Production and drainage of aqueous humor in the cynomolgus monkey (Macaca irus)

Anders Bill and Krister Hellsing

The anterior chamber fluid in cynomolgus monkeys was replaced by a solution of albumin and diodone (iodopyracet) labeled with different isotopes of iodine. After 45 to 120 minutes the chamber fluid was collected and analyzed. The same type of experiment was performed with albumin and myoglobin and with albumin and gamma globulin. In some of the experiments with albumin and myoglobin, the tissues of the eyes were also analyzed. The turnover rate constant for albumin in the anterior chamber was 0.021 minute⁻¹ at a mean intraocular pressure of 13.8 mm Hg. The turnover rate constants for diodone, myoglobin, and gamma globulin, in the same order, were 1.31, 1.023, and 0.997 times that for albumin. In five 90 minute experiments, on an average, 25 per cent (range 12 to 36 per cent) of the labeled albumin that had left the anterior chamber was present in the intraocular tissues and the tissues just outside the eye. For myoglobin the corresponding mean figure was 16 per cent. It is concluded that diodone leaves the anterior chamber both by flow and diffusion but that the proteins leave the anterior chamber essentially by bulk flow. Part of this takes place by way of unconventional routes through the anterior uvea, the suprachoroid, and the sclera. The total volume of the anterior and posterior chambers was on an average 123 μl and the rate of flow of aqueous humor into the anterior chamber was about 1 to 2 μl per minute.

In a previous paper¹ it was reported that there is a bulk outflow of anterior chamber fluid, in part by way of unconventional paths in the cynomolgus monkey when the experiment is conducted shortly after death. The purposes of the present investigation were to try to find a substance that could be used as a label for the bulk flow of aqueous humor in this animal, to determine the rate of flow, and to study the routes for outflow under in vivo conditions.

Methods

Young cynomolgus monkeys (Macaca irus) of both sexes and weighing 0.75 to 2.6 kilograms were employed. The animals were anesthetized with a barbiturate given intravenously (pentobarbital sodium, initial dose 30 to 33 mg per kilogram body weight). Small additional doses were given when required to maintain anesthesia. Heparin, 1,500 IU per kilogram body weight, was given intravenously, and a femoral artery was cannulated for blood sampling. The head of the animal was fixed, and the animal was kept warm with a heating pad. Two cannulas were fired² into the anterior chamber. The tips of the needles were rather close to each other. As shown in Fig. 1, one of the cannulas with one branch connected the anterior chamber to a pressure transducer which could be disconnected by a stopcock. Another branch of this cannula and another cannula connected the eye to a system of two Agla precision syringes. The syringes were coupled rigidly to each other on a frame in such a way that injection of a volume into the eye from one of the syringes was accompanied by withdrawal of the same volume from the anterior

From the Institutes of Pharmacology and Medical Chemistry, University of Uppsala, Uppsala, Sweden.

The present investigation was supported by a grant from the Swedish Medical Research council (Project No. Y 249), and by Research Grant B 3060 from the National Institutes of Neurological Diseases and Blindness, U. S. Public Health Service.
Fig. 1. Schematic presentation of the setup. The anterior chamber was connected through two cannulas and polyethylene tubing to a pressure transducer and a pair of syringes, A and B, which were coupled to each other on a frame.

chamber by the other. At the start of the experiments syringe A of the figure and the tubing leading to A had been filled with the radioactive solution. All other tubings had been filled with a solution similar to that in syringe A but containing no labeled molecules. After the cannulation, the intraocular pressure settled down to a steady value within 10 to 15 minutes. One milliliter of the radioactive solution was injected into the anterior chamber from A with a simultaneous withdrawal of 1 ml. from the anterior chamber with B. During the washing-through the pressure transducer was disconnected from the eye. After 45 to 120 minutes a blood sample was taken from the femoral artery, and the anterior chamber was cannulated with a third cannula. A slight pressure on the eye was produced with a finger, and the fluid dripping out was collected on filter papers that were put into polyethylene test tubes together with which they had been preweighed. The tubes were sealed and reweighed. In some of the experiments the eyes were dissected at the end. The following samples were taken: (1) conjunctiva, fat, and connective tissue from the outside of the sclera; (2) the sclera; (3) the cornea; and (4) the rest of the eye.

Care was taken to avoid contamination with anterior chamber fluid during dissection.

To determine the efficiency of the chamber fluid exchange maneuver, special experiments were performed essentially as described but with emptying of the chambers of the eye immediately after the washing through with the radioactive solution.

The test substances. Diodone (iodopyridone-N-acetic acid, 131I-diodone (iodopyridone-N-acetic acid). 131I-human serum albumin, and 131I-human serum albumin were supplied by Mr. H. Björling, AB Kabi, Sweden. Fifty milligrams of the albumin was labeled with about 1.5 mc. 125I. The reaction mixture was passed through columns of Sephadex G 25. The final protein solution contained 12 mg albumin per milliliter (measured with a biuret reaction) and 80 per cent of the original radioactivity. The labeled albumin was tested with gel electrophoresis and immune electrophoresis, and was unchanged as determined by these methods. Fifteen milligrams of the gamma globulin was labeled with 0.5 mc. 125I and then purified from the reaction mixture and analyzed.

Table I. Data of the substances under study

<table>
<thead>
<tr>
<th>Substance</th>
<th>Coefficient of diffusion $\times 10^7$, 20° C. (cm²/sec.)</th>
<th>Diameter of equivalent sphere (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diodone</td>
<td>About 42</td>
<td>About 10</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>11.3</td>
<td>38</td>
</tr>
<tr>
<td>Human serum albumin</td>
<td>6.1</td>
<td>70</td>
</tr>
<tr>
<td>Human serum gamma globulin</td>
<td>3.8</td>
<td>111</td>
</tr>
</tbody>
</table>

Diffusion coefficient and diameter of the equivalent sphere.

For the proteins the diffusion coefficients were collected from Edsall. For the diodone, which has an ionic weight of 404, the diffusion coefficient was assumed to be about the same as that for sucrose, which has a molecular weight of 342. The diameters of the equivalent spheres were calculated from Stock's formula.

Protein labeling with 125I was performed according to McFarlane.

Human serum albumin and human serum 7-S-gamma globulin were supplied by Mr. H. Björling, AB Kabi, Sweden. Fifty milligrams of the albumin was labeled with about 1.5 mc. 125I. The reaction mixture was passed through columns of Sephadex G 25. The final protein solution contained 12 mg albumin per milliliter (measured with a biuret reaction) and 80 per cent of the original radioactivity. The labeled albumin was tested with gel electrophoresis and immune electrophoresis, and was unchanged as determined by these methods. Fifteen milligrams of the gamma globulin was labeled with 0.5 mc. 125I and then purified from the reaction mixture and analyzed.
as the albumin. Its final concentration was 0.5 mg. per milliliter and approximately 65 per cent of the radioactivity was incorporated.

Ammonium sulfate precipitated horse myoglobin was supplied by Professor H. Theorell of Stockholm. It was dialyzed against 0.2M NaCl containing one drop of concentrated ammonia per liter; 15 mg. was labeled with 0.5 mc. 125 I. The reaction mixture was passed through a column of Deacidite FF. The labeled solution contained approximately 5 mg. myoglobin per milliliter, measured spectrophotometrically at 408 nm, and about 70 per cent of the original radioactivity. The labeled myoglobin was compared in gel electrophoresis with the original myoglobin. No difference could be detected.

Immediately before the experiments, the proteins to be injected were transferred from the original solutions into an aqueous replacement fluid (ABF) described by Barany. A Sephadex column G 25 was flooded with the latter solution. The column was then loaded with a small volume of the solution of labeled protein and flooded again with ARF. The proteins, which were the only large molecules in the solutions, passed the column much faster than the other constituents. As a consequence they were lifted over into the ARF. Carriers were added both for the proteins and the diodone so that in the final solution the carrier concentration was 0.2 per cent for each molecule under study.

Assay of radioactive isotopes. The samples were assayed with a two-channel gamma spectrometer with a well-type crystal, one channel taking the 0.36 mev. peak of 131 I and the other the 0.035 mev. peak of 123 I.

Results

Seven experiments were performed with emptying of the chambers immediately after the washing-through with the 131 I-albumin solution. The mean volume obtained from the eye was 123.1 ± 6.1 µl and the mean albumin concentration as compared with that in the fluid washed into the eye was 60.6 ± 3.0 per cent. As a consequence of these findings it was assumed in the experiments below that after the washing-through the mean concentration in the chamber fluid of the labeled substances was 0.6 times the concentration in the fluid washed into the eye.

The turnover rate constant for albumin. The fall in albumin concentration after its injection into the anterior chamber was observed in 21 experiments. It was assumed that the albumin left the eye exponentially so that at the end of the experiments

\[ C_{AF} = 100 \cdot e^{-KA t} \]

where \( C_{AF} \) was the final concentration of labeled albumin in the fluid collected from the eye as per cent of the initial concentration, \( K_A \), the turnover rate constant for albumin and \( t \) time in minutes. The mean value for \( K_A \) (± standard error of the mean) was 0.0205 ± 0.0016. The mean intraocular pressure was 13.8 ± 0.5 mm. Hg.

Albumin and diodone. It was assumed also that the diodone left the anterior chamber in an exponential way so that the final concentration, \( C_{DF} \), in the fluid collected from the eye was

\[ C_{DF} = 100 \cdot e^{-KD t} \]

where \( K_D \) was the turnover rate constant for diodone. Then, since the albumin and the diodone had been in the anterior chamber for the same period of time, by the end of the experiments:

\[ KD \approx e \log 100 - e \log C_{DF} / e \log 100 - e \log C_{AF} \]

Four experiments were performed. The mean value for \( \frac{K_D}{K_A} \) was 1.308 ± 0.049. At the end of the experiments the concentration of the labeled substances in the blood was less than 3 per cent of that recovered from the chambers of the eye.

Albumin and myoglobin. If the turnover rate constant for myoglobin is called \( K_M \)
the ratio, $K_M/K_A$, had a mean value of $1.0227 \pm 0.0081$ with the 0.01 confidence interval $1.0499 - 0.9955$. Nine experiments were made. At the end of the experiments the concentration of the labeled substances in the blood was less than 3 per cent of that in the fluid collected from the eye. In five of the experiments the chambers of the eye were emptied 90 minutes after the washing-through and the eyes were dissected. Table II shows that on an average 11.9 per cent of the labeled albumin originally present within the anterior chamber remained in the aqueous humor. Thus 88.1 per cent had disappeared. The apparent volume of original chamber fluid present in the intraocular tissues and in the episcleral tissues, as determined from the content of labeled albumin, was 26.5 µl. With a total chamber volume of 123 µl, this means that 21.5 per cent of the albumin originally present in the anterior chamber was recovered in the tissues investigated. Thus 24.5 per cent of the albumin that had left the anterior chamber remained in the eye or in the tissues close to the eye. For myoglobin the corresponding figure was 15.6 per cent.

**Albumin and gamma globulin.** Eight experiments were performed. If the turnover rate constant for gamma globulin is called $K_\gamma$, the mean ratio, $K_\gamma/K_A$, was 0.9973 ± 0.0065 with the 0.01 confidence interval $1.0202 - 0.9775$. At the end of the experiments the concentration in the blood of the labeled substances was less than 5 per cent of that collected from the eye.

**Discussion**

The outflow paths. It cannot be excluded that, in spite of all precautions, the fluid washed into the eye or the mechanical trauma may have opened channels which are not normally present.

The fact that myoglobin, albumin, and gamma globulin left the anterior chamber at very similar rates in spite of large differences in diffusion coefficients indicates that all the three substances were carried out of the anterior chamber mainly by bulk flow. For reasons that have been discussed previously, this does not exclude the possibility that some protein may have passed through the endothelium of Schlemm's canal by way of narrow pores that produce molecular sieving or with pinocytosis.

The volume of the channels usually thought to carry the aqueous humor into extraocular veins, where the flow of blood is rapid, is not known exactly, but can be assumed to be a few microliters. The concentration of labeled substances in the fluid in the vessels at the end of the experiments presumably was very little different from

---

**Table II.** The concentration of labeled albumin and myoglobin in the fluid collected from the eye as per cent of the original mean anterior and posterior chamber fluid concentrations

<table>
<thead>
<tr>
<th>Exper. No.</th>
<th>Time (min.)</th>
<th>Final chamber fluid concentration (%)</th>
<th>Apparent volume of chamber fluid (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Extraocular tissues</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Albumin</td>
</tr>
<tr>
<td>1</td>
<td>90</td>
<td>21.4</td>
<td>21.5</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>6.8</td>
<td>6.1</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>4.8</td>
<td>4.6</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>7.5</td>
<td>6.7</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>19.2</td>
<td>17.7</td>
</tr>
<tr>
<td>Mean</td>
<td>90</td>
<td>11.9</td>
<td>11.3</td>
</tr>
</tbody>
</table>

The table also shows the apparent volumes of original chamber fluid recovered in the tissues investigated as calculated from their contents of labeled albumin and myoglobin.
that in the anterior chamber. Because, at the end the anterior chamber, fluid concentration of labeled substances was low, only negligible quantities of labeled material could have been present within the tissues because of fluid in the aqueous veins. Nonetheless, in the experiments with dissection of the eyes, for albumin, as much as 25 per cent of the amount that had left the anterior chamber in 90 minutes was present within the tissues investigated, and for myoglobin the corresponding figure was 16 per cent. It follows that nearly all of this must have been present in the tissue fluids.

Now, since the labeled proteins appeared to leave the anterior chamber by bulk flow, and since they were recovered to a large extent in the tissues investigated, it may be concluded that the bulk flow from the anterior chamber in part went by way of unconventional routes through the tissues investigated. Such a drainage has been found previously in dead eyes. The results of those experiments indicated that anterior chamber fluid could pass out not only through the conventional paths but also through other holes in the anterior sclera as well as the posterior. To reach the holes, part of the fluid had to pass through the anterior uvea and the suprachoroid. It seems very likely that there was a similar drainage in the present experiments.

An upper limit for the drainage from the anterior chamber by way of unconventional paths cannot be determined from the present experiments because some of the labeled material drained by these routes may have left the tissues investigated before these were dissected. It is apparent, however, that in the present experiments, with a mean intraocular pressure of 14 mm. Hg, unconventional drainage accounted for on an average 25 per cent or more of the total drainage of anterior chamber fluid.

That labeled fluid passed out of the anterior chamber by way of nonconventional routes does not mean it was drained from the eye unchanged with respect to composition and volume. The low content of labeled myoglobin as compared with that of labeled albumin in the tissues investigated demonstrates one change, probably due to diffusion of myoglobulin into the blood vessels.

Leboucq has reported that in rabbits colloids could enter the anterior uvea by flow from the anterior chamber but there was no penetration into the suprachoroid; the tendon of the ciliary muscle was thought to constitute an anatomical barrier to penetration behind the anterior uvea. However, according to Fowlks and Havener, who also have reported evidence for a flow into the anterior uvea, there is no such anatomical barrier to penetration behind the anterior uvea. They reported "the continuity of aqueous-filled spaces from the anterior chamber through the full length of the ciliary body and into the suprachoroid." Since the pressure in the suprachoroid as determined in cats is lower than that in the anterior chamber, it remains to be explained why in Leboucq's experiments there was no flow into the suprachoroid.

The tendency shown by the myoglobin in the present experiments to leave the anterior chamber somewhat faster than albumin may be explained entirely by its easier penetration into the cornea. It also seems likely that myoglobin had some tendency to enter the iris capillaries, and that there was some diffusion of myoglobin into the iris.

Diodone left the anterior chamber at a rate that was, on an average, 31 per cent higher than that for albumin. This was no doubt due to two factors: a rapid diffusion into the tissues around the anterior chamber, and absorption by the blood vessels. That the former occurred seems very likely from the finding in in vitro experiments that diodone rapidly becomes distributed within the large volumes of the cornea and the anterior sclera. That the latter took place seems likely from the fact that, although diodone is actively transported out of the posterior chamber, it accumulates
within the anterior chamber after intravenous administration and in steady state reaches a concentration that is about 14 per cent of that in plasma. Diodone can thus pass through the iris vessels. It is also possible that there was some drainage of diodone from the anterior chamber caused by ultrafiltration through very small pores not permitting the proteins to enter.

The tendency of the diodone to leave the anterior chamber by diffusion makes it unsuitable for studies of the rate of flow of aqueous humor with the present technique. The proteins seem preferable although even they, no doubt, have a tendency to diffuse into the tissues round the anterior chamber and in addition may not enter all paths for aqueous drainage.

**The pore size.** In vitro experiments have demonstrated that albumin and gamma globulin pass from the anterior chamber into Schlemm's canal with very little if any molecular sieving. It was calculated that less than 10 per cent of the total drainage into Schlemm's canal passed by way of pores with an apparent diameter of less than 0.16 μ. The present results suggest that also under in vivo conditions, and when drainage into unconventional paths is taken into account, there is very little, if any, drainage through pores which produce sieving of proteins at their entrance. It is possible, however, that there are unconventional paths which do not permit any of the proteins to enter. The importance of such channels remains to be determined.

**The rate of flow of aqueous humor.** If all drainage by way of paths not permitting proteins to enter freely is neglected, and if it is assumed that there was no net gain of fluid from any of the tissues surrounding the anterior chamber, it is possible to calculate an approximate figure for the flow of aqueous humor from the posterior chamber into the anterior chamber using data for the volume of the anterior chamber and the mean turnover rate constant for albumin in all the 21 experiments. The latter was 0.021; the former can be calculated. In the experiments with emptying of the chambers of the eye immediately after the washing-through, on an average 123 μl was obtained and the concentration of labeled albumin in the fluid was about 60 per cent of that in the fluid just washed into the anterior chamber. If all of the original anterior chamber aqueous humor had been replaced by the labeled solution, the low concentration would have been caused only by the posterior chamber fluid, and the volume of the anterior chamber would have been 0.6 × 123 μl = 74 μl. The rate of flow of aqueous humor then would have been 74 × 0.021 μl per minute = 1.6 μl per minute. It seems possible that during the washing-through there was not ideal mixing in the anterior chamber and that the solution did not leave the anterior chamber strictly exponentially. With this taken into account, it is suggested that the rate of production of aqueous humor was about 1 to 2 μl per minute.

Thanks are due Miss Britta Axelsson for valuable technical assistance.

**REFERENCES**

