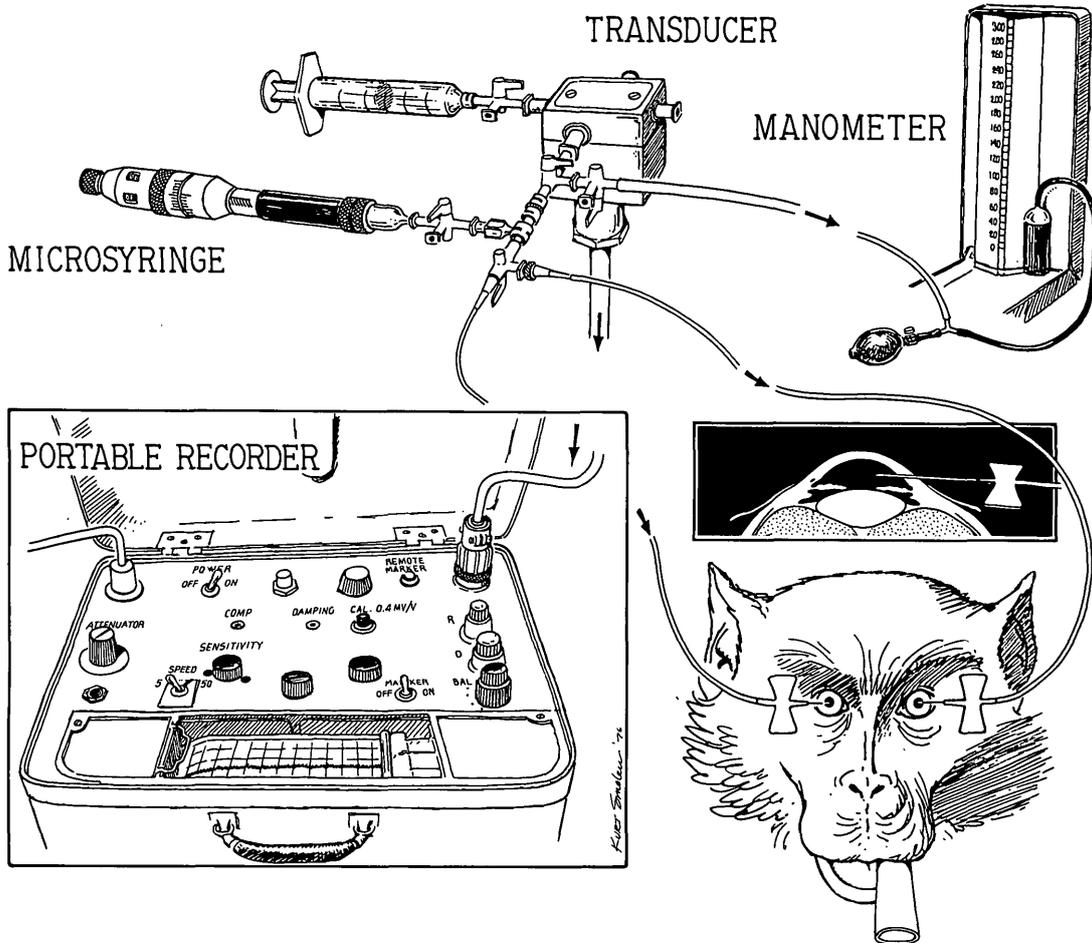


Fig. 1. A diagrammatic illustration of the apparatus used for the randomized constant-pressure infusion studies. An emphasis was placed on a simple and portable experimental setup.

to the pattern of the test and will neither show nor delimit the full potential of the eye. In the near-physiological pressure range this is probably not of consequence, but when higher (+30 mm. Hg) pressures are utilized, factors such as prior scleral stretching or the previously introduced fluid volume will significantly modify the amount of fluid an eye is capable of accepting. At increased infusion pressures the problems introduced by experimental error are magnified, and it becomes more difficult to ensure the comparability of observations.

One way to obtain a full expression of the ability of a series of eyes to accept infused fluids, while both minimizing experimental error and maximizing test sensitivity, would be to utilize a randomized data-collection design on a small population of the desired eyes. In such a protocol, repetition is necessary at each infusion pressure in order to secure an average value, thus

incidentally ensuring that the eye will be sufficiently stressed so that all but the grossest of experimental errors will have no consequence. The modified technique of constant-pressure infusion described herein was predicated upon the successful application of such a randomized protocol to the population study of a group of rhesus monkeys.

Methods and materials. Twenty-one adult male rhesus monkeys (*Macaca mulatta*) weighing 4.5 to 13 kg. were anesthetized with either phencyclidine HCl (Sernylan; Bio-Ceutic Laboratories, St. Joseph, Mo.) injected intramuscularly followed by intravenous and/or intraperitoneal sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, Ill.) or with the sodium pentobarbital alone. The proper anesthetic level was defined as one in which both corneal and testicular sensitivities were suppressed and a constant, low-level respiratory rate (16 to 26 per minute) was maintained.

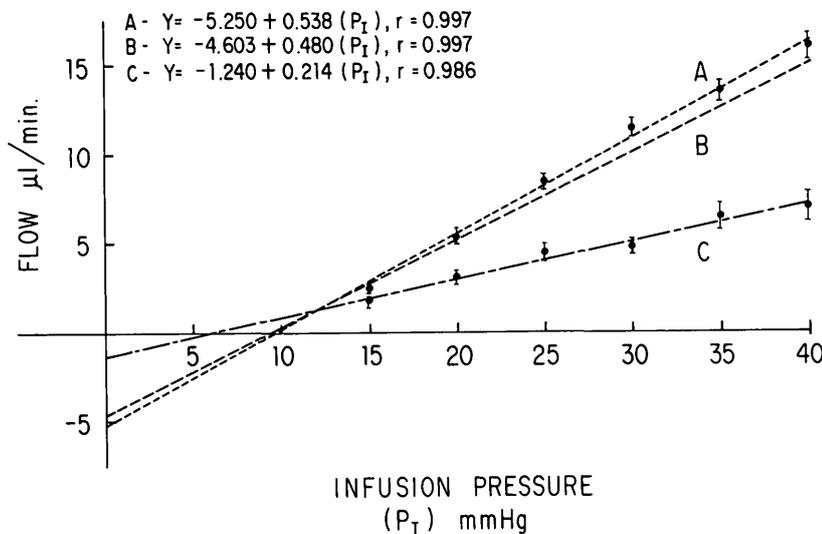


Fig. 2. Regression plots of pressure-flow means at 15, 20, 25, 30, 35, and 40 mm. Hg. The curves are extrapolated from the 15 mm. Hg values to the y-axis. Lines A ($N = 41$ each point) and C ($N = 10$ each point) represent the curves for pentobarbital and phencyclidine/pentobarbital anesthetized animals, respectively. Line B is the regression of the overall means at each point. The bars on curves A and C indicate standard error of the mean. All lines have a statistically significant fit.

Once anesthesia was obtained, the animal under study received 6 to 8 drops of 1 per cent atropine at the epiglottis and was intubated. The monkey was placed in an immobilization bag (Vac-Pac, Olympic Surgical Co., Seattle, Wash.) with its head elevated slightly above the rest of its body. The infusion apparatus (Fig. 1) included a Hewlett-Packard 267BC transducer, a Hewlett-Packard 301 single channel carrier amplifier-recorder, a Gilmont S-3200 microliter syringe (technique as described by Sears⁶), a series of three-way valves (Pharmaseal Laboratories, Glendale, Calif.) and two 25G vein sets (Pharmaseal). The fluid path from the transducer through the vein sets was sequentially filled with sterile, degassed Hanks balanced salt solution (BSS), pH 7.15, starting at the filling port of the transducer. The fluid path was sterilized prior to each use. The Hanks BSS was compounded from the stock components, adjusted in pH and filtration sterilized (GA-8, 0.2 μ pore diameter, Gelman Instrument Co., Ann Arbor, Mich.) for each series of two eyes. Once the system was intact, each eye in turn was cannulated with a 25G vein set through which Hanks BSS was slowly being infused. Eyes were cannulated in the temporal quadrant about 1 to 2 mm. anterior to the limbus. The needle was placed half-way to the near pupil margin, and the slow infusion of Hanks BSS was stopped. The cornea was gently depressed to assure that the system was pressurized, and the vein set tube was adjusted so that it put no strain on the needle

entry site. After both eyes had been cannulated, a final inspection was made for leaks, ocular trauma, and proper anesthetic level. Each eye was then covered with a methylcellulose solution (Goniogel; Muro Pharmaceutical Laboratories, Inc., Quincy, Mass.) to inhibit evaporation from the surface.

The infusion sequence was begun in either the right or left eye as determined by a coin toss. The design utilized three runs of 4 min. duration done at six different pressure levels. Each eye received an 18 run sequence with 72 min. of active infusion of fluid from the microliter syringe. The actual order in which the pressures (15, 20, 25, 30, 35, and 40 mm. Hg) were presented to a given eye on a given day were determined from a computer-generated random number table listing possible arrangements of the 18 numbers. Baseline pressures were recorded for both eyes at the start and end of each eye infusion sequence.

The raw data were collected as flow/4 min. and were then converted to flow per minute. Data analysis was primarily done on flow data because of the relative ease of calculating this value, instability of the baseline intraocular pressure as a base for a Δc value, lack of literature identification with a facility value derived from the total pressure, and standard use of the flow-pressure relationship in studies of other systems. Flow values and the derivative values of facility obtained by dividing flow by the infusion pressure,

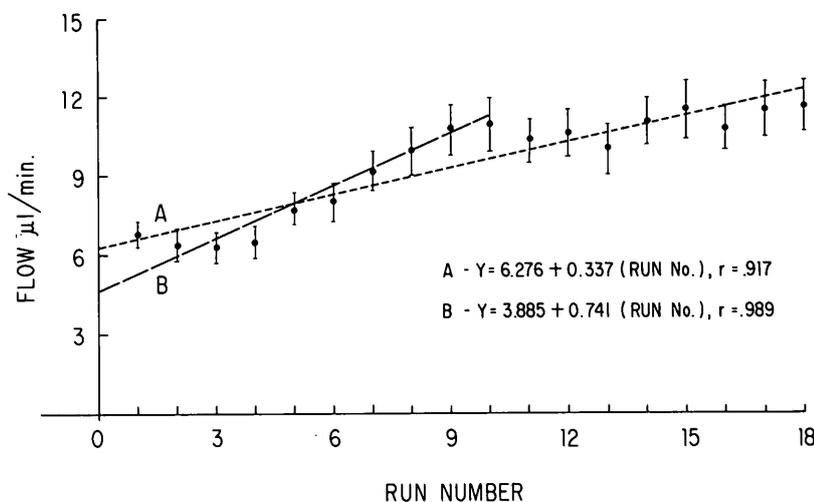


Fig. 3. Regression plot (A) of the mean flows at runs 1 to 18. Using data from pentobarbital-anesthetized animals, an across-sample compilation was made of all first observations, etc., to all eighteenth observations regardless of pressure ($N = 41$ at each point). Line A has a statistically significant linear fit. Line B, a plot of runs 2 to 10, shows that a portion of the curve has an even tighter fit and that a use of this part of the whole curve only would lead to an error in interpretation of the run-flow effect over time.

the inverse, or resistance, of that value, and Δ -facility (Δc) obtained by a comparison of the mean flows at each pressure level divided by the 5 mm. step increment were analyzed with analysis of variance (ANOVA), regression, Pearson correlation coefficient, and t tests. Predetermined significance levels of $p > 0.001$ were set for the first three tests and $p > 0.005$ was used with the t tests. All numerical data will be presented as mean flow values plus or minus the standard error.

Results. Animal age expressed as weight, anesthetic dose of sodium pentobarbital, eye as OS or OD, order in which eye is done, and date as three 1.5 month periods showed no significant effect upon flow, facility, or resistance measurements. The grand mean flows per eye were $9.143 \pm 0.259 \mu\text{l}/\text{min.}$ and $9.107 \pm 0.265 \mu\text{l}/\text{min.}$ for the right and left eyes respectively.

Infusion pressure (Fig. 2), type of anesthetic (Fig. 2), and run number (Fig. 3) all had a significant effect upon flow measurements, with 46 per cent of the flow variation assignable to these three factors. An ANOVA analysis of pressure versus flow indicated that the mean flow values ranged from $2.347 \pm 0.221 \mu\text{l}/\text{min.}$ at an infusion pressure of 15 mm. Hg to $14.555 \pm 0.671 \mu\text{l}/\text{min.}$ at 40 mm. Hg and that the flows generated by the six pressures were significantly different ($F = 61$, D.F. = 5,827) from one another and fell on a linear curve (Line B, Fig. 2). This linear relationship indicates that there is a constant Δc value ($0.490 \mu\text{l}/\text{min.}/\text{mm. Hg}$) over

the pressure range studied. Also evident in a consideration of lines A and C in Fig. 2 is a noticeable increase in the standard error of the mean with increasing infusion pressure.

Anesthetic effect, another factor introducing a significant amount of variation into the mean flow data, provided two discrete sets of flow means at each infusion pressure. The sodium pentobarbital data (Fig. 2, line A) fell in a linear sequence defined by $Y = -5.25 + 0.539 P_i$ and had a range of $2.476 \pm 0.261 \mu\text{l}/\text{min.}$ at 15 mm. Hg to $15.982 \pm 0.650 \mu\text{l}/\text{min.}$ at 40 mm. Hg with an overall mean of $9.711 \pm 0.261 \mu\text{l}/\text{min.}$ Phencyclidine HCl plus sodium pentobarbital yielded flow means of a linear sequence ($y = -1.24 + 0.214 P_i$) that was significantly different ($F = 151$, $r = 0.986$, D.F. = 1,773) from that of pentobarbital alone. The grand flow mean when the two drugs were used together was $4.612 \pm 0.172 \mu\text{l}/\text{min.}$ with a range from $1.667 \pm 0.199 \mu\text{l}/\text{min.}$ at 15 mm. Hg to $7.029 \pm 0.914 \mu\text{l}/\text{min.}$ at 40 mm. Hg. Thus, in these monkey eyes, cannulated for the first time, there was less than half as much flow per infusion sequence with phencyclidine than without it.

The third important factor, position in the sequence of eighteen runs, also showed a significant ($F = 83$, $r = 0.917$, D.F. = 17,737) positive correlation with increasing flow (Fig. 3). Even though the data do fit a linear regression, some parts of the curve showed different trends. This is especially apparent when the means at runs 2 through 10 are considered separately.

The regression line $Y = 3.885 + 0.741$ (Run No.) covering this limited part of the data has a better fit ($r = 0.989$) than that of all mean values. After run 9 no mean flow value differs from the mean flow at 9 by more than ± 1 standard error unit.

Discussion. The randomized constant-pressure infusion technique generates discrete observations upon factors affecting measured inflow. The advantages of using this procedure in combination with a wide pressure range are three-fold: (1) the data are more amenable to statistical analysis than are observations derived from the two-level technique because statistical tests are based on the premise of a random data base; (2) the technique can isolate several factors affecting flow without having the experiment specifically designed for that one factor in the study of a given population; and (3) the technique minimizes the potential for experimental error by stressing the infused eye over such a wide range of pressures and for a sufficiently long time to ensure that observations are coherent and are not the product of a changing function in the scope of the measured inflow-pressure curve. Our data show that this curve is linear over the range from 15 to 40 mm. Hg, but that the inflow-pressure curves for pentobarbital alone and phen-cyclidine plus pentobarbital are significantly different from each other. As suggested by Bárány,² the length of time that the eye has been infused with fluid also has a significant effect on flow. Investigations on the fluid dynamics of the in vivo eye have been concentrated on the collection of Δc values obtained at moderate infusion pressures and the association of morphological configurations of the meshwork and canal with fluid infusion at different pressures.⁷ Neither the in vivo nor the in vitro studies have provided a statistical analysis of measured inflow results, so it is not possible to compare those data to ours. However, the results presented here indicate that flow does not decrease with increasing pressure and thus that the anatomical changes caused by different infusion pressures may not be quantitative expressions of flow rates.

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Present address: *University of California at San Diego School of Medicine and Veterans Administration Hospital, San Diego, and **Department of Mathematics, Marquette University, Milwaukee, Wisc. Presented in part at 1974 ARVO Meeting, Sarasota, Fla. Supported by the Gainesville, Fla., Veterans Administration Hospital. Submitted for publication June 30, 1976. Reprint requests: M. Gary Wickham, Ph.D., Veterans Administration Hospital, 3350 La Jolla Village Drive, San Diego, Calif. 92161.

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Immunofluorescent studies on the trabecular meshwork in open-angle glaucoma.

M. BRUCE SHIELDS, RALPH C. MCCOY,
AND JOHN D. SHELBURNE.

The trabecular meshwork of eyes with open-angle glaucoma has been demonstrated to have an increase in gamma globulin and plasma cells, raising the question of an immunogenic mechanism in this disorder. In the present study, however, immunofluorescence assays on the trabecular meshwork of eyes with open-angle glaucoma were negative for specific immunoglobulins and for complement components that would result specifically from an antigen-antibody reaction. The study fails to provide any evidence in support of an immunogenic mechanism in open-angle glaucoma.

Over a decade ago, Becker and co-workers raised the question of an immunogenic mechanism in open-angle glaucoma by demonstrating an increase in gamma globulin and plasma cells in the trabecular meshwork of eyes with this disorder.^{1, 2} Despite growing evidence for immunogenic mechanisms in many ocular disorders, additional evi-