The aqueous humor drainage mechanism in the cynomolgus monkey (Macaca irus) with evidence for unconventional routes

Anders Bill

The aqueous in freshly killed cynomolgus monkeys was replaced by a solution of ¹²⁵I-albumin and ¹³¹I-diodone (iodopyracet). An inflow of this solution into the anterior chamber was produced by a horizontal buret placed above the eye, and at the same time the eye was continuously washed through with the radioactive solution at a high rate by a push-pull arrangement. Fluid was collected from the denuded anterior sclera in 1 minute samples over a period of 25 minutes. The same type of experiments was performed with ¹²⁵I-gamma globulin and ¹³¹I-albumin. In the effluent, albumin and gamma globulin reached an apparently steady concentration after 1 minute, while throughout the experiments the diodone concentration in the effluent remained lower than expected from a bulk flow through well-defined channels. The low concentration was due to a rapid diffusion of diodone into the tissues of the limbus region. The labeled albumin entered the uvea and the sclera in such amounts as to indicate that about 20 per cent of the labeled fluid had passed out by way of unconventional uveoscleral routes. The results suggest that more than 90 per cent of the conventional drainage was carried by pores with an apparent diameter of more than 0.16 μ.

It has been demonstrated in many ways that in the primate eye aqueous humor flows from the anterior chamber into Schlemm’s canal. It is quite clear that the aqueous first passes into the spaces of the trabecular meshwork but how it then passes through the inner wall of Schlemm’s canal is a matter of question. Much physiologic evidence¹⁻⁴ suggests that the flow occurs through pores with a diameter of 0.1 to 1.5 μ; Holmberg⁵ has in fact presented anatomical evidence for pores of this order.

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It seems possible that aqueous humor may also flow out by passage between endothelial cells⁶ and that the vacuoles observed in the endothelium of Schlemm’s canal⁷⁻⁹ are not all parts of pores, but are still involved in the drainage.

Earlier studies of the permeability of the filtering region of the primate eye have used particles of various sizes. The particles have been so large as to tend to block the smaller pathways if these have a diameter around 0.1 μ, as suggested by Huggert’s⁸ experiments with isobutyl alcohol. In the present study the permeability of the outflow paths to diodone, albumin, and gamma globulin has been investigated.

Methods

Cynomolgus monkeys (Macaca irus) weighing 0.9 to 2.6 kilograms were employed. The animals were anesthetized with Nembutal (Abbott), 30
mg. per kilogram body weight, and 1,500 IU heparin was given intravenously. The head was fixed with a headholder and the animal was then killed by an overdose of Nembutal. The superior eyelid was removed, the upper part of the eye was freed from the conjunctiva and episcleral tissue, and the superior rectus muscle was cut at the sclera. The anterior chamber was then cannulated with three cannulas connected to three pairs of Agla precision syringes and a horizontal buret as shown in Fig. 1. Syringes A1 and A2 were coupled to each other on a frame in such a way that the total volume within the two syringes was constant; B1 and B2 and C1 and C2 were similarly coupled to each other. Before the cannulation, the syringes and the tubing had been filled with a radioactive solution which filled also the buret. A1, B1, and C1 were in their filled position, A2, B2, and C2 in their unfilled position. The position of the cannulas is shown in Fig. 1. After the cannulation, 1 ml. radioactive fluid was washed through the anterior chamber with A1 and A2. The buret, placed so as to give an intraocular pressure of about 15 mm Hg, was then connected to the eye and adjusted to give an inflow of about 8 μl per minute. B1 and B2 and C1 and C2 were operated by hand to give a washing-through of the anterior chamber at a rate of 75 μl per minute. Fluid now started to appear on the sclera, usually from several places near the limbus and from one or two vessels at the insertion of the superior rectus muscle. The fluid was collected continuously in 1 minute samples on strips of filter paper. Immediately after the sampling these were put into polyethylene test tubes with which they had been weighed previously. The tubes were sealed and reweighed on a Mettler semimicro balance. After 25 minutes the cannulas were withdrawn from the anterior chamber and the anterior and posterior chambers were emptied by a slight pressure on the eye. The eye was then washed with saline, enucleated, and freed from conjunctiva and episcleral tissue. The limbus region was then excised. The preparation contained the trabecular meshwork, Schlemm’s canal, some corneal tissue, and a 2 to 3 mm wide ring of the sclera.

Some experiments were performed in which the conjunctiva was intact until the eyes were dissected. In these experiments the anterior chamber was washed through with 1 ml. of inactive fluid just before the eyes were enucleated. The chambers of the eye were then emptied. The fluid from the eye was collected to determine the efficiency of the final wash-through procedure. The concentration of labeled material in this fluid was less then 5 per cent of that in the radioactive fluid originally washed through the eye. In these experiments the following tissue samples were taken: (1) the whole conjunctiva with parts of the eyelids; (2) episcleral connective tissue and fat; (3) all of the sclera behind the equator region; (4) all of that between the equator and the limbus region; (5) the limbus preparation taken as described above; (6) the cornea; (7) the iris; (8) the ciliary body; (9) the choroid with the retina and some vitreous attached; (10) the lens; and (11) the vitreous humor. The last-mentioned preparation was contaminated with fluid that had leaked out of the other tissues during the dissection.

**Pilot experiments.** It could be expected that

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**Fig. 1.** Schematic diagram of the setup. The anterior chamber was cannulated with three cannulas, one connecting the chamber to a buret, the other two connecting the chamber to three pairs of precision syringes. A1, A2; B1, B2; and C1, C2.
after appearing on the sclera the test substances should have a tendency to diffuse back into the scleral substance. To determine whether this was a factor that might influence the results appreciably, experiments were performed in which 4 to 6 mg. of radioactive fluid was placed on the exposed sclera from a syringe. After 30 seconds the fluid was collected on a filter paper as in the previous experiments, and a new drop of fluid was placed on the same part of the sclera and collected after another 30 seconds. In all, 8 drops were placed successively and collected in each experiment. In these experiments, the animals were dead and the anterior chamber was not perfused.

The test substances. The test substances were diodone (iodopyracet) (the sodium salt of 3, 5 di-iodo-4-pyridone-N-acetic acid), human serum albumin, and human gamma globulin. 131I-human serum albumin and 125I-diodone were obtained from the Radiochemical Centre, Amersham, England, which also supplied the 125I used in the labeling of albumin and gamma globulin. The molecular size of the three substances is given by the radii of their equivalent spheres: for gamma globulin 56 Å, for albumin 35 Å, and for diodone about 5 Å. The protein labeling with 125I was performed as described elsewhere.11 The basic solution in which the test substances were dissolved was that described by Barany.1- Carriers were added to give a 0.2 per cent concentration of the substances under study in the solution washed into the eye.

Spaces. For brevity, in the following, the albumin space of a tissue sample is defined as the amount of labeled albumin in counts per minute in the tissue divided by the concentration of labeled albumin in counts per minute per microliter in the anterior chamber fluid. The diodone and gamma globulin spaces are defined similarly.

Assay of radioactive isotopes. The samples were assayed for 131I and 125I in a two-channel gamma spectrometer with a well-type crystal.13 The counting error in the analysis of the fluid samples collected on filter paper was less than 0.5 per cent, that for the analysis of the tissue samples less than 5 per cent for samples with an apparent volume of the anterior chamber fluid of more than 0.5 μl.

Results

Two pilot experiments, one with 131I-diodone and 125I-albumin, and one with 131I-albumin and 125I-gamma globulin, were performed in which fluid was placed on the sclera from a syringe and collected. Diodone was lost so rapidly from the fluid that in the first samples it had a concentration of only 60 per cent of that expected from the albumin content of the samples. After eight samples had been collected, the diodone concentration still was only 88 per cent of that expected.

The gamma globulin content in the control experiments was not different from that expected from the albumin content. The technique of collecting the fluid appearing on the sclera continuously was a consequence of these findings. Its chief disadvantage was a considerable and uncontrolled loss of water from the filter paper caused by evaporation from a large surface.

Albumin. In all, ten experiments were performed with intracameral injection of radioactive fluid and sampling from the sclera, four with 131I-diodone and 125I-albumin, and six with 131I-albumin and 125I-gamma globulin.

The mean pressure found to give an inflow into the eye of 8 μl per minute was 18 mm. Hg.

Fig. 2 presents the albumin concentration in the effluent as compared with that in the fluid washed into the eye. The figure shows that after 1 minute the mean albumin concentration was about 110 per cent of the

Fig. 2. The concentration of the labeled albumin in the effluent collected on the sclera as per cent of the concentration in the fluid washed through the eye. Connected dots represent mean values; vertical bars through the dots, the standard errors of the means.
activity in the inflow fluid. There was a considerable scatter of the values but there was no detectable tendency for the concentration in the effluent to increase or decrease with time. In the first sample the albumin concentration was on an average 86 per cent of that in the subsequent samples. Since the mean intraocular pressure during the first minute was much less than 18 mm. Hg, the outflow from the eye was less than 8 µl per minute, and the figure 86 per cent then indicates that the volume of the dead space of the outflow paths with a rapid flow was about or less than 1 µl. The scatter of the data and the inexactness of the flow data make it impossible to give a more precise value.

**Albumin and diodone.** The effluent from the sclera had a lower diodone concentration than expected from the albumin content throughout the whole period of time studied, as shown in Fig. 3. The diodone and albumin spaces of the limbus preparation were 24.3 ± 0.7 and 5.9 ± 0.5 µl, respectively (arithmetic mean ± standard error of the mean). The mean weight of the preparation was 50.3 mg.

Five experiments were performed without damaging the conjunctiva before the eye was dissected. The diodone space of the limbus preparation in the experiments was 19.3 ± 1.0 µl. The mean weight of the preparation was 54.0 mg. Table 1 shows that in these experiments both diodone and albumin penetrated into all the other tissue samples collected and in different proportions. The mean albumin space per 100 mg. posterior sclera was 12.6 µl; for the anterior sclera the corresponding figure was 13.4. The smaller diodone space in the experiments with intact conjunctiva indicates that with the conjunctiva removed some diodrast entered the limbus preparations by diffusion from the outside, as could be expected from the pilot experiments.

![Fig. 3. The ratio concentration of labeled diodone in effluent/that in the anterior chamber fluid divided by the ratio labeled albumin concentration in the effluent/labeled albumin in the anterior chamber fluid.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932953/)

**Table I.** The albumin and diodone spaces of the tissues in five experiments. The quotients diodone space/albumin space for the tissues and the weights are also presented.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Albumin space (µl) (M ± SEM)</th>
<th>Diodone space (µl) (M ± SEM)</th>
<th>Diodone space/albumin space (M ± SEM)</th>
<th>Weight (mg) (M ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctiva</td>
<td>2.8 ± 0.5</td>
<td>13.0 ± 2.1</td>
<td>4.8 ± 0.3</td>
<td>518 ± 38</td>
</tr>
<tr>
<td>Extraocular tissue</td>
<td>9.4 ± 0.7</td>
<td>22.7 ± 3.3</td>
<td>2.4 ± 0.3</td>
<td>1,087 ± 91</td>
</tr>
<tr>
<td>Posterior sclera</td>
<td>11.5 ± 2.5</td>
<td>31.7 ± 4.7</td>
<td>1.6 ± 0.2</td>
<td>91 ± 11</td>
</tr>
<tr>
<td>Anterior sclera</td>
<td>12.6 ± 2.2</td>
<td>38.7 ± 5.1</td>
<td>1.6 ± 0.2</td>
<td>99 ± 12</td>
</tr>
<tr>
<td>Limbus preparation</td>
<td>3.9 ± 0.5</td>
<td>19.3 ± 1.0</td>
<td>5.2 ± 0.6</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>Cornea</td>
<td>0.5 ± 0.04</td>
<td>10.6 ± 0.7</td>
<td>23.4 ± 2.8</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>Lens</td>
<td>0.4 ± 0.08</td>
<td>0.9 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>92 ± 10</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>5.4 ± 0.5</td>
<td>10.6 ± 1.0</td>
<td>1.9 ± 0.05</td>
<td>71 ± 9</td>
</tr>
<tr>
<td>Choroid, retina, vitreous humor</td>
<td>7.3 ± 1.0</td>
<td>5.4 ± 1.1</td>
<td>0.7 ± 0.05</td>
<td>713 ± 128</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>2.6 ± 0.5</td>
<td>4.0 ± 1.4</td>
<td>1.5 ± 0.5</td>
<td>1,026 ± 72</td>
</tr>
<tr>
<td>Iris</td>
<td>2.2 ± 0.04</td>
<td>2.8 ± 0.1</td>
<td>1.3 ± 0.08</td>
<td>14 ± 1</td>
</tr>
</tbody>
</table>

*M*, arithmetic mean; *SEM*, standard error of the mean. For the definition of spaces see text.
Albumin and gamma globulin. Fig. 4 presents the results of six experiments with gamma globulin and albumin. The values for the 1 minute samples were used to give mean concentrations during 5 minute periods. For each period the ratio concentration of labeled gamma globulin in effluent/that in the filtering fluid was divided by the ratio concentration of labeled albumin in effluent/that in the filtering fluid. The mean value of all thirty 5 minute periods was 0.9982 ± 0.0015. The 0.01 confidence interval for the mean ranged from 0.9940 to 1.0024. The mean albumin and gamma globulin spaces of the limbus preparation were 2.88 ± 0.31 μl and 3.40 ± 0.33 μl, respectively. The mean weight of the preparations was 28.9 mg. The mean ratio gamma globulin space/albumin space was 1.25 ± 0.036. The limbus preparations in the experiments were smaller than those in the previous ones and as a consequence also the albumin spaces were smaller.

Discussion

The outflow paths. The fact that the diodone molecules appeared in a low concentration in the filtrate and that at 25 minutes the diodone space in the limbus region was much higher than the corresponding albumin space indicates that on their way out the small diodone molecules could enter not only the conventional paths for aqueous drainage but also large spaces in the limbus preparation. The results reported by Berggren suggest that in rabbits also a similar mechanism delays the outflow of small molecules.

In the present experiments about 80 per cent of the total outflow of anterior chamber fluid was drained by conventional routes, that is, about 6.4 μl per minute. The diodone loss from the fluid on its way out, as calculated from this flow and the concentration data of Fig. 3, corresponded to the diodone content of 32 μl anterior chamber fluid, while the mean diodone space in the limbus preparation was only 24.3 μl. This means that about 7 μl must have been lost to the cornea in front of the limbus region and to the sclera behind. The loss into the scleral tissue may in part have been due to diffusion from the scleral surface; this is clear from the pilot experiments, in which diodone was found to enter the sclera rapidly.

The fact that also in the episcleral tissues and in the conjunctiva the diodone space was larger than the albumin space in spite of the losses to the sclera indicates that diodone leaked out of the episcleral and conjunctival vessels much easier than albumin.

For the proteins the concentration in the effluent appeared to reach a steady level within 1 minute and the volume of the dead space in the outflow channels was of the order of less than 1 μl. In the limbus preparations taken after 25 minutes' perfusion with intact conjunctiva, the mean albumin content corresponded to that in 4 μl anterior chamber fluid. There was thus more albumin than expected from the volume of the outflow paths for rapid flow. This may have been due to adsorption of albumin in the outflow paths, retention in the filter due to steric effects, flow through very slow and circuitous routes, or to penetration of albumin into the scleral sub-.
stance. With regard to the latter possibility, it is known that in rabbits albumin can pass out through the sclera after suprachoroidal administration.\textsuperscript{14} It then seems very likely that it can penetrate the sclera also in front of the iris root, directly from the trabecular meshwork, or by way of the iris root or as a result of leakage through the walls of the canals carrying aqueous humor.

The way in which large amounts of the albumin reached the sclera far behind the iris root is not clear in detail. Diffusion is much too slow a process to be considered as the reason for the high and nearly equal albumin concentration in the two sclera preparations. Flow into intrascleral blood vessels communicating with Schlemm's canal also cannot explain the high concentration of labeled material since a similar accumulation of anterior chamber fluid in the sclera has also been observed in the living monkey eye.\textsuperscript{11} The presence of much albumin and diodone in the anterior uvea and in the preparation containing the choroid indicates that there was a flow of anterior chamber fluid into the anterior uvea and from there into the sclera, in part by way of the choroid and the suprachoroid. The relatively low diodone content of the posterior sclera and the choroid is consistent with the possibility that the fluid passing through the anterior uvea, the choroid, and the suprachoroid toward the posterior sclera lost more diodone than albumin to the anterior uvea and the anterior sclera (Table I). A pressure difference between the anterior chamber and the suprachoroid, which is a prerequisite for a flow, has been reported by van Alphen.\textsuperscript{15}

Table I shows that in the experiments with intact conjunctiva the content of labeled albumin in the conjunctiva and in the episcleral tissues corresponded to that in about 12 µl anterior chamber fluid. A considerable part of this must have been located within the aqueous veins and the blood vessels. This means that little of the labeled albumin that had entered unconventional paths had passed out of the sclera into the episcleral tissue during the 25 minute period. Thus, if diffusion of albumin is neglected, the sum of the various other albumin spaces of Table I, 46 µl, represents close to the total amount that had left the anterior chamber by unconventional routes. Hence, it appears that of 200 µl (25 x 8) aqueous humor leaving the anterior chamber about 20 per cent went into the unconventional drainage paths, and the flow into Schlemm's canal was thus of the order of 6 µl per minute. It is clear from the assumptions made that this calculation gives only the order of magnitude of the uveoscleral drainage, and they apply only to the cynomolgus monkey under the conditions of study. Preliminary experiments in rabbits have failed to show a flow of anterior chamber fluid into the ocular tissues behind the anterior uvea.

It was clear from the analyses of the limbus preparations that in the experiments with albumin and gamma globulin they contained more gamma globulin than albumin, while in the effluent there was no significant difference in concentration between the two. The findings do not conflict with each other because the total amounts retained were very small fractions of the total filtered. The mechanism responsible for the difference in albumin and gamma globulin spaces in the limbus preparation is not clear. It may have been adsorption or steric effects such as can be produced by bulk flow through a composite filter with relatively coarse pores at the entrance and smaller pores in the deeper layers.

The pore size. The data for the effluent from the eye can be used to set an upper limit for the importance of very small pores for the drainage into Schlemm's canal. This has a bearing on the use of albumin as a tracer of bulk flow of water by way of conventional routes. It should be mentioned that the calculations are tentative and based on many assumptions.

From studies of the passage of different water-soluble molecules through nonbiological membranes it is well known that diffusion and filtration through water filled pores may be restricted even if the radius
of the pore is many times that of the molecule in question. This is due in part to steric hindrance at the entrance to the pores and in part to friction between the molecules and the pore walls. Renkin and Pappenheimer and Grotte have treated this problem for isoporous membranes. For diffusion into a pore with radius \( r \), molecules with radius \( a \) are supposed to enter the pore only through an area limited by a circle with radius \( (r - a) \), and the relationship between the coefficient for restricted diffusion \( D' \) and that for free diffusion \( D \) when also frictional resistance is taken in account, is

\[
\frac{D'}{D} = \left( \frac{r - a}{r} \right)^2 \left[ 1 - 2.104 \left( \frac{a}{r} \right) + 2.09 \left( \frac{a}{r} \right)^3 - 0.95 \left( \frac{a}{r} \right)^5 \right]. \tag{1}
\]

The rate of transport of a radioactive substance in counts per minute per second through an isoporous membrane due to diffusion, \( T_D \), according to Fick’s law is then:

\[
T_D = D' A_p \frac{C_1 - C_2}{A_x}, \tag{2}
\]

where \( A_p \) is the total pore area permitting free movement of water into the membrane in square millimeters, \( C_1 \) the concentration of labeled material in counts per minute per microliter on one side of the membrane, and \( C_2 \) that on the other side; \( A_x \) is the length of the pores in millimeters.

During filtration the molecules in question can enter the same limited part of each pore. Ferry has calculated that, if this were the only reason for molecular sieving, the concentration in the filtrate, \( C_a \), and that in the filtering fluid, \( C_b \), would be related to each other as:

\[
C_a = C_b \left[ 2 \left( \frac{r - a}{r} \right)^2 - \left( \frac{r - a}{r} \right)^4 \right]. \tag{3}
\]

If, during filtration, frictional resistance is taken into account, as suggested by Renkin and Pappenheimer, but diffusion is neglected for a moment, the sieve constant, \( S \), defined as \( C_a/C_b \), becomes:

\[
S = \left[ 2 \left( \frac{r - a}{r} \right)^2 - \left( \frac{r - a}{r} \right)^4 \right] \left[ 1 - 2.104 \left( \frac{a}{r} \right) + 2.09 \left( \frac{a}{r} \right)^3 - 0.95 \left( \frac{a}{r} \right)^5 \right]. \tag{4}
\]

As a consequence, the rate of transport of a labeled substance with filtration, \( T_F \), through a membrane is

\[
T_F = S \times Q_F \times C, \tag{5}
\]

where \( Q_F \) is the flow in microliters per second.

The total rate of transport through the membrane of the labeled substance is, of course, influenced also by diffusion, and the situation is complicated by the fact that during filtration the concentration gradient in the pores is no longer linear. The effect of the flow on the rate of transport through the pores caused by diffusion is to reduce it.

In the present experiments the flow into Schlemm’s canal was about 6 \( \mu \)l per minute, or 0.01 \( \mu \)l per second. The intraocular pressure that produced this outflow was about 18 mm Hg. If it is assumed that the pressure in Schlemm’s canal in the dead animals was 3 mm Hg, the facility due to outflow into Schlemm’s canal is 0.4 \( \mu \)l per minute mm Hg, a value quite common in living monkeys. How much of this facility can have been caused by small pores? The calculation will first be done for 200 \( \AA \) pores, that is, pores with a radius of 100 \( \AA \) = 0.01 \( \mu \)m. Poiseuille’s law gives the facility of a single 20 \( \mu \) long pore with a diameter of 200 \( \AA \) at 20° C. as \( 1.56 \times 10^{-12} \) \( \mu \)l per minute mm Hg. Thus, \( 1.28 \times 10^{10} \) such pores would be required to give 5 per cent of the conventional outflow path facility. The total area of the pores would be 4.02 square millimeters. If it is assumed that the remaining 95 per cent goes by way of large pores with no sieving, the effect on the composition of the total outflow of the sieving produced by the small ones can be calculated as follows: The diffusion constant for albumin at 20° C. is \( 6.1 \times 10^{-5} \) square millimeters per second, and according to equation 1 the value of \( D'/D \) for \( a = 35 \) \( \AA \) and \( r = 100 \) \( \AA \) is 0.15, and according to equation 3 the value for \( S = 0.23 \). The rate...
of transport of labeled albumin in counts per minute per second, caused by flow through the small pores, $T_{FS}$, according to equation 4, is:

$$T_{FS} = 0.23 \times 0.05 \times 0.1 \times C_A$$

where $C_A$ represents the concentration in the anterior chamber fluid. The rate of transport caused by flow through the wide pores, $T_{FW}$, is:

$$T_{FW} = 0.95 \times 0.1 \times C_A$$

and, if the interaction between flow and diffusion is neglected provisionally, the rate of transport due to diffusion through the small pores, $T_D$, according to 2 is:

$$T_D = 0.15 \times 6.8 \times 10^{-5} \times 4.02 \times C_A / 0.02$$

where $C_E$ is the concentration in the effluent. The transport due to diffusion through the large pores can be neglected since the total area for diffusion in these pores is very small. The concentration in the effluent is then:

$$C_E = \frac{T_{FS} + T_{FW} + T_D}{0.1}$$

This treatment is slightly different from that of Renkin and Pappenheimer and Grotte. They assumed that the diffusion rate was proportional to $D'/D$ but to $S$. The previous equations give $C_E = 0.9622C_A$ and $T_D = 0.00007C_A$. As pointed out previously, diffusion could be expected to have played a smaller role than in the above calculations because of the interaction between diffusion and filtration. From equations given by Manegold and Solf it was calculated that the influence of filtration on diffusion in fact was considerable; the gradient for diffusion from the entrance of the pores to their middle was reduced by about 50 per cent, which means that the transport due to diffusion through the small pores was even more reduced. However, since the total transport due to diffusion was very small, a reduction in diffusion rate had very little effect on the filtrate; thus, if the diffusion was totally neglected, $C_E = 0.9615C_A$.

The same calculations as above for gamma globulin gave $C_E = 0.9539C_A$. If diffusion was neglected the value of $C_E$ was 0.9538$C_A$.

The ratio, $C_E/C_A$, for gamma globulin divided by the same ratio for albumin gave 0.9914 with the effect of filtration on diffusion neglected, and 0.9920 if all diffusion was neglected. The pore length in the narrow parts of the conventional outflow paths is not known; it may be shorter than 20 $\mu$.

The same calculations as above were therefore made for pore lengths of 0.5 $\mu$ and 10 $\mu$, and these gave almost the same ratios. Because all the figures were outside the 0.01 confidence interval found in the present experiments, it appears that there was not as much as 5 per cent drainage of aqueous humor into the canal of Schlemm through 5 to 20 $\mu$ long 0.02 $\mu$ pores. Similar calculations for other pore sizes show that drainage of as much as 10 per cent by way of 0.04 to 0.16 $\mu$ pores with a length of 5 to 20 $\mu$ would cause concentration ratios outside the 0.01 confidence limits.

In all the theoretical cases treated the calculations showed that if the fluid reaching Schlemm's canal from the anterior chamber has a lower concentration of albumin than that in the anterior chamber, the difference is less than 4 per cent.

Pores with a diameter of less than 0.02 $\mu$ permit very little passage of proteins, and the data for albumin and gamma globulin therefore give no information about such pores. However, McEwen has calculated the filtering surface of Schlemm's to be about 11 square millimeters. If 50 per cent of the whole resistance to flow from the anterior chamber into Schlemm's canal is located within the endothelium, and it is assumed that as much as 1.1 square millimeters of the filtering surface is occupied by 0.01 $\mu$ pores with a mean length of 1 $\mu$, it can be calculated that these could carry only 4 per cent of the total bulk flow into Schlemm's canal. Longer pores and pores with smaller diameters occupying the same surface of course would drain less. The fluid drained by 0.01 $\mu$ pores would con-
tain very little protein because of sieving but, since there is no mixing between the fluid in the deep layers of the trabecular meshwork and that in the anterior chamber, it can be presumed that most of the protein which was excluded from the small pores would be carried out by flow through larger pores in the neighborhood, or with pinocytosis, which would make the effect of the small pores undetectable. It should be pointed out that this last-mentioned mechanism in fact may be responsible for the close parallelism in outflow between albumin and gamma globulin that was observed. Since the trabecular meshwork and the endothelium of Schlemm's canal constitute a composite filter, only apparent pore sizes can be determined.

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