The mode of action of pilocarpine on outflow resistance in the eye of a primate (Cercopithecus ethiops)

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Facility of outflow in the eye of the vervet monkey Cercopithecus ethiops was studied by constant-rate and by constant-pressure infusion. Facility increased greatly after the injection of 2 to 3 µg of pilocarpine into the anterior chamber or after the application of 0.2 to 0.3 mg, to the cornea. The effect on facility consisted of 2 components. One component was reversed in 3 to 5 minutes by 1 mg. per kilogram intravenous atropine, the time it took for the atropine to relax pilocarpine-induced accommodation. The other component was reversed only slowly by intravenous or even intracameral atropine. The slowly reversed component was thought to represent a pilocarpine action directly on the endothelial cells of the outflow system, possibly a histamine-like action on the endothelial wall of Schlemm's canal.

Pilocarpine is a curious drug. Among ophthalmologists it is thought of as a miotic, as a purely cholinergic substance attacking the smooth muscle cells directly. However, things are more complicated. For instance, pilocarpine dilates the pupil of the rat and the guinea pig (but not that of cat, rabbit, or primate); it competes with other cholinergics; and it can exert an atropine-like action under certain conditions. Above all it does not look like a cholinergic drug. I dare say that no pharmacologist or pharmaceutical chemist could have predicted a cholinergic action from the structure of pilocarpine (Fig. 1). One would have expected an action related to histamine and, in fact, there are histamine-like effects of pilocarpine. Apart from stimulating ganglia and the adrenal medulla in low concentration, it has vascular actions in the cat and dog resembling those of histamine. We will return to this later.

Pilocarpine was first isolated in 1875. As a remedy in glaucoma it was first mentioned in 1877 in the very last sentence of a 91 page paper by Adolf Weber of Darmstadt, the father of applanation tonometry. The title of the paper was "Die Ursache des Glaucoms" but he spent the last few pages warning against the danger of eserine and ended up by saying, in effect: "There is another drug, pilocarpine, which I can recommend much more warmly . . . and I hope that it will make iridectomy unnecessary in many cases and help others where the effect of operation is not sufficiently marked."
In warning against eserine in glaucoma Weber certainly was a little too pessimistic. He had used it for several years in an amazing variety of ophthalmologic conditions including corneal ulcers but had not published anything on it when a one-page preliminary note by Ludwig Laqueur from Strasbourg appeared in June, 1876. Laqueur, who suffered from the disease himself, enthusiastically proposed the use of 0.3 to 0.5 per cent serine in glaucoma. Weber immediately wrote a rather lengthy report of his experience with the drug,\(^5\) warning against its indiscriminate use in glaucoma. This report appeared in the last 1876 issue of v. Graefe's Archiv and already in the next issue he proposed pilocarpine. Thus, the two drugs on which glaucoma therapy has rested for so long a time were proposed almost simultaneously and for many years they reigned unopposed. Their usefulness in glaucoma has usually been considered to be due to their effect on the iris sphincter and the ciliary muscle. There is little doubt that the miotic action proper is the important one in angle-closure glaucoma. But in open-angle glaucoma it is not clear how pilocarpine or for that matter any drug improves outflow facility. That miosis is not essential has been known for a long time. Armaly and co-workers\(^{10,11}\) presented tonographic evidence that voluntary ciliary muscle contraction by accommodation improved facility in the human eye, but there are also experiments suggesting that this is not the whole story. Shaffer\(^{12}\) has reported that facility is less improved by voluntary accommodation than by the same amount of pilocarpine-induced accommodation, and Ballintine\(^{13}\) has repeatedly stressed his clinical impression that there is a difference between eserine and pilocarpine; he believes that with eserine the effect on facility runs parallel with the effect on accommodation while with pilocarpine an effect is obtained on facility in doses which affect accommodation but little.

This is where the matter stands at the moment. It is possible but not proved that pilocarpine acts at several different levels.

Evidently the matter was worth looking into from the ophthalmologic point of view as well as from the pharmacologic, and since the generosity of the United States Public Health Service made it possible for me to work with large numbers of monkeys I decided to look into the mode of action of pilocarpine in a primate species.

**Plan of investigation**

The simple plan of the experiments was based on the fact known to every pharmacologist, that the cholinergic smooth-muscle effects of pilocarpine are quickly reversed by a sufficient dose of atropine. Thus, take an eye under the influence of pilocarpine. Record facility of outflow. Give a huge dose of atropine intravenously. If the effect of pilocarpine is due to its influence on the ciliary muscle or on smooth muscle in vessels carrying blood, the pilocarpine effect on facility will disappear within a few minutes. If it does not, pilocarpine has another point of attack on facility.

**Choice of primate species**

Pilocarpine is a miotic in all monkeys mentioned below, but different doses are needed to affect facility in different species. Thus in the green vervet *Cercopithecus ethiops*, in the baboon *Papio anubis*, in the mangabey *Cercocebus albigena* and in the...
Fig. 2. Diagram of infusion system used for facility determinations. Appropriate stopcocks allow either constant-pressure infusion from reservoir or constant-rate infusion from motor-driven syringe. Drugs are injected from micrometric syringe.

redtail *Erythrocebus patas*, all of them African, a few micrograms of pilocarpine injected into the anterior chamber have a marked effect on facility. This is not so in the two macaques tested, the Indian rhesus, *Macaca mulatta*, and the Javanese cynomolgus monkey, *Macaca irus*. In 6 individual rhesus monkeys I found no certain effect of doses up to 12 \( \mu \)g in spite of maximal miosis and in the cynomolgus the dose of 20 \( \mu \)g was needed to obtain a moderate effect. The bulk of my experiments were done in the green vervet and, when nothing else is expressly stated, the data to be presented here derive from about 130 individuals of this species. The constant-rate infusions were made in East African monkeys, the constant-pressure ones in West African animals.

Methods

The monkeys were anesthetized with intravenous veterinary Nembutal, 25 to 30 mg per kilogram body weight, and kept warm with an electric heating pad. Under these conditions mean blood pressure was about 80 to 100 mm Hg. Quite a number of experiments were lost because of nystagmus. Other anesthetics were tried but were no better. The only remedy was to keep anesthesia really deep. The anterior chambers were cannulated with branched needles, 0.45 mm. outer diameter, by means of the needle gun described by Sears.\(^{14}\) Sometimes topical anesthesia with lidocaine was used. One of the branches of each needle was connected by narrow polythene tubing to an Agla micrometric syringe for drug or saline injections. The other branch was connected by similar tubing to a manifold leading to a strain gauge pressure recorder and to sources for infusion fluid. Two types of infusions were made to record facility: constant rate and constant pressure. For constant rate, Agla syringes actuated by a constant rate motor were used. They were set to deliver identical amounts to both eyes, as a rule close to 5 \( \mu l \) per minute. The increase in steady-state pressure in millimeters of mercury per microliter per minute inflow rate was a measure of outflow resistance. The pressure rise due to needle resistance was deducted. For constant-pressure infusion a small reservoir, a polythene "thimble" suspended on a force transducer, could be set at different levels above its eye. A narrow polythene tubing connected the thimble with the manifold. The weight of the thimble with its content of remaining infusion fluid was recorded and facility estimated from the difference in rate of inflow at two different levels. Appropriate corrections for the resistance of the perfusing needle and tubing were made. The infusion fluid was 0.9 per cent NaCl unless otherwise stated. Pilocarpine hydrochloride and atropine sulfate were used and are identified without mentioning the anion in the following. The doses given refer to the salts.

Fig. 2 is a diagram showing the arrangement.

Results

1. Constant-rate infusions. Fig. 3 shows a constant-rate infusion experiment demonstrating the effect of 2 \( \mu \)g of pilocarpine injected into the anterior chamber. First an infusion lasting 13 minutes was given into both untreated eyes. Then the pilocarpine in 10 \( \mu l \) of saline was injected into
Table I. Effect of 2 μg pilocarpine HCl into the anterior chamber of vervet monkeys

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Body weight (Kg.)</th>
<th>Resistance ratio, experimental eye/control eye</th>
<th>Ratio B/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Before pilocarpine</td>
<td>B. After pilocarpine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>1.2</td>
<td>1.44</td>
<td>0.39</td>
</tr>
<tr>
<td>49</td>
<td>3.2</td>
<td>0.90</td>
<td>0.34</td>
</tr>
<tr>
<td>56</td>
<td>3.6</td>
<td>0.86</td>
<td>0.33</td>
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<td>35</td>
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<td>0.47</td>
</tr>
<tr>
<td>58</td>
<td>1.9</td>
<td>1.73</td>
<td>0.90</td>
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<td>4.8</td>
<td>0.88</td>
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</tr>
<tr>
<td>50</td>
<td>2.5</td>
<td>0.81</td>
<td>&lt; 0.44*</td>
</tr>
<tr>
<td>36</td>
<td>4.8</td>
<td>0.89</td>
<td>&lt; 0.60*</td>
</tr>
</tbody>
</table>

*Pressure in pilocarpine eye still falling when infusion was stopped.

the experimental eye and the same volume of saline into the control eye. Two later infusions showed that the resistance of the experimental eye was reduced to about half and that it was still low one hour after the pilocarpine.

There are several features of this experiment which merit discussion. The resting pressure was low even for an anesthetized monkey. Under our conditions the average pressure in 135 eyes was 8.9 mm Hg (as against about 20 mm. in light ether anesthesia). The rise on the first infusion of 4.77 μl. per minute (as it happens to be in this experiment) corresponded to a resistance of 1.93 and 2.14 mm. Hg/μl x min. in the experimental and control eyes, respectively, and was slightly higher than the geometric mean 1.63 derived from 116 eyes.

As usual the resistance of the control eye diminished during the course of the experiment, and the rise caused by successive infusions decreased. This drop was smaller than average in the experiment illustrated. In 35 eyes, where a second infusion was made without any intervening treatment of the eye but after varying amounts of first infusion, the mean resistance obtained during the first infusion was 1.91 ± 0.15 mm. Hg/μl x min. as against 1.46 ± 0.1 during the second. The coefficient of correlation between first and second was 0.81 and the coefficient of regression of the second on the first was 0.56. In some experiments resistance dropped very markedly during the infusions, down to below 50 per cent of the original value.

The degree of pilocarpine effect during this experiment was rather typical for the dose. In 8 experiments with 2 μg, performed in a similar manner, the values...
shown in Table 1 were obtained. The eyes illustrated in Fig. 3 belonged to No. 49.

The time course of the pressure rises when infusion starts were not strictly exponential but can be approximately characterized by the time needed for half the rise to take place: 2.7 and 3.3 minutes, respectively, in the first period. Unexpectedly, but regularly, the half-time for return to resting pressure after the infusion was much shorter, 1.3 and 1.1 minutes, respectively, in the first period. Now, if the volume compliance of the globe and the outflow resistance had been constant in the pressure range covered, the curves would have been strict exponentials and their half-times would have been proportional to the volume compliance and the outflow resistance and equal for the ascending and descending phases. There was a good correlation \( (r = 0.73) \) between resistance and half-time of the descending phase looked quite different (Fig. 4B). This remains unexplained but indicates the presence of some complicating factor probably affecting the time course of the descending phase.

The importance of the time constant becomes evident if one regards a typical atropine experiment (Fig. 5). After the first infusion of saline into both eyes, 3 \( \mu \)g of pilocarpine is given into one anterior chamber. The effect on resting pressure is not significant but that on resistance is dramatic. Intravenous atropine in the very high dose of 1 mg per kilogram is then given. It reverses the pilocarpine effect and the pressure in the pilocarpine-treated eye rises gradually after a few minutes of latency.

Let us consider how the time course of this latter rise would have looked under the simplest assumptions, that atropine rapidly and completely reverses the effect of pilocarpine after a certain latency, and that volume compliance and outflow resistance are pressure independent.

As soon as the reversal has taken place, then, the eye is back in its untreated condition. Under continued infusion the pressure will move toward its old steady state and this movement will be governed by a time constant characteristic of the untreated eye (Fig. 6). Comparing Figs. 5 and 6, it is evident that the time constant for the untreated eye is much less than that for the pilocarpine-treated eye. This is because the volume compliance is higher in the untreated eye than in the pilocarpine-treated eye.
and 6 we see that the real time course of recovery of pressure after atropine looks different from the simple quasi-exponential one we would have expected from the simplified assumptions and the actual behavior of the control eye. The probable explanation is that pilocarpine effect is not reversed rapidly and completely by the atropine but more gradually. However, the discrepancy is not large enough to make this quite evident and the experiment is typical in not proving anything one way or the other.

In Fig. 7 where one can also see the rapid drop after pilocarpine, a slow recovery after atropine is more clear-cut. It is seen to have continued between the first and second infusions. In Fig. 8 where the resistance of the control eye is not sustained under continuous infusion, the reversal of the pilocarpine effect evidently is quite slow. The same can be seen in Fig. 9, a quick effect of a huge dose of pilocarpine and a very slow recovery after atropine.

Clear-cut slowness of this kind was not
the rule in our experiments. Of about 20 experiments with this protocol only 7 showed such slowness of recovery that the presence of a gradual effect of atropine was suggested. In the remaining cases, recovery was not markedly slower than would be expected from the time constant of the untreated eye. In the 6 experiments where recovery was slowest relative to the time constant of the eye, the average resistance was 2.3 mm. Hg/μl × min⁻¹, in the 6 fastest the average resistance was 1.0 mm. Hg/μl × min⁻¹. We will return to this difference later.

We thus had evidence suggesting that at least part of the pilocarpine effect on facility could be reversed only slowly by atropine, the process taking many minutes. However, this slow part was not demonstrable in every experiment.

From general pharmacologic experience one would not expect antagonism between atropine and pilocarpine to develop so slowly. In ordinary experiments with isolated smooth-muscle strips from cat or rabbit intestine a contact time of 3 minutes was ample for atropine to exert its relaxing effect against spasm induced by a 50 times higher concentration of pilocarpine. It seemed probable that relaxation of the ciliary muscle by atropine or the contraction of pilocarpine-dilated vessels should be equally prompt.

To make doubly sure, however, I have tried to see directly how fast pilocarpine-induced accommodation was relaxed by intravenous atropine. Since this was difficult to do in eyes with a needle in the cornea (without excluding cornea refraction by immersion), it was necessary to introduce the pilocarpine through the intact cornea. Preliminary experiments similar to that shown in Fig. 10 indicated that the dose of 200 μg contained in 10 μl and applied to the cornea about one hour before the experiment has approximately the same effect on facility as 2 μg injected into the anterior chamber. In 6 experiments with constant-rate infusion, the average resistance on the side treated with 200 μg was 48 per cent of that on the control side (range 24 to 70 per cent) and in 4 experiments with constant-pressure infusion the values were 28 to 36 per cent. Consequently this dose was applied to 9 intact eyes and after one hour 1 mg. per kilogram atropine was given
intravenously. The refraction was followed by retinoscopy. The pupil widened sufficiently within about 1 minute. Since it was difficult to do the retinoscopy fast enough during the phase where refraction changed quickly, the individual curves were rather irregular, but when the 9 experiments were averaged, as shown in Fig. 11, it became evident that the ciliary muscle behaved similarly to other smooth muscles: pilocarpine was quickly antagonized by a sufficient dose of atropine. Within 3 to 5 minutes the pilocarpine effect on accommodation was reversed. There was no reason to believe that the effect of pilocarpine on vascular smooth muscle would behave very differently.

Pilocarpine applied to the cornea 1 hour before the infusion had the opportunity to act on intraocular structures a much longer time than in our regular experiments. It was therefore of interest to study what happened to facility after intravenous atropine in eyes treated with pilocarpine in this manner. Fig. 12 shows the results.

The lowermost curve represents 5 eyes

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**Fig. 8.** Slow reversal of pilocarpine effect by intravenous atropine.

**Fig. 9.** Slow reversal of pilocarpine effect by intravenous atropine.
in 5 monkeys. Four of these had received 0.2 and one 0.3 mg. pilocarpine on the cornea one hour before the experiment. In 4 of them retinoscopy was successfully performed on the contralateral, non-cannulated eye during the pressure recording, and these values were included in the average refraction curve. Atropine was injected intravenously at zero time and the curve shows the average pressure during infusion of 5 μl per minute.

The middle curve is the refraction curve also shown in Fig. 11, illustrating how accommodation was relaxed.

The topmost curve is obtained from 7 other monkeys. These eyes had not received any treatment except the topical lidocaine for cannulation. It is seen that the normal eye did not change its resistance despite the sudden cycloplegia. But also the pilocarpine-treated eye showed only a small pressure rise as accommodation relaxed, corresponding to a resistance rise of 0.15 unit. The main part of the pressure difference between the two groups of eyes remained. Part of this difference admittedly could have been due to the fact that the eyes belonged to different groups of monkeys. But all of it could not be due to this cause. If the pilocarpine effect had been completely reversed by the atropine, it would mean that its size would have been only about 0.15 unit. Since the dose of pilocarpine reduced the resistance by an average of 60 per cent, the average resistance of this group of 7 eyes would have been about 0.4 unit. This is lower than any single eye of 116 I have studied,15 their range being from 0.73 to 4.09. It is quite certain therefore that a considerable part of the pilocarpine effect remained unreversed even at the end of the 12 minutes of observation in these experiments.

A slow reversal of the pilocarpine effect could also be seen if atropine was injected directly into the anterior chamber. In Fig. 13 the enormous dose of 15 μg per eye is seen not to affect the control eye and only very slowly to bring the pilocarpine-treated eye up to control level. Similar, very slow pressure rises were seen in 2 more experiments with this protocol, while in 2 experiments the rise was no slower than could be expected from the half-time of the eye and in 3 experiments the result was questionable.

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**Fig. 10.** Effect of pilocarpine applied to the cornea demonstrated by constant-rate infusion. Verbet, 4.6 kilograms. Infusion 5 μl per minute. Experimental, 200 μg pilocarpine at ~60 minutes on the cornea.

**Fig. 11.** Reversal by intravenous atropine of accommodation induced by 0.2 mg. pilocarpine placed on the cornea about 60 minutes earlier. Data from 8 monkeys, 1 contributing both eyes at 5 day interval. Experimental values for each animal were plotted and values for whole minutes derived by interpolation. The curve shows the average of these interpolated values. Refraction zero has arbitrarily been assigned to the asymptote.
Fig. 12. Eye pressure during constant-rate infusion and refraction changes after intravenous atropine. The refraction curve is that of Fig. 11. Note rapid disappearance of myopia with only small increase in resistance after atropine.

Fig. 13. Slow effect of 15 μg atropine injected into the anterior chamber of both eyes on resistance of pilocarpine-treated eye.

Experiments like Fig. 13 exclude the extremely improbable possibility that the slowly reversible action of pilocarpine is on smooth muscle not accessible from the bloodstream.

2. Constant-pressure infusions. Until now we have concentrated on evidence for the presence of a slowly reversible component in the pilocarpine response. This has been clearly demonstrated in some experiments but may be present in many more, hidden behind the intrinsic slowness of eye pressure as an index of outflow resistance. And if a slow response can hide, this must be doubly true of any quickly reversible component, for instance, due to pilocarpine-induced accommodation. A hint of the presence of such a component was seen in Fig. 12 where pressure rose a little as accommodation relaxed. In order to disentangle the situation, however, we need a method to study quick changes in resistance. Constant-pressure infusion is such a method.

The advantage of constant-pressure infusion in certain situations depends on the fact that the viscoelastic time constant of the eye is determined by the product of volume compliance and a resistance which comprises the ordinary outflow resistance in
parallel with the resistance of the perfusion system $R_p$. In constant-rate infusion $R_p$ is virtually infinite, the machine delivers at a rate independent of the counterpressure in the eye. In this case the properties of the eye itself govern the situation. In constant-pressure infusion, $R_p$, the resistance between the fluid container and the eye can be made quite low and the time constant correspondingly short. Thus, with constant-pressure infusion, quick changes in outflow resistance should be resolvable. It seems that a limiting factor with this system is the imperfect elasticity of the sclera, which in our monkeys takes about 1 minute to adjust its volume to a new pressure. Whether or not blood volume changes in the uvea also occur in this first minute is not clear.

To demonstrate the time resolution attainable with constant-pressure perfusion, Figs. 14 and 15 show the latency of an intracameral pilocarpine effect and an intravenous atropine effect, respectively. In these experiments the drugs were given during perfusion at 12.5 mm. Hg above the original resting level of the eye. Fig. 14 shows that already 2 minutes after the beginning of the injection of 1.5 $\mu$g pilocarpine the rate of inflow started to accelerate. In Fig. 15 there is a slowing of the inflow beginning about 4 minutes after intravenous atropine.

Infusion rate at only one pressure level can give quite misleading results if rate of secretion in the eye changes. Therefore, for really quantitative studies, we have used alternating periods of 2 pressures, 7.5 mm. Hg apart, usually placing the lower one 5 mm. Hg above the original resting level of the eye. The minimum duration at each pressure was 4 minutes. The difference between the inflow rate at the two pressures gives a facility, $\Delta$ flow/$\Delta$ pressure. Thus each period of infusion in each eye affects 2 facility values, one preceding and one following the period. The method presupposes constant conditions over the 8 minutes of each pair of periods. Evidently it is only a simplification of the slope-plotting method of Becker and Constant.17
Fig. 16 shows a section of a record with the two-pressure method. The figures are the facilities derived from the just preceding breaks in the curves. It is seen that, when pressure was lowered, 7 minutes after pilocarpine, the slope of the pilocarpine-treated eye became nearly nil. The slope difference was large, the facility definitely increased, but the drug had increased secretion so that actually inflow from the perfusion system at a low pressure was smaller than it was before the drug was given. Evidently it is not quite safe to use single-pressure methods. The two-pressure method moreover allowed an extra check: The resting pressure where slope would be zero could be calculated by extrapolation from the two slopes at the two pressures. In successful experiments it was reasonably constant.

When the two-pressure method was applied in 16 vervet experiments with intracameral injection of 1.5 to 3 µg of pilocarpine, a quick pilocarpine effect on facility was seen in 15 of them. Intravenous atropine caused a quick reversal in almost all. Only in 2 of them was the reversal virtually complete, however (Fig. 17). The incompleteness in the majority of experiments (Fig. 18) might have been due to the presence of a slowly reversible component. But, as a rule, no actual slow decline in facility was discernible. Instead, on continued infusion, the experimental and the control eye usually showed gradually increasing facility, sometimes ending with facilities several times larger than the starting values. Changing the 0.9 per cent NaCl as infusion fluid to Ringer's solution containing 24 mg. per 100 ml. CaCl₂ and 42 mg. per 100 ml. KCl did not improve matters.

Only when we changed to Ringer's solution containing 75 mg. glucose per 100 ml. solution and shortened the period of infusion by preapplication of 200 µg pilocarpine to the cornea one hour before the experiment did we succeed in demonstrating the slow component in reversal with this method too (Fig. 19). A systematic study of the influence of the composition of the infusion fluid evidently would be worthwhile.

Discussion

Two components in the pilocarpine effect on facility in normal vervet eyes have been demonstrated. The one component disappears in a few minutes after intravenous atropine. This time course is similar to that of the disappearance of accommodative spasm caused by pilocarpine. There is little reason to doubt that this component of pilocarpine action is due to an effect of the drug on the ciliary muscle and only indirectly on the trabecular meshwork. The other component is only slowly reversible by intravenous atropine. Thus it very probably is due to a direct pilocarpine effect on the structures forming the out-

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Fig. 15. Latency of action of intravenous atropine recorded with constant-pressure infusion. Ordinate, microliters of saline left in reservoir. Both eyes kept at 12.5 mm. Hg above resting level. The facility of the control eye had increased during the early part of the experiment but did not react to atropine.
flow resistance. The slowness with which even intracameral atropine reverses the effect indicates that smooth muscle is not responsible for this effect. The only other possibility seems to be the endothelial cells, most importantly those forming the wall of Schlemm’s canal.

If pilocarpine reduces the resistance of the endothelial wall of the canal, the effect of this component of its action on over-all facility evidently will depend on the fraction of total resistance residing in the endothelium from the start. If the major resistance is in the fibrous meshwork, changes in the endothelial resistance will have little over-all effect. Thus, if in prolonged perfusion the endothelial wall has reacted with leakiness even before pilocarpine was given, its resistance will be low and the major part of the resistance perhaps reside in the fibrous meshwork. Pilocarpine will then induce a clear quickly reversible effect by ciliary muscle action on this latter structure but no noticeable slowly reversible one. The findings in Section 1 that when a slowly reversible effect was seen the resistance was more than twice as high as when it was not seen may have this explanation.

It was easier to demonstrate the slowly reversible effect if pilocarpine was applied to the cornea 60 minutes before the infusion than if it was injected into the chamber only shortly before atropine was given. There are two factors which might be of importance here. The preapplication experiments involved much shorter infusions, with presumably better conservation of endothelial starting resistance, and the drug had much more time to act. It is possible that the slowly reversible effect takes time to develop fully.

The nature of the slowly reversible change caused by pilocarpine is not clear. It could be a general shrinking or swelling of cells, affecting the passage of fluid in several places, presumably most efficiently in the transcellular passages of Holmberg.18 But it is also possible that pilocarpine has a histamine-like action here and that it opens new passages altogether by producing a “leaky canal of Schlemm.”
The fact that the slow effect is reversible by atropine does not militate against a histamine-like action. Atropine in high concentrations is quite unspecific.

Histamine affects vascular endothelium in two ways. It may cause the cells to swell and it causes them to separate. That this separation is actually the basis of the vascular leakiness caused by histamine has recently been beautifully shown by Majno and Palade. If pilocarpine were to cause a swelling of the endothelial cells along the route of the aqueous, resistance could hardly be lowered. But if the drug were to cause separation between the cells of the wall of Schlemm's canal (which after all is a small vein) an increase in facility might result.

There is some tonographic evidence from the human eye compatible with such an action on the endothelial wall.

Scheie and collaborators have published a diagram which indicates that the use of miotics (presumably mainly pilocarpine) increases facility in patients who have chronic simple glaucoma by the same amount, independent of the starting value.

Linner has recently compared the additional facility induced by pilocarpine in 80 normal eyes (mean C = 0.27) and in 32 eyes with chronic simple glaucoma (mean C = 0.11). Pilocarpine, 3 per cent, was given twice with an interval of one hour; tonography was done one hour after the last instillation. Facility increase was somewhat lower in glaucoma —0.026 ± 0.0071— than in normal eyes —0.048 ± 0.0056. The difference is not statistically significant.

It is interesting to note that similar findings have been made by Becker with two strong cholinesterase inhibitors, echthiophate and demecarium.

If miotics open new pathways across the endothelium independent of the old ones and if endothelial resistance dominates over all other resistance components in the majority of cases, constant addition of facility is what one would expect.

Thus we are back to the question: How large is the relative contribution of endothelial resistance? There is probably no single answer, either in normals or in
Fig. 19. Gradual reversal of the effect of pilocarpine applied to the cornea by 1 mg. per kilogram intravenous atropine. Constant-pressure infusion with Ringer’s solution containing 75 mg. per 100 ml. glucose. A rapid atropine effect is also seen.

Glaucoma. To begin with glaucoma, there may perhaps be a majority of eyes with chronic simple glaucoma in which endothelial resistance constitutes the dominant part, but this is not necessarily so in cases where considerable hyalinization of the fibrous trabeculae has occurred. And in the normal eye it seems possible that the homeostatic mechanism regulating eye pressure plays on endothelial resistance since it cannot very well play on accommodation. If this is so one would expect considerable variation in the relative importance of endothelial resistance even in normal eyes.

A hint of the size of endothelial resistance can possibly be gained from the size of the very marked lowering of resistance sometimes observed during infusion, especially in constant-pressure infusions. It seems improbable that homeostasis is responsible for this resistance drop, but since, whatever its mechanism, it cannot very well be due to shifting ciliary muscle pull, the size of the drop indicates the importance of the state of the endothelium for resistance.

We still have to consider the action of pilocarpine on the resistance of the fibrous meshwork. It is tempting to ascribe the easily demonstrated, quickly reversible component in pilocarpine action to changes in this structure brought about by ciliary muscle pull. If a considerable resistance resides in the fibrous meshwork, contraction of the ciliary muscle could change it by pulling the scleral spur inward and separating the trabecular lamellae. But since the endothelial wall of the canal cannot disengage from the rest of the meshwork, ciliary muscle pull will also affect this layer. It is therefore not yet permissible to estimate the relative importance of endothelial wall resistance and fibrous meshwork resistance from the relative sizes of the slowly reversible and the quickly reversible pilocarpine effects in the single case. Part of the quickly reversible effect may be an action on endothelial resistance. But if this should prove not to be the case, very interesting conclusions as to the site of resistance in individual cases of glaucoma could be drawn from the difference or lack of difference in effect between pilocarpine and accommodation or between pilocarpine and a miotic without endothelial action. Whether such a miotic exists is still an open question.

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