The elimination of red cells from the anterior chamber in vervet monkeys (Cercopithecus ethiops)

Anders Bill

The elimination of $^{11}$Cr-labeled red cells from the anterior chamber was compared with that of $^{125}$I-labeled albumin in in vitro and in vivo experiments. A reservoir was used in the in vitro experiments to give an inflow into the anterior chamber of about 5 µl per minute. The concentration of labeled material in the anterior chamber was maintained at a steady level with syringes coupled in push-pull. The concentration of red cells in the fluid collected on the denuded sclera increased with time. Over a 10 minute period the average amount of red cells recovered was 71 ± 6 per cent of that expected from the amount of labeled albumin. In in vivo experiments with the eyes at their spontaneous pressures, 5 to 10 per cent red cells in the anterior chamber fluid gave a pressure rise of about 6 mm Hg over a 60 minute period. The amount of labeled albumin recovered in the general circulation after 1 hour corresponded to that in about 50 µl anterior chamber fluid. The amount of labeled red cells recovered in the blood was 61 ± 5 per cent of that expected if the red cells had passed out of the eye without selective retention in the filtering structures. It is pointed out that addition of red cells to the anterior chamber fluid may become useful in experimental studies, e.g., on the relationship between eye pressure and rate of aqueous humor formation and in studies on the mode of action of drugs that influence the resistance of the drainage paths for aqueous humor.

In enucleated human eyes rigid particles with a diameter of 1.5 µm can pass through the trabecular meshwork into the canal of Schlemm. In rabbits, man, and cattle during the elimination of chromium-phosphate particles from the anterior chamber, particles with a diameter of more than 0.1 µm tend to be retained in the filtering region, and it is possible that even 0.1 µm particles are to some extent held back. Clinically, it is well known that red cells can leave the anterior chamber, and it has been demonstrated that this is mainly due to flow of intact cells into the general circulation. The cells seem to be squeezed through the narrow paths that connect the chamber angle with the canal of Schlemm. Burton has shown that theoretically the elasticity of red cells makes it possible for them to pass intact through long 2.5 µm pores. With his mathematics it can be calculated that with pore lengths of less than 20 to 25 µm red cells can pass undamaged through even narrower pores.

In Hörven's studies it was found that up to 81 per cent of the erythrocytes injected into the anterior chamber of human
eyes passed out of the eye intact within one day. No more detailed data seem to be available on the degree of restriction to the passage of red cells that is exerted by the filtering structures.

In monkeys albumin passes out from the anterior chamber with the aqueous humor into the general circulation without any detectable restriction. The purpose of the investigation reported here was to compare the rate of elimination of red cells with that of albumin to elucidate to what extent red cells are retained by the filtering tissues in the chamber angle of the monkey eye.

Methods

In vivo experiments. Vervet monkeys (Cercopithecus aethiops) of both sexes and weighing 1.8 to 2.3 kilograms were employed. The animals were anesthetized in the cages by intramuscular sodium methohexitol (Brietal sodium, Eli Lilly & Company), 20 mg. per kilogram body weight. A tail vein was cannulated for intravenous injections and a femoral artery was cannulated for blood pressure measurements and sampling of arterial blood. After 15 to 20 minutes, the Brietal anesthesia became too light and pentobarbital sodium was injected intravenously. Several small doses were given in such a way that the arterial blood pressure was not depressed. Heparin, 1,500 I.U. per kilogram per body weight, was given intravenously to prevent clotting. A needle gun was used to cannulate one eye with three cannulas. One cannula connected the eye to a pressure transducer and a reservoir that was continuously weighed. The other two cannulas connected the anterior chamber to 2 syringes connected in push-pull in such a way that fluid could be washed through the eye from one syringe to the other without significant changes in intraocular pressure or volume. The volume of the fluid in the syringes and the tubes connecting them was 1.5 ml. The reservoir was first connected to the eye and adjusted to give an intraocular pressure of 10 mm. Hg during the rest of the experiment. The reservoir was then disconnected. The syringes contained 51Cr-labeled red cells and 125I-labeled human serum albumin in isotonic saline. The cells constituted 5 to 10 percent of the total volume. The concentration of albumin was about 0.1 percent. After the eye had attained its spontaneous resting pressure, the contents of the syringes and the anterior chamber were mixed continuously for 1 hour. To prevent sedimentation of the red cells in the syringes, these were rotated. Sedimentation of the red cells in the anterior chamber was prevented by adjusting the cannulas in such a way that there was a continuous movement of the fluid in the lower part of the anterior chamber.

Samples of the fluid in the syringes were taken after the first mixing and 30 and 55 minutes later. One hour after the first mixing the labeled substances were washed out of the anterior chamber with the syringes the contents of which had been replaced by inactive fluid. Blood samples were taken, and in 3 experiments the animal was killed and the eye and the episcleral tissues were dissected. In 5 experiments 0.3 ml. of the radioactive fluid that had previously been washed through the anterior chamber was injected intravenously. Thirty minutes later new blood samples were collected; the animals were killed by an overdose of pentobarbital sodium and the eyes and the episcleral tissues were dissected.

In vitro experiments. The eye was removed and placed on a bed of cotton wool. The anterior chamber was cannulated with three cannulas connecting it to an arrangement of syringes similar to that in the previous experiments and to a reservoir. The episcleral tissues were removed from the eye. The intraocular pressure was set at 30 mm. Hg with the reservoir. The content of the anterior chamber was then mixed continuously with 51Cr-labeled red cells and 125I-labeled albumin with the syringes. The volume of the fluid in the syringes and the tubes connecting them with the anterior chamber was 3 ml. Fluid that appeared on the sclera from several opened blood vessels was collected on filter papers every 2 minutes. For technical reasons all fluid that emerged could not be collected. The height of the reservoir over the eye was adjusted to give an inflow into the eye of about 5 μl per minute. After 30 minutes the fluid in the syringes was washed out of the anterior chamber and the eye was dissected. Samples of the fluid in the syringes were collected after the first mixing and at the end of the 30 minute period with labeled material in the anterior chamber fluid.

Solutions. In each experiment the red cells to be infused into the anterior chamber were obtained from the animal to be used. The cells were incubated for 30 minutes with sodium chromate (51Cr), washed three times with the basal solution of the experiments, and then again suspended in that solution. The labeling of human serum albumin with 125I was performed by Dr. K. Hellring. In all experiments before the protein was used, any radioactive iodine not bound to protein was removed by gel filtration with Sephadex G-25.

Assay. Two-channel gamma spectrometry was used to analyze blood and tissue samples for 35S and 51Cr. The radioactivity of the samples was determined after drying. All samples were small and of similar size which made corrections for
absorption of radiation in the samples unnecessary. All blood samples and samples of anterior chamber fluid were counted long enough to make the counting error less than 2 per cent. For most tissue samples the error was less than 5 per cent.

The content of red cells in the fluids used was determined by a microhematocrit centrifuge, 9,000 g for 5 minutes.

Calculations.

In vivo experiments. Because of differences in the concentration of red cells in large and small blood vessels and to leakage of albumin from the capillaries, it could be expected that the labeled red cells and the labeled albumin that passed out of the anterior chamber became distributed in different volumes. The intravenous injection of the labeled substances in some of the experiments served to give approximate values for the virtual spaces that had the same concentration of labeled red cells and albumin as whole blood collected from the femoral artery. It can be presumed that the 30 minute spaces determined were very similar to the pertinent spaces, that is those after 1 hour with almost constant rate of intravenous administration of labeled red cells and labeled albumin.

The 30 minute distribution space for red cells, \( V_{cr} \), in each of the experiments with an intravenous injection of labeled material was:

\[
V_{cr} = \frac{Q_{cr}}{C_{cr, ti}}
\]

where \( Q_{cr} \) was the amount of \( ^{51} \text{Cr} \)-labeled red cells in counts per minute injected intravenously, \( C_{cr, ti} \) and \( C_{cr, w} \) were the concentrations of labeled red cells in counts per minute per gram in the blood samples collected 60 and 90 minutes after the commencement of the perfusion of the anterior chamber.

The distribution space of the red cells was expressed as the per cent of the body weight. The average percentage was \( F_{cr} \). The average percentage for the distribution volume of labeled albumin, \( F_{al} \), was determined analogously.

In each experiment, at the end of the hour with labeled material in the anterior chamber, the volume of red cells from the anterior chamber fluid that appeared to be present in the general circulation \( A_{r, ti} \) was:

\[
A_{r, ti} = \frac{F_{cr} \cdot B.W. \cdot C_{cr, w}}{C_{cr, ti}} \cdot 100
\]

where \( C_{cr, ti} \) and \( C_{cr, w} \) were the concentration, in counts per minute per microliter cell-free anterior chamber fluid, \( C_{cr, ti} \) and \( C_{cr, w} \) were the concentrations of labeled red cells and cell-free fluid, respectively, in the anterior chamber fluid.

The corresponding volume, \( B_{rc, ti} \), that would have been recovered in the general circulation if there had been no selective restriction to the cells in the filtering region was calculated with the labeled albumin data:

\[
B_{rc, ti} = \frac{Q_{al, ti}}{C_{al, ti}} \cdot \frac{100}{C_{al, w}}
\]

In vitro experiments. The concentration of \( ^{51} \text{Cr} \) in counts per minute per microliter red cells in the anterior chamber fluid during the experiments; this fall was of only a few per cent due to the large external volume with which the anterior chamber fluid was mixed continuously.

The volume of cell-free anterior chamber fluid, \( A_{r, ti} \), that was recovered in the general circulation including parts of the extravascular albumin space, was:

\[
A_{r, ti} = \frac{F_{al} \cdot B.W. \cdot C_{al, w}}{C_{al, ti}} \cdot 100
\]

where \( C_{al, w} \) was the concentration, in counts per minute per gram of labeled albumin in the blood sample collected 60 minutes after the first mixing of anterior chamber fluid and the contents of the syringes. \( C_{al, ti} \) was the concentration of labeled albumin, in counts per minute per microliter cell-free anterior chamber fluid.

The volume of cell-free anterior chamber fluid recovered in the ocular and episcleral tissues \( A_{r, ti} \) was:

\[
A_{r, ti} = \frac{Q_{al, ti}}{C_{al, ti}} \cdot \frac{100}{C_{al, w}}
\]

In vivo experiments. Six experiments were performed. Fig. 1 shows that at the start the concentration of red cells in the fluid emerging on the sclera was 35 to 70 per cent of that in the anterior chamber. In most experiments the concentration increased with time. The average value during the first 10 minutes was determined in
Red cells in the anterior chamber

Fig. 1. The anterior chambers in enucleated eyes from vervet monkeys were perfused with \( ^{51} \text{Cr} \)-labeled red cells and \( ^{125} \text{I} \)-labeled albumin. The fluid passing out from the anterior chamber via the episcleral veins was collected. The ratio labeled red cells in counts per minute to labeled albumin in counts per minute in each sample, \( R_S \), was divided by the same ratio for the anterior chamber fluid, \( R_t \). The resulting ratios of ratios in 6 experiments are shown.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Labeled red cells (pl)</th>
<th>Cell-free fluid (pl)</th>
<th>Labeled red cells (pl)</th>
<th>Cell-free fluid (pl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.99</td>
<td>9.5</td>
<td>0.098</td>
<td>3.2</td>
</tr>
<tr>
<td>2</td>
<td>3.64</td>
<td>17.3</td>
<td>1.02</td>
<td>9.1</td>
</tr>
<tr>
<td>3</td>
<td>5.76</td>
<td>21.8</td>
<td>0.85</td>
<td>8.7</td>
</tr>
<tr>
<td>4</td>
<td>2.47</td>
<td>20.5</td>
<td>0.69</td>
<td>14.3</td>
</tr>
<tr>
<td>5</td>
<td>0.93</td>
<td>8.6</td>
<td>0.69</td>
<td>14.3</td>
</tr>
<tr>
<td>6</td>
<td>1.77</td>
<td>14.1</td>
<td>0.35</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Table I

The apparent content of labeled red cells from the anterior chamber fluid and cell-free such fluid in the ciliary body and in the choroid-retina preparation in 6 in vitro experiments. The anterior chamber had been perfused for 30 minutes with \( ^{51} \text{Cr} \)-labeled red cells and \( ^{125} \text{I} \)-labeled albumin.

each experiment. The mean value of these averages was 70.8 ± 5.3 per cent. Table I shows that large amounts of labeled material were recovered in the ciliary body and the choroid-retina preparation.

Living eyes. Eight experiments were performed. The mean arterial blood pressure at the start of the experiments was 80.8 ± 5.8 mm. Hg. There was no significant change during the experiments. In the experiments with an intravenous injection of labeled material the 30 minute distribution space for \( ^{125} \text{I} \)-albumin was 7.1 ± 0.5 per cent of the body weight. For \( ^{51} \text{Cr} \)-labeled red cells the 30 minute distribution space was 4.6 ± 0.4 per cent of the body weight or 65.0 ± 2.4 per cent of the 30 minute distribution space of labeled albumin. Table II shows that the volume of red cells from the anterior chamber that was recovered in the general circulation and in the ocular and episcleral tissues was lower when calculated from the \( ^{51} \text{Cr} \) data than expected from the \( ^{125} \text{I} \) data. This indicates that also in living eyes the red cells tended to be retained within the anterior chamber. The
Table II

<table>
<thead>
<tr>
<th></th>
<th>Labeled red cells, observed (μl)</th>
<th>Labeled cell-free fluid, observed (μl)</th>
<th>Labeled red cells, expected (μl)</th>
<th>Labeled red cells, observed/expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>General circulation</td>
<td>2.1 ± 0.21</td>
<td>45.8 ± 3.9</td>
<td>3.43 ± 0.29</td>
<td>0.61 ± 0.05</td>
</tr>
<tr>
<td>Ocular and episcleral tissues</td>
<td>0.69 ± 0.13</td>
<td>10.5 ± 1.7</td>
<td>0.79 ± 0.13</td>
<td>0.87 ± 0.12</td>
</tr>
</tbody>
</table>

The apparent content of red cells from the anterior chamber fluid and cell-free such fluid in the general circulation and in the ocular and episcleral tissues after 1 hour of perfusion of the anterior chamber with 51Cr-labeled red cells and 131I-albumin. The contents of red cells expected if there had been no selective restriction to the cells in the filtering region of the eye are also shown and the ratio observed/expected. All limits are ± the standard error of the mean.

Outflow of red cells was on an average about 35 per cent lower than expected from the outflow of albumin. During these experiments the intraocular pressure rose from a steady level of 10.6 ± 1.7 mm. Hg at the start to 16.2 ± 1.7 mm. Hg at the end. In 3 of the experiments the concentration of 51Cr in plasma at 60 minutes was determined. It was less than 4 per cent of the concentration of 51Cr in whole blood. Almost all of the 51Cr thus remained within the red cells after these had passed from the anterior chamber into the general circulation.

Discussion

In the in vitro experiments at least two factors may have contributed to give a rising concentration of red cells in the fluid collected on the sclera. First, with time most small pores through the filtering region may have been occluded by red cells. Second, since the fluid in the region of the intertrabecular space most probably could not be adequately circulated from the anterior chamber, it seems likely that with time the concentration of red cells within this space rose. As a consequence, after some time, the fluid at the entrance of the most narrow part of the outflow routes, which is probably the wall of Schlemm's canal, may have had a higher concentration of red cells than the fluid in the anterior chamber. The results indicate that to get a clear picture of the size distribution of the pores that function under normal conditions, it will be necessary to investigate in pairs a small particle that can pass out with no retention with increasingly larger ones which can be suspected to be retained in the filter. In addition, in such studies, if the larger particle tends to be stopped in the filtering region, it is the initial outflow that is of major interest.

In the in vivo experiments after 60 minutes with red cells in the anterior chamber, the total content of labeled red cells in the blood was 61 ± 5 per cent of that expected from the content of labeled albumin. In the in vitro experiments the total outflow from the anterior chamber during the first 10 minutes was about the same as that during 60 minutes in the in vivo experiments, that is about 50 μl. The average red cell content in fluid collected on the sclera during this time was 70.8 ± 5.3 per cent of that in the anterior chamber. The in vivo and the in vitro experiments are not quite comparable because of the higher rate of uveoscleral flow after death, but it is of interest to note that the difference between the average values in the in vivo and the in vitro experiments was moderate and not statistically significant which suggests that the size distribution of the functional pores immediately after death is not very different from that in vivo. In the in vitro experiments considerable amounts of red cells were recovered in the ciliary body and in the choroid-retina preparation, which indicates that the red cells can enter the uveoscleral routes for aqueous humor drainage. If and to what extent red cells...
can pass through these routes into the episcleral tissues could not be ascertained. In the in vivo experiments with labeled cells there was very little flow into the uveoscleral routes both of labeled cells and labeled albumin which was most probably due to a tone in the ciliary muscle. When contracted, the ciliary muscle prevents the flow through the uveoscleral routes to a very large extent. In addition the red cells may have tended to occlude also these channels.

Most probably two factors contributed to raise the intraocular pressure in the in vivo experiments: partial blockade of the outflow routes and a high effective viscosity of the fluid passing out. The ability of red cells to raise the intraocular pressure has already been used to study the effects of a pressure rise on aqueous humor dynamics. It seems possible that addition of red cells to the anterior chamber fluid may become useful also in studies on the mode of action of drugs that influence the resistance of the drainage paths of aqueous humor. For example, drugs which tend to widen the pores will serve to reduce the tendency to red cell retention and maybe also reduce the effective viscosity of the fluid containing red cells.

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REFERENCES