In conclusion, we confirm the stability with age of the SCE as a general rule but add some second thoughts because of two exceptions. Although, the SCE evidently shows remarkable recovering properties after retinal disease, it may not always be resistant against the ravages of age. Further follow-up of the same group of subjects during the next decades of life may yield more insight into possible senile deterioration of the receptor architecture.

**Key Words**
age, fovea, photoreceptor and optical properties, Stiles-Crawford effect

**References**


**Timolol Decreases Aqueous Humor Flow But Not Na⁺ Movement From Plasma to Aqueous**

**Thomas H. Maren, David R. Godman, Bruno M. Pancorbo, and Betty P. Vogh**

**Purpose.** To determine whether the well-known effect of timolol in reducing ocular pressure and aqueous humor (AH) flow is a function of reduced Na⁺ movement from plasma to aqueous. Previously, the authors have shown this to be the case for carbonic anhydrase inhibitors.

**Methods.** The rate of appearance of $^{22}$Na in rabbit posterior aqueous was measured 1 to 3 minutes after the intravenous injection (time $T$) of the isotope. One hour before this, the animals received one of the following: two drops of 0.5% timolol, two drops of 3.5% pilocarpine, or 25 mg/kg intravenous methazolamide. At 1 minute ($T + 1$), a posterior chamber sample was taken; 2 minutes later ($T + 3$) a second sample was removed from the fellow eye. The rate constant of sodium accession is simply the difference between the two counts/2 minutes. Aqueous flow was measured by dilution of sulfacetamide marker as described previously.

**Results.** The rate constant ($k_{in}$) for sodium entering the posterior chamber was $0.036 \pm 0.004$ minute$^{-1}$ ($n = 17$). Corresponding to previous findings, methazolamide (25 mg/kg intravenous) reduced this to $0.023 \pm 0.005$ minute$^{-1}$ ($n = 14$). Conversely, timolol (two drops of 0.5% solution) had no effect on $k_{in}$, which measured $0.037 \pm 0.004$ minute$^{-1}$ ($n = 12$). Similarly, as expected, pilocarpine had no effect on $k_{in}$ (0.035 ± 0.003 minute$^{-1}$). Control flow was 3.9 $\mu l$/minute ± 0.4; after timolol, 2.5 $\mu l$/minute ± 0.1; after methazolamide, 2.4 $\mu l$/minute ± 0.2; after pilocarpine, 3.6 $\mu l$/minute ± 0.2. These are converted to rate constants by dividing by volume of posterior aqueous (60 $\mu l$). The
control rate constant for fluid entry was 0.065 minute\(^{-1}\), 1.8-fold higher than for sodium.

Conclusions. A central dogma of the formation of AH (and cerebrospinal fluid) is that fluid moves isotonically from plasma to AH or cerebrospinal fluid and, therefore, that rate constants \(k_m\) for fluid and for sodium are approximately the same. In the authors’ hands, the fluid constant was modestly higher than for sodium. This holds for normal function and also for the reduced \(k_m\) for fluid and sodium after carbonic anhydrase inhibition. The \(k_m\) for neither flow nor sodium was affected by pilocarpine. Surprisingly, however, timolol, which reduces flow, had no effect on Na\(^+\) entry. Invest Ophthalmol Vis Sci. 1997;38:1274–1277.

The passage of Na\(^+\) into the aqueous humor (AH) has been a curiously neglected subject. The literature until 1955 was reviewed in the context of sodium as a marker for measurement of fluid formation in aqueous and cerebrospinal fluid.\(^1\) A few years later, with the advent of the systemic carbonic anhydrase inhibitors, the passage of radioactive sodium from plasma to aqueous was studied in the rabbit. This work\(^2-5\) is reviewed,\(^5\) showing (surprisingly) no effect of acetazolamide on Na\(^+\) transport, even though the known was known to affect rate of formation of aqueous. This posed a dilemma, until analyses of the data\(^5\) showed either that samples were not taken from the posterior chamber or the time of sampling (15 to 60 minutes) was too long (close to equilibrium) to measure initial rates.

Sampling from the posterior chamber at 1 to 5 minutes after intravenous injection of \(^{22}\)NaCl into dogs yielded a rate constant \(k_m\) of 0.044 minute\(^{-1}\), close to the 0.042 minute\(^{-1}\) approximated for fluid formation.\(^5\) The \(^{22}\)Na\(^+\) was reduced to 0.031 by systemic acetazolamide. There is an equivalent value of 0.017 minute\(^{-1}\) in the monkey, reduced to 0.009 by acetazolamide.\(^5\)

In neither of these nor in any study, however, is there a critical experimental comparison of the sodium and flow rate constants. This is particularly surprising because it is generally held that the \(\beta\)-blockers and carbonic anhydrase inhibitors reduce AH secretion. Accordingly, we compared, in rabbit, timolol with methazolamide, adding pilocarpine to exemplify a drug that does not reduce aqueous flow.

MATERIALS AND METHODS. Male New Zealand White rabbits weighing 2.2 to 3 kg were used. The experimental procedures conform to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Research was conducted in facilities accredited by the American Association for Accreditation of Laboratory Animal Care.

An intramuscular injection of 0.7 ml of a solution containing 1.5% xylazine and 10% ketamine–hydrochloride was given. One intravenous and one intraarterial catheter were placed in the femoral vein and artery for injection and sampling, respectively. After this, pentobarbital (30 mg/ml) was given intravenously as needed. One of the following drugs was given: two drops 0.5% timolol in each eye, two drops 3.5% pilocarpine in each eye, or 25 mg/kg methazolamide intravenously. Approximately 50 minutes after drug dosage, 0.2 ml of 10,000 U/ml sodium heparin was given intravenously to prevent blood clotting at sampling time. With the rabbit lying on its side, eye facing upward, the eyelids were fastened open by the weight of small hemostats attached to nearby fur. One drop of 0.5% proparacaine (Alcaine; Alcon, Fort Worth, TX) was placed on the cornea and left for 3 minutes, then carefully absorbed onto a cotton swab with no pressure on the cornea. One hour after drug dosing, 5 \(\mu\)Ci of \(^{22}\)NaCl was given intravenously (\(T = \) time 0). It had been determined that this was well mixed in 1 minute. Two operators worked together to ensure accurate timing. For posterior aqueous collection, a device was made from the shaft of a 23-gauge needle connected to a 20-cm length of PE-50 tubing. Experimenter 1 grasped the rectus muscle with fine forceps while the tip of the needle was placed perpendicular to the surface of the eyeball 2 to 3 mm below the limbus, near the muscle. At \(T + 50\) seconds, the needle was rotated gently to initiate entry. After \(~2\) mm of the needle had penetrated, its angle was changed to allow an approach parallel to and just under the iris. Pulling the needle back slightly allowed posterior aqueous to flow into the tubing at \(T + 70\) seconds. Either 20 \(\mu\)l of fluid or 30 seconds were allowed, then the needle was withdrawn. Right after the hemostats were removed and the rabbit turned to the other side, the process was repeated for the fellow eye at \(T + 3\) minutes. The actual times of sampling were recorded and the latter plotted on a log scale to normalize the values to 1 and 3 minutes.

One milliliter samples of blood were collected from the arterial catheter at \(T + 1, +3, +5, +10\) minutes by experimenter 2. Collected blood was spun for 10 minutes, and 100 \(\mu\)l of plasma was removed from each sample for isotope counting. The AH samples were discharged into preweighed scintillation vials.

Calculations of rate constants for sodium were as follows. Counts per minute per milliliter of plasma at \(T + 1\) were standardized at 100. \(T + 3\) on this scale was 64 to 70, mean 67, for all experiments. The average plasma concentration for the 1- to 3-minute period was taken as \((100 + 67)/2 = 84\). In control animals, the aqueous at \(T + 1\) minute was 11, and at \(T + 3\) was 17. The \(k_m\) then could be calculated for the 2-minute period between \(T1\) and \(T3\) as

\[
\frac{3 \text{ min aq} - 1 \text{ min aq}}{\text{mean plasma concentration between 1–3 min}} = \frac{17-11}{84}/2 \text{ min} = 0.036 \text{ min}^{-1}
\]

This is described in more detail elsewhere.\(^5\)
A recent careful work yields 3.5 \text{ ml/minute}. West Point, PA) and Pilocarpine (Pilocar; Iolab, Claremont, CA) were used from the standard pharmaceutical preparations.

### RESULTS.

Table 1 lists the effects of the three drugs on sodium access (columns 2, 3, 4A, and 5) and flow of AH (column 4). In terms of rate constants, compare column 2 with column 4. Each drug has a different pattern. Methazolamide reduces both processes, timolol only the flow, and pilocarpine neither. The methazolamide result duplicated what was found in the dog\(^8\) and surely fits the large literature showing no effect on sodium, and none is observed.\(^7\)

Methazolamide was obtained from American Cyanamid Company (Pearl River, NY); Timolol (Merck, West Point, PA) and Pilocarpine (Pilocar; Iolab, Claremont, CA) were used from the standard pharmaceutical preparations.

### DISCUSSION.

Our main purpose was to find whether timolol showed the same effects, linking sodium movement and flow of aqueous, that we have found for the carbonic anhydrase inhibitors and that support the "central dogma" relating these two processes.\(^9\) This was not found; there was a flow reduction but no sodium effect. This implies that the concentration of newly transported sodium into the aqueous would rise after timolol compared with that of control animals and with that of carbonic anhydrase inhibitors. This was the case (Table 1, column 6). However, these data show anomalously low calculated Na\(^+\) concentrations in aqueous for control animals and methazolamide quite different from those for dog or monkey,\(^5\) in which the calculated Na\(^+\) concentration in aqueous was the same as that in plasma. This only can be resolved by further experiments.

There seems no doubt about the effect of timolol in reducing flow,\(^8\) but the dissociation between flow and sodium movement is not explained readily. It appears as if aqueous channels, perhaps in the ciliary muscle, are affected, without any effect on the primary (electrolyte) secretion.\(^8\) The patterns observed differ from that following the prostaglandins, which reduce ocular pressure with no effect on flow.\(^10,11\)

Our results with pilocarpine, showing no effect on flow, agree with earlier studies in humans.\(^12\) Sodium accession, also showing no change, had not been studied previously.

In conclusion, the carbonic anhydrase inhibitors appear to be the only class of drugs studied thus far that lowers sodium transport from plasma to AH. Table 2 summarizes the effects of "antiglaucoma" drugs on sodium and flow movement in aqueous.

### Key Words

aqueous humor, carbonic anhydrase, methazolamide, pilocarpine, sodium movement, timolol

* Dr. Anders Bill suggests that the larger value for flow over sodium may be caused by diffusion of the ion into the vitreous. With timolol, diffusion of Na\(^+\) between stroma and posterior chamber may be increased by dilation of intercellular spaces or tight junctions, whereas net fluid movement is decreased.
Local Inhibition of Natural Killer Cell Activity Promotes the Progressive Growth of Intraocular Tumors

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Purpose. To study the effect of aqueous humor (AH)-mediated inhibition of natural killer (NK) cell activity on intraocular tumor progression.

Methods. Two NK-sensitive tumors, RMA-S lymphoma and OCM-3 uveal melanoma, were tested in vitro for susceptibility to NK cell-mediated lysis in the presence or absence of AH in conventional cytotoxicity assays. Various numbers of RMA-S and OCM-3 tumor cells were injected either subcutaneously or intracamerally into C57BL/6 severe combined immunodeficiency mice and BALB/c nude mice respectively and tumor growth was monitored. The role of NK cell-mediated cytotoxicity in controlling tumor growth was confirmed by depleting NK cells in severe combined immunodeficiency mice by administering antiasialo GM1 antibodies before subcutaneous tumor injection.

Results. AH significantly inhibited NK cell-mediated lysis of RMA-S and OCM-3 tumor cells in vitro and in vivo. NK sensitive RMA-S (1 × 10⁶ cells) and OCM-3 tumors (1 × 10⁶, 5 × 10⁶ cells) were rejected after subcutaneous injection in C57BL/6 mice, whereas the same or even lower numbers of cells grew progressively in the eye. In vivo NK cell depletion resulted in progressive growth of subcutaneously injected RMA-S tumors at a dose rejected by mice with normal NK cell activity.


The intraocular microenvironment is an immunologically privileged site in which tissue and tumor allografts escape immunologic rejection and destruction. The intraocular milieu is endowed with a multitude