SECRETION AND RATE OF FLOW OF AQUEOUS HUMOUR IN THE CAT*

BY

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KNOWLEDGE of the means by which substances enter and leave the eye, usually referred to as "aqueous-humour dynamics", is essential for an understanding of the factors contributing to the control of the intra-ocular pressure.

In non-living systems, when a membrane separates two concentrations of a substance in a solution to which the membrane is permeable, this substance travels across the membrane until the concentrations on either side are equal; chemical equilibrium is then said to exist. In the living state this type of equilibrium is seldom found; instead, a dynamic equilibrium occurs in which there is no further change in concentration of the solute across the membrane or barrier in spite of a difference of concentrations. An equilibrium of this nature is generally referred to as a "steady-state". The concentration of urea in the aqueous humour, for example, is less than in the blood plasma, which indicates that, so far as this substance is concerned, a chemical equilibrium does not exist.

It is agreed that small quantities of aqueous humour are continually being formed and eliminated from the eye. In contrast to the complex problem of the formation of the individual components of the aqueous humour, elimination is thought to consist of a simple drainage in bulk, i.e., a unidirectional flow, involving no separation of its constituents. Experimentally, the determination of this rate of drainage has presented difficulties. The problem of maintaining the eye in a physiological condition precludes measurement of the rate of flow of the aqueous humour by the direct introduction of a cannula. The rate at which a slowly penetrating substance accumulates in the aqueous humour and the nature of the distribution ration in the steady-state may be used, with certain assumptions, to derive the approximate rate of flow of the aqueous humour, as has been shown by Bárány and Kinsey (1949). In this connection, the ratio of the concentration of a substance in the aqueous humour and in the water of the plasma when the concentration in the aqueous humour is no longer rising, is generally known as the equilibrium ratio ($R_{eq}$). The rates of accumulation of cysteine and ascorbic acid have been studied from this viewpoint in the present work.

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METHODS

Experiments were carried out on cats weighing approximately 7 lb., nembutal solution being used as the general anaesthetic. Neutralized, isotonic solutions of ascorbic acid and cysteine hydrochloride were injected intravenously in order to maintain raised concentrations in the blood plasma. Owing to rapid loss of cysteine through the kidneys it was found necessary to ligature the renal arteries. Experimental results were rejected if the samples of aqueous humour contained significant amounts of protein.

Analytical.—Solutions of ascorbic acid and cysteine were neutralized to pH 7.2 and diluted to an isotonic concentration immediately before the experiment. Ascorbic acid was estimated by a modification of the electrometric microtitration procedure of Mindlin and Butler (1938) in which a solution of 2.6 dichlorophenol-indophenol is used as oxidant. Cysteine was estimated by an application of a standard iodate technique. Although not specific for cysteine, this method was considered very suitable for the present study where marked increases in concentration of this substance in the blood plasma and the aqueous humour were being measured. The method comprised an initial precipitation of protein by 22 per cent. sulphosalicylic acid solution followed by the oxidation of the protein-free sample with an excess of standard iodate solution. The excess iodate was then used to liberate iodine from a potassium iodide solution which was estimated using a standard thiosulphate solution. Using the electrometric titration apparatus, the end point of the microtitration was determined colorimetrically with a starch solution as indicator. Estimation of either ascorbic acid or cysteine by the techniques described could be completed within 15 minutes of the withdrawal of the blood sample, which enabled the operator to keep a constant check on the concentration in the plasma, and, when necessary, to adjust the rate of intravenous injection.

The extent to which these substances are bound or adsorbed to the protein was investigated using a dialysis chamber as described by Davson and others (1949a, b), isotonic saline being employed as the equilibrating fluid. In neither case could any significant degree of absorption on to the plasma proteins be demonstrated.

Theoretical.—The composition of the aqueous humour at any given time is determined by a balance between the rate at which substances enter and leave the aqueous humour. It is obvious that the rate at which a substance accumulates in a given time is given by the product of the constant of penetration \((k_{in})\) and the concentration in the plasma, less the product of the constant of penetration outwards \((k_{out})\) and its concentration in the aqueous humour.

In the case of a given solute this relationship may be expressed as a simple equation:

\[
\frac{dC_{aq}}{dt} = k_{in} C_{pl} - k_{out} C_{aq}
\]

(1)

At the steady-state equilibrium

\[
\frac{dC_{aq}}{dt} = 0;
\]

hence

\[
k_{in} \frac{C_{aq}}{C_{pl}} = k_{out} \frac{C_{aq}}{C_{pl}} = R_{eq}.
\]

By substituting for \(k_{out}\) in equation (1) we have:

\[
\frac{dC_{aq}}{dt} = k_{in} (C_{pl} - \frac{C_{aq}}{R_{eq}})
\]

which, on integrating between the limits of \(T_{1}\) and \(T_{2}\), yields the equation:

\[
k_{in} = \frac{R_{eq}}{T_{2} - T_{1}} \ln \left( \frac{R_{eq} C_{pl} - C_{aq}}{R_{eq} C_{pl} - C_{aq}} \right) \text{ if } C_{pl}\text{ be constant.}
\]
AQUEOUS HUMOUR IN THE CAT

In the present study $R_{eq}$ and $k_{in}$ have been determined, and $k_{out}$ then calculated with the use of the above relationship between $k_{in}$, $k_{out}$, and $R_{eq}$.

It follows that, for a substance entering the eye by a simple diffusion process, $R_{eq}$ will be independent of the concentration of the substance in the plasma. A simple diffusion process may thus be recognized by this fact. In contrast, for other modes of entry, whether by active transfer or partly by active transfer and partly by diffusion, $R_{eq}$ will be determined not only by the concentration of the substance in the blood plasma but also by the capacity of the secretory mechanism.

The above equations must be considered to represent the transfer mechanism as a first approximation only, for it involves assumptions which are not necessarily strictly true (Langham, 1949). In the present work, the limitations of the equations have been much reduced by using it only to compare $R_{eq}$ for two substances entering the aqueous humour at a similar rate.

RESULTS

The concentration of ascorbic acid in the aqueous humour of the cat varies between 0.5 and 2.0 mg. per 100 ml., and the normal plasma values between 0.5 and 1.0 mg. per 100 ml. In the rabbit, on the other hand, the concentration in the aqueous humour is some thirty times higher than in the plasma (Kinsey, 1947). Thus, although the concentration of ascorbic acid in the aqueous humour of the former animal is very much less than that found in the rabbit, there is evidence of accumulation on a smaller scale, the equilibrium ratio having a value equal to or even greater than unity. The iodate method showed that the aqueous humour of the cat contains an equivalent of 1.0 to 2.0 mg. of cysteine per 100 ml. (ten experiments). Blood samples were always taken before the commencement of the experiment in order to make the necessary adjustment. Corrections in the case of the aqueous humour were made by taking an average value of 1.5 mg. per 100 ml. The equilibrium distribution ratios for cysteine and ascorbic acid are recorded in Tables I and II. The first sample of aqueous humour was withdrawn at the end of $3\frac{1}{2}$ hrs and the second after $4\frac{1}{2}$ hrs. The results for cysteine indicate that the

<table>
<thead>
<tr>
<th>Plasma Range</th>
<th>$R_{eq1}$</th>
<th>$R_{eq2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>23—28</td>
<td>0.58</td>
<td>0.52</td>
</tr>
<tr>
<td>30—35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>35—40</td>
<td>0.41</td>
<td>0.38</td>
</tr>
<tr>
<td>40—45</td>
<td>0.45</td>
<td>—</td>
</tr>
<tr>
<td>70—75</td>
<td>0.43</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Mean: 0.43 ± 0.03 (9)

Results in the tables are expressed in mg. per cent. Averages are expressed as an arithmetic mean plus the standard error for $n$ number of experiments (in brackets).
value of the equilibrium ratio is independent of the concentration of cysteine in the plasma, a finding suggestive of a simple diffusion process. In contrast, the equilibrium value was found to decrease with a rise in the concentration of ascorbic acid in the plasma. This result suggests an entry both by active transfer and by diffusion. The capacity of the former process is readily saturated, thus allowing the process of simple diffusion to govern the value of the equilibrium ratio when the concentration of ascorbic acid in the plasma is high.

The coefficients of transfer from the plasma to the aqueous humour ($k_{in}$) were derived from short-term experiments, the first aqueous humour being withdrawn after 20 minutes and the second after 40 minutes (Table III). High concentrations of ascorbic acid in the plasma were used in these experiments to minimize the contribution of secretory activity. Values of $k_{out}$ were derived from $k_{in}$ and $R_{eq}$.

### TABLE III

COEFFICIENTS OF TRANSFER ($k_{in}$) FROM PLASMA TO AQUEOUS

<table>
<thead>
<tr>
<th>Ascorbic Acid</th>
<th>Cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9 x 10^{-3}</td>
<td>11.2 x 10^{-3}</td>
</tr>
<tr>
<td>4.5</td>
<td>8.9</td>
</tr>
<tr>
<td>1.3</td>
<td>11.3</td>
</tr>
<tr>
<td>3.9</td>
<td>12.5</td>
</tr>
<tr>
<td>1.4</td>
<td>14.8</td>
</tr>
<tr>
<td>2.1</td>
<td>12.0</td>
</tr>
<tr>
<td>2.2</td>
<td>—</td>
</tr>
<tr>
<td>Mean: 2.86 ± 0.51 (8)</td>
<td>Mean: 11.8 ± 3.1 (6)</td>
</tr>
</tbody>
</table>
Measurement of the rates at which substances in solution enter and leave the aqueous humour, and a more accurate knowledge of the relative distribution of such substances between the aqueous humour and the blood plasma in the steady-state, have led to a conception of the dynamics of the aqueous humour in which secretion or active transfer into the eye, and a flow in bulk of fluid out of the eye, play important roles. The low ratios found for cysteine and ascorbic acid at the steady-state when the concentration of ascorbic acid in the plasma is high lend further support to this view.

Whilst water passes readily from the tissues bounding the anterior and posterior chambers of the eye into the aqueous humour, solutes enter mainly from the ciliary region. Substances strongly soluble in water (e.g., ions) are thought to pass into the posterior chamber mainly through the intercellular spaces of the ciliary process by diffusion, or by active transfer across the cell membranes. "Active transfer" may be defined as the passage of a substance across a biological membrane in such a way that work is done to perform the transfer. It might be noted in this connection that the term "secretion" is often used to describe active transfer, although formerly it was more generally restricted to describe the expulsion of a substance from a cell in which it had been synthesized. In its most simple form active transfer may be recognized as a movement of a substance across a biological membrane against a concentration gradient, resulting in an increase of the equilibrium ratio above that demanded by a simple diffusion process, or a Gibbs-Donnan membrane equilibrium. This is illustrated in the rabbit, in which ascorbic acid injected into the blood stream passes into the aqueous humour although the concentration is higher than in the plasma (Bietti, 1935; Kinsey, 1947; Langham, 1950). In the resting state the equilibrium ratio may be as high as 30, but with an increase in the concentration of ascorbic acid in the plasma this value tends to decrease, a fact which suggests a saturation of the secretory process. In the present work on the cat, the value of the equilibrium ratio was found to decrease from a value greater than unity in the resting state (0.5 to 1.0 mg. per 100 ml. blood plasma) to approximately 0.26 when the concentration in the plasma was maintained at 50 to 60 mg. per 100 ml. This result suggests that the entry of ascorbic acid is dominated by secretion at low, and by diffusion at high, concentrations in the plasma.

The suggestion that substances may enter the aqueous humour of the cat by either simple diffusion, active transfer, or a combination of both, provides further confirmation of the complexity of the blood-aqueous barrier. This is in agreement with the work of Davson and others (1949a, b), who, on the basis of extensive dynamic studies
on the cat, concluded that the barriers separating the intra-ocular fluid from the blood plasma possess a selectivity capable of discrimination on the basis of chemical structure as well as of molecular size. They found that, in general, nitrogen-containing molecules entered the eye more slowly than similar-sized non-nitrogenous molecules, e.g., the monosaccharides enter the eye more quickly than glycine and urea. The observation by Davson and others (1948) that sodium chloride is in a higher concentration in the aqueous humour of the cat than is demanded by the Gibbs-Donnan equilibrium is of interest in that it provides further evidence of secretory activity across the barrier.

It is established that there is a circulation of the aqueous humour into and out of the eye, but less equivocal are the conclusions concerning a flow in bulk from the anterior chamber. In the absence of a through-and-through circulation of the aqueous humour, the distribution ratio for a molecule which is not metabolized, such as urea, would equal unity, and for an electrolyte its value would be determined by the demands of the Gibbs-Donnan membrane equilibrium. In the rabbit, Kinsey and Grant (1942) confirmed that urea has a low distribution ratio (i.e., in steady-state conditions the concentration of urea in the aqueous humour is less than in the plasma), and then used it to calculate the rate of flow or bulk drainage.

More recently, these results have been confirmed by Bárány and Kinsey (1950), who demonstrated that the slowly penetrating non-metabolized molecules, p-amino hippuric acid, rayopake, and diodrast, leave the aqueous humour of the rabbit at the same rate as the sodium ion. Further evidence for the concept of bulk drainage in the rabbit has recently been reported by Weekers and Prijot (1950) and Greaves and Perkins (1951), who have demonstrated the presence of aqueous veins in this animal.

A calculation of the rate of flow of the aqueous humour from a knowledge of the constant of entry and equilibrium ratio involves making an assumption about the manner by which the fluid lost by flow and bulk drainage is replaced in the aqueous humour. Recent investigators have assumed that the rate of replacement may be calculated as the product of a factor $\alpha$ and the rate of drainage (Palm, 1948; Bárány and Kinsey, 1950); following this assumption it may be deduced that the limits of $\alpha$ are from 0 to $R_{eq}$ for a substance that enters by diffusion. On this basis the present results have been used to compute rates of flow:

$$k \text{ flow for ascorbic acid} = 0.0093 - 0.0124$$

$$k \text{ flow for cysteine} = 0.012 - 0.022$$

The average volume of the aqueous humour of the cat is 1.0 ml., which means that the rate of drainage is between 10 and 20 mm.$^{3}$ per minute. This represents between 1 and 2 per cent. of the volume of the anterior chamber per minute, a rate of flow corresponding to
the value of 1.1 per cent. per minute found by Bárány and Kinsey (1950) in the rabbit. The average volume of the aqueous humour of the rabbit is 0.25 ml., and it is therefore of interest that although the volumes of the aqueous humour in the two species are widely different, the percentage turnover rate is approximately the same.

It is suggested, on the basis of the present experimental work, that the formation of the aqueous humour of the cat depends on secretory activity in addition to a filtration-diffusion mechanism, while loss of aqueous humour occurs partly through drainage in bulk and partly through simple diffusion. The rate of flow of the aqueous humour under these conditions will be determined by the patency of the drainage routes as well as by the hydrostatic, osmotic, and secretory pressures acting across the barrier. Analogously, the intra-ocular pressure will be determined by the balance between the formation and loss of the aqueous humour.

**SUMMARY**

1. A study has been made of the relation between the equilibrium ratio ($R_{eq}$) and the concentration of ascorbic acid and cysteine in the plasma in the cat. These results suggest that cysteine enters the aqueous humour by simple diffusion, while the entry of ascorbic acid is determined by active transfer for low concentrations of ascorbic acid in the blood plasma, and by diffusion at high concentrations in the blood plasma.

2. The low equilibrium distribution ratios found for both cysteine and ascorbic acid add further proof to the conception of a flow of aqueous humour in the cat.

3. The rate of flow of the aqueous humour has been calculated from a knowledge of the equilibrium ratio and the coefficient of transfer from the blood plasma to the aqueous humour.

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**REFERENCES**


Secretion and Rate of Flow of Aqueous Humour in the Cat

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