Influence of intraocular pressure and some drugs on aqueous flow and entry of cycloleucine into the aqueous humor of vervet monkeys (Cercopithecus ethiops)

Per-Erik Wålinder and Anders Bill

The entry of \(^{3}H\)-cycloleucine from blood into the anterior chamber and the rate of aqueous humor formation were studied in vervet monkeys. \(^{3}H\)-cycloleucine was given parenterally. The anterior chamber was perfused with a buffer solution containing \(^{131}I\)-albumin or \(^{14}C\)-inulin. The rate of aqueous humor formation was calculated from the dilution. The rate of entry of \(^{3}H\)-cycloleucine was estimated from the concentration in the perfusate. A rise in intraocular pressure of 10 and 20 cm. of water reduced the concentration of cycloleucine in the anterior chamber perfusate by 25 and 32 per cent, respectively; the corresponding effect on the rate of aqueous humor formation was an average reduction by 0.05 and 0.04 \(\mu\)L per minute per millimeter of Hg, respectively. Acetazolamide 10 and 100 mg. per kilogram of body weight intravenously reduced the concentration of \(^{3}H\)-cycloleucine in the perfusate by 10 and 26 per cent, respectively, and the rate of formation by 30 and 80 per cent, respectively. Pilocarpine (10\(^{-4}\)M) in the infusion fluid reduced the concentration of \(^{3}H\)-cycloleucine in the perfusate by 21 per cent and the rate of formation on an average by 1.1 \(\mu\)L per minute. These effects were abolished by atropine (3 \(\times\) 10\(^{-4}\)M). Norepinephrine (6 \(\times\) 10\(^{-4}\)M) reduced the concentration of cycloleucine in the perfusate by 29 per cent but tended to stimulate the rate of aqueous humor formation. Norepinephrine (3 \(\times\) 10\(^{-4}\)M) had similar effects. A rise in intraocular pressure and acetazolamide 100 mg. per kilogram had similar effects on the rate of entry of \(^{14}C\)-AIB and \(^{3}H\)-cycloleucine. It was concluded that the reduced rate of entry of \(^{3}H\)-cycloleucine, observed after a rise in intraocular pressure, acetazolamide, and pilocarpine, was likely to be due to a reduced rate of aqueous humor flow from the posterior into the anterior chamber, whereas the effect of norepinephrine was due to reduced diffusion of \(^{3}H\)-cycloleucine mainly across the anterior surface of the iris.

Key words: aqueous humor formation, aqueous humor inflow, aqueous humor outflow, intraocular pressure increase, cycloleucine, pharmacodynamics, acetazolamide, atropine, pilocarpine, norepinephrine, perfusion, monkeys.

From the Department of Experimental Ophthalmology at the Wallenberg Laboratory and the Department of Pharmacology, University of Uppsala, Uppsala, Sweden.

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The lens, part of the cornea, and the trabecular meshwork receive their requirements of amino acids from the aqueous humor. Deficiency of essential amino acids in the diet causes cataract and corneal vascularization. It can be expected that, if for some other reason, the movement of amino acids from plasma into the aqueous humor is reduced, this may also have harmful effects on the intraocular structures mentioned.

In a previous investigation, a method was described for the simultaneous study of the rate of aqueous humor formation and the passage of amino acids from blood into the anterior chamber. In the present study, it is shown that increased intraocular pressure and some drugs reduce both the rate of aqueous humor formation and the passage of a nonmetabolizable amino acid, cycloleucine, into the anterior chamber.

Methods

The method used was described and discussed in a previous paper. The results to be described here were obtained in the same monkeys.

Intraocular pressure. This pressure was changed by altering the level of the outlet. The change in pressure was read on the electromanometer.

Administration of drugs. Acetazolamide and a control substance Cl 13850 (N-t-butyl acetazolamide), which is closely related to acetazolamide but has negligible carbonic anhydrase inhibitory activity, were injected intravenously. Acetazolamide was given as a 10 per cent solution with a pH of about 10.8; Cl 13850 was given as a 1 per cent solution with a pH of about 9.0; Cl 13850 was given as a 10 per cent solution with a pH of about 9.0; Cl 13850 was given as a 5 per cent solution with a pH of about 10.8.

Pilocarpine, atropine, and norepinephrine were added to the infusion fluid.

The following drugs were used: sodium acetazolamide (Diamox) and Cl 13850 (both from American Cyanamid Company), norepinephrine (Norexadrin conc., Astra, Södertälje, Sweden), pilocarpine hydrochloride, and atropine sulfate. The norepinephrine solution contained per milliliter: 1 mg. norepinephrine, 1 mg. tartaric acid, 1 mg. sodium pyrosulfite, 1 mg. methyl p-hydroxybenzoate, 7 mg. sodium chloride, and distilled water to make 1 ml.

Different types of experiments. (A) In 14 monkeys the passage of 14C-AIB from blood into the anterior chamber was studied; in 12 of them the rate of aqueous humor formation was calculated simultaneously from 14C-inulin data, which were obtained by means of small amounts of radioactivity. The influence of the following experimental changes were studied: (1) intraocular pressure raised 10 or 20 cm. of water, (2) acetazolamide and Cl 13850 given intravenously, (3) pilocarpine followed by atropine, both added to the infusion fluid, and (4) norepinephrine added to the infusion fluid. (B) In 2 monkeys the simultaneous passage of 1H-cycloleucine and 14C-AIB from blood into the anterior chamber was studied. The influence of intraocular pressure raised 20 or 40 cm. of water and that of acetazolamide was studied. (C) In 5 monkeys the rate of aqueous humor formation was calculated, with large amounts of 14C-inulin in one eye and large amounts of 14C-inulin in the other. The influence of acetazolamide was studied.

Each eye was usually exposed to more than one experimental change and, thus, was used for several test periods: e.g., first, the intraocular pressure was increased once or twice in each eye; then norepinephrine or pilocarpine (followed by atropine) was added to the infusion fluid of one eye. The first test period usually began 2 to 3 hours after the infusion was started. The above changes were induced in one eye at a time, the other eye being used as a control.

In experiments with acetazolamide, 10 mg. per kilogram of body weight was first tested, then 100 mg. per kilogram was given at least 2 hours later.

To ensure that an approximate steady level was maintained before an experimental change was induced, small "test samples" of the perfusate were assayed during the experiment. These samples were not treated with Hyamine.

At a rate of infusion of 11 and 22 µL per minute, 100 and 200 µL samples, respectively, of the effluent were taken, and this gave about 3 samples every half hour during the perfusion. However, when the intraocular pressure was raised, only 1 to 2 samples per half hour were obtained since more fluid then left the eye via its conventional outflow pathways. Sometimes smaller samples were taken during the pressure rise.

Treatment of data on aqueous flow and concentration of cycloleucine. The mean value for the rate of aqueous humor formation and for the concentration of cycloleucine in the effluent during the one hour period preceding each test period were used as the pretest values. In experiments with large amounts of 14C-inulin or 131I-albumin, the rate of aqueous humor formation could be calculated with a small experimental error, and the values for the rate of aqueous humor formation were given as a percentage of the pretest value. The change in the concentration of cycloleucine was also given as a percentage of the pretest value. These data were plotted against time in a
In the experiments with small amounts of \(^{14}\)C-inulin, the values for aqueous flow had large random errors. The 2 to 3 values obtained during each half hour of a test period were therefore pooled. The changes are given as microliters per minute deviation from the pretest value. The data thus obtained for each half hour period in different animals were averaged. These means ± S.E.M. are shown in the figures.

**Influence of an increase in intraocular pressure.**

*Rate of aqueous humor formation.* An increase in the intraocular pressure by 10 and 20 cm. of water above the initial level reduced the rate of aqueous humor formation as calculated from \(^{14}\)C-inulin data (Fig. 1). The mean reduction during the period 30 to 60 minutes after the increase was 0.7 and 1.5 μL per minute, respectively, while during the 60 to 90 minute period it was 0.4 and 0.6 μL per minute. Only the reduction of 1.5 μL was significant. This value was, however, observed at a time when steady state may not have been attained.

When the pressure was lowered to the initial level, the calculated rate of formation returned to the previous value after a temporary overshoot. The overshoot was probably due to volume changes in the eye and not to a changing rate of formation.

*SH-cycloleucine.* An increase in the intraocular pressure by 10, 20, and 40 cm. of water above the initial level reduced the steady-state concentration of cycloleucine in the effluent by 25, 32, and 52 per cent, respectively. The values are the mean reductions during the period 60 to 90 minutes after the increase in pressure. The mean values in each group were significantly below the control values (p < 0.01).

When the pressure was lowered to the initial level, the concentration of cycloleucine returned to its previous starting value after a temporary overshoot.

*\(^{4}\)H-cycloleucine.* An increase in the intraocular pressure by 10, 20, and 40 cm. of water above the initial level reduced the steady-state concentration of cycloleucine in the effluent by 25, 32, and 52 per cent, respectively. The values are the mean reductions during the period 60 to 90 minutes after the increase in pressure. The mean values in each group were significantly below the control values (p < 0.01).

When the pressure was lowered to the initial level, the concentration of cycloleucine returned to its previous starting value after a temporary overshoot.

*\(^{14}\)C-AIB.* Fig. 2 shows that the influence of an increased intraocular pressure on the concentration of AIB in the effluent was
Fig. 1. Influence of the intraocular pressure on the rate of aqueous humor formation as calculated from $^{14}$C-inulin data and on the concentration of cycloleucine in the effluent. The pressure was raised 10 ( ), 20 ( ), and 40 ( ) cm. of water above the initial level at the first zero time and then lowered to the initial level at the second zero time. The change in rate of formation is expressed in µL per minute and the concentration of cycloleucine is in per cent of the mean value during one hour before the pressure rise. Values are M ± S.E.M.; 7/5 denotes 7 eyes of 5 monkeys and so on. The rate of infusion was 38 µL per minute in the experiments with a pressure rise of 40 cm.; otherwise it was 22 µL per minute.

similar to that of cycloleucine. A pressure rise of 20 cm. of water gave a mean decrease of 37 and 45 per cent (2 experiments), and a rise of 40 cm. gave a mean decrease of 52 per cent. Values are from the periods 60 to 105 and 60 to 90 minutes, respectively, after the pressure rise.

Influence of acetazolamide at constant intraocular pressure.

Aqueous humor formation. In each of these experiments, the intraocular pressure was maintained at a steady level, at an average of 10.9 mm. of Hg. The rate of aqueous humor formation, calculated from $^{14}$C-inulin data in experiments with small amounts of $^{14}$C-inulin, was not significantly influenced by acetazolamide (10 mg. per kilogram of body weight), whereas 100 mg. per kilogram given after 4 to 5 hours of perfusion reduced the flow by 1.5 µL per minute during the period 30 to 60 minutes after the injection (Fig. 3). The reduction was significant ($p < 0.01$). Expressed as a percentage of the pretest value, 2.61 µL per minute, the reduction was 57 per cent. In 5 eyes of 3 monkeys, the effect of 100 mg. per kilogram was studied for 150 minutes; the rate of formation was reduced by 2.1 ± 0.5 and 0.8 ± 0.8 µL per minute (M ± S.E.M.) at the period 30 to 60 and 120 to 150 minutes, respectively, after the drug was given. The effect of 100 mg. per kilogram was about the same, whether given after 4 to 5 hours of perfusion or after 10 to 14 hours of perfusion.

When the rate of aqueous formation was calculated from data where high concen-
The pressure was increased at time zero by 20 cm. of water in the 2 eyes of one monkey (-). The rate of infusion was 22 μL per minute. Individual values are shown as filled and unfilled circles. In 4 eyes of 2 monkeys (----) the pressure rise was 40 cm. of water. The rate of infusion was 38 μL per minute. Values are M ± S.E.M. The intraocular pressures were lowered to the initial levels at 105 (△) and 90 (▽) minutes, respectively. The changes are expressed as in Fig. 1.

The mean reduction in aqueous formation in the same experiments 40 to 60 minutes after acetazolamide 100 mg. per kilogram was about 75 per cent when calculated from 131I-albumin data, and about 80 per cent in the 2 experiments with 14C-inulin.

14C-AIB. In 2 eyes of one monkey, acetazolamide 100 mg. per kilogram reduced the concentration of AIB in the effluent by 20 and 31 per cent 60 minutes after the injection.

3H-cycloleucine. Thirty minutes after the injection of acetazolamide 10 mg. per kilogram, the concentration of cycloleucine in the effluent (Fig. 3) was reduced by an average of 10 per cent. The reduction was significant (p < 0.01). The effect lasted about one hour.

The average reduction in concentration of cycloleucine caused by 100 mg. per kilogram acetazolamide was 26 per cent during the period 30 to 60 minutes after the injection. The reduction was significant in all groups from 20 to 120 minutes (p < 0.01). In the series of 5 eyes studied for 150 minutes, as mentioned, the concentration of cycloleucine was reduced by 35 ± 5 and 32 ± 7 per cent (M ± S.E.M.) at 45 and 150 minutes, respectively, after the drug was given. The effect of 100 mg. per kilogram was the same whether given after 4 to 5 hours of perfusion or after 10 to 14 hours of perfusion (Fig. 3).

35Cl 13850. Cl 13850 at 100 mg. per kilogram had no obvious effect in 4 eyes of 2 monkeys; the rate of aqueous humor formation increased by 0.3 ± 0.3 μL per minute (14C-inulin data), and the concentra-
tration of cycloleucine in the effluent increased by 4 ± 8 per cent; the values are the means ± S.E.M. of 4 eyes in 2 monkeys during a period 30 to 90 minutes after the injection. Acetazolamide 100 mg. per kilogram injected 2 hours after CI 13850 had about the same effect as in previous experiments; the rate of aqueous humor formation was reduced by 1.4 ± 0.2 μL per minute, and the concentration of cycloleucine was reduced by 23 ± 4 per cent during the same period.

**Influence of pilocarpine and atropine at constant intraocular pressure.** In these experiments the average intraocular pressure was kept at 12.1 mm. of Hg. Pilocarpine (Fig. 5) at a concentration of 10^{-4}M (20 μg per milliliter) in the infusion fluid reduced the rate of aqueous humor formation by 1.1 μL per minute, as calculated from 14C-inulin data (average from 30 to 90 minutes after the drug had reached the eye). The concentration of cycloleucine in the effluent fell by an average 21 per cent during the same period. The decrease in 3H-cycloleucine was significant (p < 0.01) in the groups at 20, 30, 45, 60, and 105 minutes. The reduction in the rate of aqueous formation, calculated from the 14C-inulin data in the group at 45 minutes, was almost significant (p < 0.05).

Intense miosis appeared in just a few minutes after pilocarpine had reached the eye.

When atropine, 3 x 10^{-5}M (10 μg per milliliter), was infused after pilocarpine, it rapidly abolished the effects of pilocarpine. Both the rate of aqueous humor formation calculated from 14C-inulin data and the concentration of cycloleucine returned to the levels before administration of pilocarpine. The size of the pupil increased gradually, and after 20 to 30 minutes the diameter was 3 to 4 mm. In the control eyes it was 2 to 3 mm.

No general effects, such as changes in blood pressure or salivation, were observed during infusion of the drugs.

**Influence of norepinephrine at a constant intraocular pressure.** In these experi-
Fig. 5. The influence of pilocarpine $10^{-4}$ M followed by atropine $3 \times 10^{-5}$ M on the rate of aqueous humor formation as calculated from $^{14}$C-inulin data and on the concentration of cycloleucine in the effluent. The drugs were added to the infusion fluid. Pilocarpine reached the eye at the first zero time and was followed by atropine 105 to 150 minutes later at the second zero time. The changes are expressed as in Fig. 1. Values are $M \pm S.E.M.$, 8 eyes in 8 monkeys received pilocarpine, 7 eyes also received atropine. The rate of infusion was 22 $\mu$L per minute.

Fig. 6. Influence of norepinephrine $3 \times 10^{-4}$ M on the rate of aqueous humor formation as calculated from $^{14}$C-inulin data and on the concentration of cycloleucine in the effluent. The drug was added to the infusion fluid and reached the eye at zero time. The changes and values are expressed as in Fig. 1. Five eyes in 5 monkeys were studied. The rate of infusion was 11 $\mu$L per minute. The pH of the infusion fluid was 5.8.

The simultaneous effect on the rate of aqueous humor formation, calculated from data obtained with small amounts of $^{14}$C-inulin, was an increase of 0.6 $\mu$L per minute. The increase was not, however, significant.

In the foregoing experiments, the pH was 5.8 in the infusion fluid; in the following ones, the pH was 7.0.

Norepinephrine $6 \times 10^{-5}$M (10 $\mu$g per milliliter) reduced the concentration of cycloleucine in the effluent by 17 per cent (Fig. 7) and increased the rate of aqueous humor formation by 0.4 $\mu$L per minute,
Fig. 7. Influence of norepinephrine and acetazolamide on the rate of aqueous humor formation and on the concentration of cycloleucine in the effluent. Norepinephrine (10 μg per milliliter) in the infusion fluid reached the eye at zero time; acetazolamide (100 mg per kilogram) was given intravenously at 120 minutes and an increased concentration of norepinephrine (50 μg per milliliter) reached the eye at 220 minutes. The changes are expressed as in Fig. 1. Six eyes in 3 monkeys were studied. The rate of infusion was 11 μL per minute. The pH of the infusion fluid was 7.0.

during the period 60 to 105 minutes after the drug had reached the eye. The effect on the concentration of cycloleucine was significant (p < 0.01) in the groups at 50 and 120 minutes. The increase in rate of formation was not significant. It is to be noted that, in these experiments, the values at 10 and 20 minutes were 112 and 111 percent of the mean value one hour before the drug had reached the eye. The drug was given after 2 hours of perfusion, and at that time the rate of aqueous humor formation and the concentration of cycloleucine in the effluent had been rising slowly for some time.

The true effect of acetazolamide (100 mg per kilogram) given during the infusion of norepinephrine (10 μg per milliliter) was difficult to estimate, since the rate of formation and the concentration of cycloleucine had not attained a steady level even 100 minutes after acetazolamide was given, when norepinephrine (50 μg per milliliter) started to reach the eye (at 220 minutes, Fig. 7). However, it is probable that the value of the rate of formation at 225 minutes and the value of the concentration of cycloleucine at 240 minutes were not influenced by the higher dose of norepinephrine. If these values are used to estimate the effect of acetazolamide, the rate of formation was reduced from +0.4 (under norepinephrine, 10 μg per milliliter) to −1.4 μL per minute. These values are the deviations from the pretest value. The concentration of cycloleucine was reduced from 83 to 51 percent of the pretest value, which is a reduction of the same order of magnitude as when acetazolamide was given alone. There was little additional effect of norepinephrine 50 μg per milliliter.
Discussion

Increased intraocular pressure, as well as acetazolamide given intravenously and intracameral pilocarpine, reduced the rate of entry of cycloleucine from the blood into the anterior chamber. Increased pressure and these drugs also reduced or tended to reduce the rate of aqueous humor formation. Intracameral norepinephrine reduced the concentration of cycloleucine in the effluent but tended to raise the rate of aqueous humor formation.

Effects of changes in the intraocular pressure. A rise in intraocular pressure decreases the rate of aqueous humor formation. In facility determinations this decrease contributes a pseudofacility component. 4, 5 This has been estimated as 0.087 ± 0.034 (M ± S.E.M., n = 11) μL per minute per millimeter of Hg in the vervet. 5 The reduction seems to depend on the arterial blood pressure, and in both cynomolgus and vervet monkeys with a mean arterial pressure at 85 to 90 mm. of Hg it is about 0.06 μL per minute per millimeter of Hg. 7, 8 Calculations by Goldmann 9 and measurements by Kupfer and Brubacker 10 have given a value of 0.06 μL per minute per millimeter of Hg for human eyes. The very uncertain mean steady-state values in the present experiments were 0.05 and 0.04 μL per minute per millimeter of Hg for 10 and 20 cm. of water pressure rise, respectively, i.e., about the same as in the previous experiments with a higher degree of accuracy. In all previous experiments in man and monkey, it could not be determined whether the reduction in flow rate was due to a reduction in the rate of flow from the posterior chamber via the pupil or to changed ultrafiltration from or to the tissues surrounding the anterior chamber. The rate of aqueous formation discussed was a net rate equal to the rate of the total bulk outflow from the anterior chamber minus any rate of inflow from an external system. Experiments in cats, 11 however, indicate that the rate of flow via the pupil is reduced by a rise in pressure. In the experiments reported here, a rise in intraocular pressure produced a marked reduction in the entry of cycloleucine into the anterior chamber. This makes it possible to analyze somewhat further the way in which the net formation of aqueous was reduced. Use is made of a previously discussed equation:

\[ F \cdot C_h + G = (r_1 + F) \cdot C_a \]  

(1)

where G is the amount of cycloleucine that enters the anterior chamber via diffusion and ultrafiltration, probably mainly via the iris (G is expressed in micromoles per minute); C_p, C_a, and C_h are the concentrations of cycloleucine in plasma, anterior chamber fluid, and the posterior aqueous that enters via the pupil, respectively (concentrations expressed in moles per liter). F is here the rate of aqueous flow via the pupil, and r_1 is the rate of infusion in μL per minute.

It is assumed that G is mainly due to diffusion and is not influenced by the change in intraocular pressure, and it is further assumed that C_h is approximately equal to 1.19 × C_p (see previous report 8) and also independent of pressure. Then, the change in concentration of cycloleucine in the effluent caused by increased pressure can be due only to decreased F. With the values given in Table I, the reduction amounts to 0.10 μL per minute per millimeter of Hg for a rise of 10 cm. of water, and 0.07 μL per minute per millimeter of Hg for a rise of 20 cm.

Reasonable agreement is present between these values for the reduction in flow via the pupil and the present and previous ones for the reduction in the net rate of aqueous humor formation. This suggests that the reason for the reduction in the net formation of aqueous is either a decrease in the rate of secretion—defined as the net fluid movement from the ciliary processes into the posterior chamber—or a change in the exchange of fluid between the posterior chamber and the vitreous.

For a discussion of pressure-sensitive secretion from the ciliary processes, see Bill. 12

The G/C_p values shown in Table I were calculated from equation 1 under the as-
Influences on aqueous flow and cycloleucine

Table I. Influence of a rise in intraocular pressure and of drugs on the rate of aqueous humor formation and on the concentration of cycloleucine in the effluent

<table>
<thead>
<tr>
<th>Experimental change</th>
<th>Rate of aqueous humor formation ( \mu L/\text{minute} )</th>
<th>Concentration of (^{3}H)-cycloleucine in the effluent, per cent of that in plasma</th>
<th>No. of eyes/monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Test</td>
<td>Control Test</td>
<td>Control Test</td>
<td></td>
</tr>
<tr>
<td>Intraocular pressure increased by 10 cm. water</td>
<td>1.28</td>
<td>13.3</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>1.84</td>
<td>15.2</td>
<td>10.3</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>10 mg/Kg.</td>
<td>2.20</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>100 mg/Kg.</td>
<td>2.61</td>
<td>15.8</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>10^{-4} M</td>
<td>2.16</td>
<td>15.6</td>
</tr>
<tr>
<td>Atropine</td>
<td>3 \cdot 10^{-5} M</td>
<td>2.19</td>
<td>15.9</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>6 \cdot 10^{-5} M</td>
<td>1.86</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>3 \cdot 10^{-4} M</td>
<td>2.68</td>
<td>27.2</td>
</tr>
</tbody>
</table>

Formation rate was calculated from \(^{14}C\)-inulin data obtained in the same eyes as the \(^{3}H\)-cycloleucine data. "Control" values are the average mean values during the one hour period before the experimental change. The "Test" values are the average mean values observed when the experimental change had given a new steady state (see Results). \(G/C_p\) values are calculated as described in the discussion of "effects of change in the intraocular pressure." The rate of infusion was 11 \( \mu L \) per minute in the experiments with norepinephrine, otherwise it was 22 \( \mu L \) per minute.

sumption that the true flow changes were those observed with \(^{14}C\)-inulin.

A rise in intraocular pressure of 20 or 40 cm. of water had similar effects on the concentration of AIB in the effluent as on that of cycloleucine. The effect of a 20 cm. rise on the concentration of cycloleucine in the effluent has been observed also in a rabbit experiment.\(^2\)

**Acetazolamide.** The effect of acetazolamide on the rate of aqueous humor formation was thoroughly reviewed by Maren.\(^13\) In the present experiments, 10 mg per kilogram of body weight was found to lower the net formation rate by about 30 per cent. This value is estimated from \(^{125}I\)-albumin and \(^{14}C\)-inulin data in experiments in which high amounts of radioactivity were used. In the experiments with low activities, no significant effect could be seen. One hundred milligrams per kilogram had a stronger and more prolonged effect on the rate of formation than 10 mg per kilogram.

It has been discussed as to whether acetazolamide reduces the rate of formation of aqueous humor by an effect on the secretion or for some other reason. Recently, it was suggested that the reduction of aqueous humor formation is due to vasoconstriction in the uvea.\(^14\) Vasoconstriction of the iris vessels should reduce the amount of cycloleucine that enters the anterior chamber by diffusion. The \(G\) values are highly uncertain (Table I) but show no consistently reduced rate of diffusion of cycloleucine across the anterior surface of the iris after acetazolamide. The \(G/C_p\) value fell at 10 but rose at 100 mg per kilogram.

Cl 13850 is a structural analogue of acetazolamide, but it has negligible carbonic anhydrase inhibitory effects. Since it had no obvious effect on the rate of aqueous formation or on the passage of cycloleucine into the anterior chamber, it is likely that the effects of acetazolamide obtained were due to carbonic anhydrase inhibition, and that they were not unspecific effects caused, e.g., by the fairly high
pH of the solutions given intravenously.

Previous reports have demonstrated that supramaximum doses of acetazolamide reduce the rate of aqueous humor formation by 50 to 60 per cent. In those of the present experiments in which the effect was determined with the highest degree of accuracy, 100 mg. per kilogram acetazolamide reduced the rate of formation by almost 80 per cent (Fig. 4). The tendency to a more pronounced effect in the present experiments may be explained by the fact that the intraocular pressure was kept at a constant level, whereas in previous estimates the intraocular pressure was allowed to fall, and a fall in pressure tends to increase the rate of formation.

The drop in the net rate of aqueous formation, as well as that in the rate of entry of cycloleucine into the anterior chamber, strongly suggest that the rate of aqueous secretion was reduced to about the same extent as the net rate of aqueous humor formation.

**Pilocarpine and atropine.** In a previous investigation in cynomolgus monkeys, pilocarpine was found to reduce the rate of aqueous humor formation and to raise the intraocular pressure. It could not be clarified whether the former effects were altogether due to the latter.

Edwards, Hallman, and Perkins recently reported an increased rate of aqueous humor formation after instillation of 4 per cent pilocarpine into the conjunctival sac. The rate of aqueous humor formation was defined as equal to the outflow into a reservoir, when the intraocular pressure was adjusted to the level of the recipient venous pressure. With this definition, a change in the uveoscleral flow is interpreted as a change in rate of formation. Pilocarpine reduces the rate of drainage by uveoscleral routes, and may therefore produce a rise in aqueous flow when this is defined as by Edwards and co-workers. In addition, pilocarpine tends to raise the recipient venous pressure. In the experiments of Edwards and associates, this may have caused some backflow into the anterior chamber from the intrascleral veins.

In the present experiments, pilocarpine (10^-4M) in the anterior chamber fluid was found to produce a probably significant reduction in the rate of aqueous humor formation at a constant intraocular pressure calculated from ^14C-inulin data.

The passage of cycloleucine into the anterior chamber was also reduced. It is probable that the decrease in passage of cycloleucine into the anterior chamber was due mainly to a reduced rate of aqueous flow from the posterior into the anterior chamber, that was secondary to a reduced rate of aqueous secretion in the ciliary processes. Pilocarpine has an effect on the ciliary processes has been shown by Berggren, who found that the drug inhibits the secretion of fluid by surviving rabbit ciliary processes. Pilocarpine also influences the accumulation of iodo-pyract and iodide by isolated ciliary processes. On the other hand, pilocarpine has little effect on the accumulation of AIB by isolated rabbit ciliary processes.

The finding that atropine abolished the effect of pilocarpine suggests but—because of the high dose used—does not prove that this effect of pilocarpine is a cholinergic one. Atropine in eyes without pilocarpine has no significant effect on the rate of aqueous humor formation, which suggests that, at least in anesthetized monkeys, there is practically no spontaneous cholinergic suppression of the rate of aqueous humor formation.

**Norepinephrine.** Recent experiments suggest that norepinephrine slightly stimulates the net rate of aqueous humor formation. Such an effect was also seen in the present experiments although the change was not significant. In addition, in the experiments reported here, the drug reduced the concentration of cycloleucine in the effluent: 10 µg per milliliter by 17 per cent and 50 µg per milliliter by 27 per cent, counted from the pretest period. In the series where 10 µg per milliliter was studied, the concentration of cycloleucine was rising during the pretest period, and a truer value of the reduction caused by norepinephrine is obtained if the reduc-
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The calculation of G gave values less than zero (Table I). A low G value suggests that the rate of diffusion of cycloleucine into the anterior chamber was reduced, probably as a result of vasoconstriction of the iris vessels. The reduced area of the iris may also have contributed to the low G value. Since a G value cannot be below zero, the present negative values may be due to the experimental error or to the assumptions made when calculating G not being entirely valid.

The effects of norepinephrine (10 μg per milliliter) and acetazolamide (100 mg per kilogram) on the entry of cycloleucine into the anterior chamber and on the rate of aqueous humor formation were roughly additive. Since both drugs were probably given in supramaximal doses, this suggests different points of attack. This is in good agreement with the hypothesis that acetazolamide reduced the rate of aqueous secretion, and norepinephrine reduced the rate of entry of cycloleucine via the iris due to vasoconstriction.

Concluding remarks. The nonmetabolizable amino acids, cycloleucine and AIB, are extensively used in the study of the movement of amino acids. They are known to be transported by the same mechanisms and to move in the same way as natural amino acids; in the eye it has been demonstrated that they compete with several natural amino acids for the mechanism of entry from blood to aqueous humor. Thus it is probable that the effects on the entry of cycloleucine and AIB into the anterior chamber found in the present experiments would also apply to the entry of natural amino acids.

An increase in the intraocular pressure reduced the entry of cycloleucine and AIB into the anterior chamber. This suggests that at least part of the concentration deficit of amino acids in the anterior aqueous humor in eyes with glaucoma is due to a reduced rate of entry.24

In the experiments with acetazolamide, higher doses were given than those used in man. Despite this, the experiments indicate that a dose which reduces the rate of aqueous humor formation also reduces the entry of amino acids.

Pilocarpine (10^-4M) in the anterior chamber fluid reduced the entry of cycloleucine. Unpublished observations in rabbits of the penetration of 3H-pilocarpine into the anterior aqueous humor showed that about this concentration is attained in the anterior aqueous 20 minutes after local instillation of a 2 per cent solution in the conjunctival sac. The secretion of aqueous humor into the posterior chamber is the net effect of several mechanisms. Active transport of any ion in any direction can be presumed to be accompanied by water flow. Very low concentrations of pilocarpine stimulate the accumulation of iodopyracet by the ciliary processes, probably by stimulating the mechanism which, in vivo, transports iodopyracet from the posterior chamber into the ciliary processes. The rate of net water flow from surviving rabbit ciliary processes is reduced by pilocarpine.17 It is then possible that the reduction in flow from ciliary processes into the posterior chamber that was indicated by the pilocarpine experiments reported here was, in fact, due to stimulation of a transport from the posterior chamber into the ciliary processes of an ion accompanied by water.

Although norepinephrine is not used in the treatment of glaucoma, the present findings indicate that other adrenergic drugs, e.g., epinephrine, may reduce the entry of amino acids by diffusion during the vasoconstrictive phase. However, this effect will probably be compensated for during the following vasodilative phase.

The present experiments thus show that both a high intraocular pressure and some drugs used in the treatment of glaucoma
reduce the rate of entry of amino acids into the anterior chamber. In glaucoma, however—when a drug reduces the intraocular pressure by increasing the facility of outflow—the pressure drop will tend to enhance the rate of aqueous humor formation, and thus the net effect of the drug may even be to increase the rate of entry of amino acids if the pressure drop is large enough.

The clinical significance of a reduction in the concentration of amino acids in the aqueous humor is not established. However, a general deficiency in amino acids causes cataract and corneal vascularization in animals, and it seems possible that a low concentration of amino acids in the aqueous humor might damage ocular structures also in the human eye.

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