THE MECHANISM OF AQUEOUS HUMOR FORMATION INFERRED FROM CHEMICAL STUDIES ON BLOOD-AQUEOUS HUMOR DYNAMICS

By V. EVERETT KINSEY and W. MORTON GRANT

(From the Howe Laboratory of Ophthalmology, Harvard Medical School, Boston)

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Perhaps no problem in ocular physiology has received so much attention or been discussed so vehemently as the question of whether the aqueous humor is formed by ultrafiltration (dialyzation) or by some secretory process. One of the chief arguments cited for or against the ultrafiltration as opposed to the secretion theory has been based upon the equilibria which the constituents of the aqueous humor form with the blood. Strong support has been gained for the ultrafiltration hypothesis from the fact that with several notable exceptions (K, PO_4, SO_4) the electrolyte distribution does not vary appreciably from that required by the Gibbs-Donnan equation for static equilibria.

On the other hand, it has always been assumed that the ultrafiltration hypothesis was incompatible with equilibrium ratios of freely diffusible substances which are appreciably less than 1, unless some special assumptions, such as utilization within the anterior chamber or selective reabsorption were invoked to account for the deficiency. Examples of compounds which appear to be present in lower concentrations in the aqueous humor than in the blood are phosphate and sulfate and such freely diffusible non-electrolytes as urea, uric acid, creatinine, sucrose, raffinose, and probably glucose. With the possible exception of the last named, there is no evidence that appreciable utilization of any of these compounds occurs within the anterior chamber nor that they are selectively reabsorbed.

For reasons which will be given subsequently the authors do not believe that it is permissible to draw any conclusions concerning the mechanism of aqueous formation merely from the aqueous humor-blood equilibria ratios. Neither would it appear possible to estimate directly the relative rates of transfer of different substances from the blood to the anterior chamber from such ratios alone. Some of the additional data deemed necessary for the solution of these problems will be presented later.

The purpose of this paper is twofold: first, to point out mathematically the quantitative implications of the ultrafiltration and secretory hypotheses, and secondly, to analyze the experimental data already presented (1, 2) on the basis of such theoretical formulations, with the object of determining (*a*) the relative rates of transfer of substances into the anterior chamber, and (*b*) which hypothesis, if either, is supported by these data.

If aqueous humor is assumed to form either as a result of ultrafiltration or of some secretory phenomenon, there appear, in general, to be three separate processes[1] which could account for the equilibria between aqueous humor and blood. They are:

1. Aqueous constituents separately may enter the anterior chamber from the blood by ultrafiltration where, under static conditions[2] they would accumulate until the concentration in the aqueous reaches that in the blood. In this instance the process would presumably obey Fick's law, *i.e.* the rate of transfer would be proportional to the difference in concentration on the two sides of the barrier or barriers, and would eventually lead to an equilibrium ratio of 1.0. (It is this process only, which is assumed, presumably, by those who use the ratios below 1.0 as evidence against the ultrafiltration theory.)

2. Aqueous constituents may enter the anterior chamber by ultrafiltration and escape by another mechanism; *e.g.*, flow.[3] It will be shown later that such a mechanism could account for any equilibrium ratio below 1.0.

3. Aqueous constituents may enter the anterior chamber from the blood by secretion; in this instance it is obviously necessary that there be some leakage out of the anterior chamber. Such leakage would presumably occur by a different route, and might be either a flow or conceivably another secretory process. In either instance the simplest assumption concerning the rate of leakage of any substance out of the anterior chamber is that it is proportional to its concentration in the aqueous humor. It will be shown that this process also could account for equilibrium ratios less than 1.0 and in addition could lead to equilibrium ratios above 1.0.

In summary, an aqueous constituent may form a static equilibrium with the blood by a process of ultrafiltration, or it may enter by ultrafiltration or secretion and leave the anterior chamber by means of a flow. Moreover, final equilibrium ratios below 1.0 could be accounted for by either the ultrafiltration or secretory hypothesis under the conditions set forth under paragraphs 2 and 3, *i.e.* some net flow (not exchange) out of the anterior chamber, furthermore

[1] It is, of course, possible that some combination of these processes, or others, may be the actual one involved in aqueous formation, but the point which we desire to emphasize especially is not how the aqueous is necessarily formed, but some of the additional factors which must be considered before inferring from analytical procedures what is the mechanism for aqueous formation.

[2] Static conditions are here assumed to be those in which there is no flow, or so called through and through circulation resulting in transfer of aqueous humor to outside the anterior chamber. The only loss of material from the aqueous under static conditions would occur by means of exchange such as occurs in dialysis.

[3] For the present purpose it does not matter whether the aqueous constituents are considered to enter through the ciliary body, the iris or elsewhere; nor does it matter whether they leave as a flow through Schlemm's canal or elsewhere so long as the site of entrance and exit are different.

such ratios are not readily explicable by either hypothesis under static conditions.

While many investigators have recognized the possibility of flow out of the anterior chamber, and have designed experiments to measure its rate, no one to our knowledge has pointed out the effect such a flow would have on the equilibrium ratio for any particular aqueous constituent, other than to call attention to the fact that if there is a flow the Gibbs-Donnan equilibrium would not prevail (Robertson, Ridley, etc.).

Let us consider now the situation as outlined in paragraph 2 above; *viz.*, materials enter the anterior chamber from the blood by filtration and in addition leave by a flow. Under these conditions substances would enter the aqueous at a rate proportional to the difference in concentration in the blood and anterior chamber, whereas, if they leave the anterior chamber by a flow process they would do so at a rate proportional to the actual concentration in the anterior chamber. In this simple case it is assumed that the blood level is constant, that the diffusion rates are rapid compared with the rates of transfer, and that the ratio of the area of the blood-aqueous barrier to the volume of the anterior chamber remains constant for different eyes. Moreover, it is assumed that the relative ease with which water may move across the barrier in either direction, as shown in (3), permits the volume, hence the intraocular pressure, to remain essentially constant despite loss of fluid by means of flow.

The relation of the concentrations reached in the anterior chamber to the inflow and outflow may be seen from the equations which follow. Where

A = amount of constituent present in aqueous humor,
C_1 = concentration in blood,
C_2 = concentration in aqueous at a given time t_1,
k' = coefficient of transfer out of anterior chamber by flow,
k_2 = coefficient of transfer from blood to anterior chamber,
t = time,
V = volume of the anterior chamber,

The rate of change of amount of a given constituent in the anterior chamber =

$$\frac{dA}{dt} = k_2(C_1 - C_2) - k'C_2 \tag{1}$$

$C_2 = A/V$, whence

$$\frac{d(VC_2)}{dt} = V\frac{dC_2}{dt} = k_2C_1 - (k_2 + k')C_2$$

Integrating between the limits $t = 0$ to $t = t_1$ and rearranging, we have

$$C_2 = \frac{k_2}{k_2 + k'}C_1\left[1 - e^{-\left(\frac{k_2+k'}{V}\right)t_1}\right] \tag{2}$$

Equilibrium will occur when: $t = \infty$

$$\frac{C_2}{C_1} = \frac{k_2}{k_2 + k'} \tag{3}$$

From Equation 3 it is apparent that equilibrium ratios less than 1 may result for any freely diffusible substance without making any assumption other than that there is some flow out of the anterior chamber; *viz.*, $k' \neq 0$. As suggested above, it follows that unless definite proof is forthcoming to show that there is no flow out of the anterior chamber, it appears that the ultrafiltration hypothesis can account for equilibrium ratios less than 1 and therefore need not be discarded because of experimental findings of such ratios.

Similarly, measurements of the equilibrium ratio do not give any indication of the rate of transfer of a substance from the blood into the anterior chamber; they give only the overall effect of net transfer by ultrafiltration into the anterior chamber and leakage out by flow.

In a similar manner let us now consider the situation when substances are presumed to enter the anterior chamber from the blood by a secretory process and leak out of the anterior chamber by a process of flow as described above under paragraph 3. It is again assumed, as the simplest case, that the concentration of the material in the blood remains at a constant level, and that the rate of secretion is proportional to the concentration present in the blood. All symbols have the same significance as before. The rate of change of amount of a given constituent in the anterior chamber equals

$$\frac{dA}{dt} = k_2 C_1 - k' C_2 \tag{4}$$

$$\frac{d(VC_2)}{dt} = V\frac{dC_2}{dt} = k_2 C_1 - k' C_2$$

Integrating between the limits from $t = 0$ to $t = t_1$, and rearranging we have

$$C_2 = \frac{k_2}{k'} C_1 \left(1 - e^{-\frac{k'}{V} t_1}\right) \tag{5}$$

and at $t = \infty$

$$\frac{C_2}{C_1} = \frac{k_2}{k'} \tag{6}$$

In this instance too, the effect of a flow out of the aqueous is to complicate the situation, thereby making it impossible from the equilibrium ratio to determine either the rate of transfer from the blood to the anterior chamber, or to infer whether the transfer occurs as a result of filtration or secretion.

It should be evident that, since the blood-aqueous barrier is more permeable to water than to solutes constant volume conditions will be maintained and the effect of flow out of the anterior chamber is to reduce the quantity of solutes

below that which would be present were there no flow. This reduction in the quantity of solutes present will have no effect on the rate of secretion, which, by hypothesis is dependent solely on the concentration in the blood, but will have an effect on the rate of filtration which is dependent upon the difference in concentration between the aqueous humor and the blood and *a fortiori* on the concentration in the aqueous, since the concentration in the blood is assumed to be constant.

The question now arises whether it is possible from any experiments involving analyses of blood and aqueous humor to determine either the transfer rate from the blood to the aqueous, or to infer by what process the transfer is taking place. It seems that experiments involving analyses of blood and aqueous humor can permit such inferences to be drawn provided that the concentration of a given substance in the anterior chamber and in the blood is known at different periods of time following its introduction into the blood.

It is apparent that when k' (coefficient of out flow) is small, relatively high concentrations in the anterior chamber could be obtained from relatively small values of k_2 (coefficient of transfer in), while if k' is large, relatively high values of k_2 would have to be assumed to account for the same experimental data.

If, for a given ratio of concentration in the aqueous to that in the blood, a value of k' is assumed, the corresponding value of k_2 may then be calculated. Moreover, on assuming a different value of k', other values of k_2 will be found. Hence it is possible for any given ratio of concentrations to plot k_2 against k'. At another time, when the ratio of the concentration in the aqueous to that in the blood is different, a new plot will be found, the slope of which will differ from the first one. If, therefore, we are to arrive at values for k' and k_2 which are to be reasonably satisfactory for all of the experimentally observed concentration ratios, the specific values for k' and k_2 must be selected which are found at the intersection of all of the plots.

The procedure just outlined was applied to concentration ratios in which the concentration of the substance under consideration was assumed to be at a constant level in the blood. From an experimental standpoint this condition is difficult to obtain, but if continuous records are made of the concentration in the blood, it is still possible although quite laborious to apply the same method of treatment to the resultant data, provided an equation can be developed which will describe the experimental data representing the concentration in the blood. Since the blood concentration varied for different test materials, as described in the preceding papers, it was necessary to derive several different equations in order to treat the data in the manner outlined above. These equations along with the methods used in applying them to the solution of the general problem will be described forthwith.

The concentration in the blood of radioactive isotopes of sodium and chloride (see (1)), and lithium (preceding paper) was found to increase to a maximum

and remain essentially stationary throughout the observation period. A good fit for the data may be had from the following equation:

$$C_1 = C(1 - e^{-k_1 t_1}) \tag{7}$$

where C is the asymptotic blood constant and represents the maximum concentration level approached by the blood. K_1 is the coefficient of transfer from peritoneal cavity to blood; the other symbols have the same meaning as before.

By substituting for C_1 in equation 1 which describes the rate of change of concentration in the anterior chamber when the transfer is assumed to take place by filtration and takes into account a flow out of the anterior chamber, we have:

$$\frac{dA}{dt} = k_2[C(1 - e^{-k' t_1}) - C_2] - k'C_2 \qquad \text{or}$$

$$\frac{dC_2}{dt} = \frac{k_2}{V}[C(1 - e^{-k' t_1}) - C_2] - k'C_2$$

Integrating from $t = 0$ to $t = t_1$

$$C_2 = k_2 C\left[\frac{1}{k_2 + k'}\left(1 - e^{-\frac{k_2+k'}{V}t_1}\right) - \frac{1}{k_1 V - k_2 - k'}\left(e^{-\frac{k_2+k'}{V}t_1} - e^{-k_1 t_1}\right)\right] \tag{8}$$

Again when $t = \infty$

$$\frac{C_2}{C} = \frac{k_2}{k_2 + k'}$$

In a similar way the equation may be derived for treating the data on the basis of secretion—the condition outlined under paragraph 3 above. The resulting equation is:

$$C_2 = k_2 C\left\{\frac{1}{k'}\left(1 - e^{-\frac{k' t_1}{V}}\right) - \frac{1}{k_1 V - k'}\left(e^{-\frac{k' t_1}{V}} - e^{-k_1 t_1}\right)\right\} \tag{10}$$

If, as was the case for the SCN and Br experiments, the level of the test substance in the blood reaches a maximum and then falls, the solution of the problem becomes more laborious, but still may be handled quantitatively with the aid of the following equation which empirically describes the concentration in the blood within the time limits investigated.

$$C_1 = C(1 - e^{-k_1 t_1}) - k_3 t_1 \tag{11}$$

where k_3 represents the coefficient of loss from blood. By substituting again for C_1 in both Equation 1 (ultrafiltration) and Equation 4 (secretion) and integrating, we arrive at the following relation:
for ultrafiltration:

$$C_2 = \left(\frac{k_2 C}{k_2 + k'} + \frac{k_2 k_3 V}{(k_2 + k')^2}\right)\left(1 - e^{-\left(\frac{k_2+k'}{V}\right)t_1}\right) - \frac{k_2 C}{k_1 V - k_2 - k'}\left(e^{-\left(\frac{k_2+k'}{V}\right)t_1} - e^{-k_1 t_1}\right) - \frac{k_2 k_3}{k_2 + k'} t_1 \tag{12}$$

and for secretion:—

$$C_2 = \left(\frac{k_2 C}{k'} + \frac{k_2 k_3 V}{(k')^2}\right)\left(1 - e^{-\frac{k' t_1}{V}}\right) - \frac{k_2 C}{k_1 V - k'}\left(e^{-\frac{k' t_1}{V}} - e^{-k_1 t_1}\right) - \frac{k_2 k_3}{k'} t_1 \qquad (13)$$

It is interesting to note that if the concentration in the blood is falling rapidly, *i.e.* k_3 is large compared with k' the concentration in the anterior chamber may reach or even exceed that in the blood, even though the equilibrium ratio is ordinarily below 1. Clearly, no quantitative inference as to the rate of transfer or mechanism involved can be drawn from observations that do not include continuous determinations of the concentration of the test substance in the blood, and treatment of the data which does not consider the existence of outflow from the anterior chamber.

As reported in the preceding paper the blood concentration in the urea experiments was maintained constant throughout the period of the investigation. However, urea differs from the other substances which will be analyzed quantitatively in that some is present initially in the anterior chamber; *i.e.*, $C_2 \neq 0$ at $t = 0$, but $= C_I$. In this instance the integration is still carried out between the limits $t = 0$ and $t = t_1$ remembering, however, that at $t = 0\ C_2 = C_I$ with the result that on the basis of the ultrafiltration hypothesis:

$$C_2 = \frac{k_2}{k_2 + k'}\left[C\left(1 - e^{-\left(\frac{k_2 + k'}{V}\right) t_1}\right)\right] + C_I e^{-\left(\frac{k_2 + k'}{V}\right) t_1} \qquad (14)$$

On the basis of the secretion hypothesis:

$$C_2 = \frac{k_2}{k'} C\left(1 - e^{-\frac{k' t_1}{V}}\right) + C_I e^{-\frac{k' t_1}{V}} \qquad (15)$$

RESULTS

Fig. 1 shows the result of calculating the coefficient of transfer (k_2) for sodium from the experimental data (reported in (1)) for various assumed values of k' on the basis of the ultrafiltration hypothesis. Fig. 2 shows the results of analogous calculations made on the basis of the secretory hypothesis. The broken line, in each case, represents the values of k_2 and k' which would be required by the ratio of concentration in the anterior chamber to that in the blood under equilibrium conditions. The value used, 0.90, was obtained by estimation from the sodium curve as shown in Fig. 1 of (1) and appears to be of the same order of magnitude as that reported by others from chemical studies.

It is at once apparent from Fig. 1 that a reasonable fit of the experimental data (measured concentration in the anterior chamber and in the blood) is found only when k_2 is equal to about 3.5, and k' equal to something less than $\frac{1}{2}$. With two exceptions all of the ratios give values for k_2 which vary only from 3.0 to 4.4 for this value of k', whereas with slightly greater assumed rates of flow it may be seen that k_2 varies tremendously for the different experimental points.

From Fig. 2, it is clear that once again the best fit is found when k_2 is about 3.5, but in this instance k' is almost 4.0.

The results from the chloride experiments have been calculated in a similar manner and the plots are shown in Fig. 3 for the ultrafiltration and secretion hypotheses. Since the equilibrium ratio for chloride is approximately 1.03 the slope of a line showing the relation of k_2 to k' under equilibrium conditions would

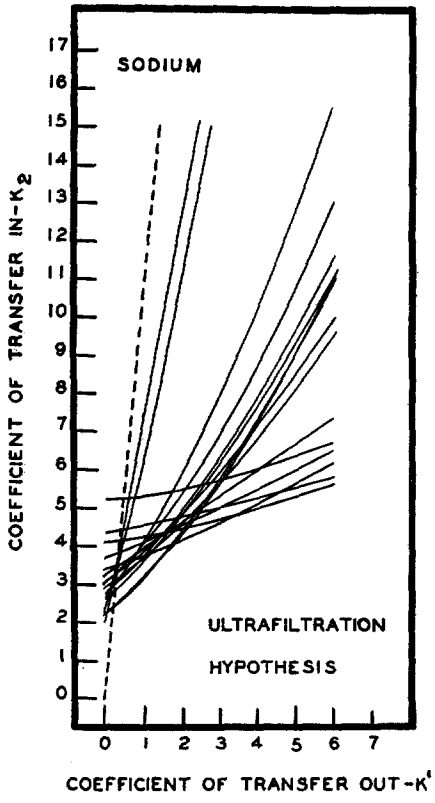

FIG. 1

be negative on the basis of ultrafiltration, and since factors other than possible flow of aqueous out of the anterior chamber presumably would be needed to account for this slope, no line has been shown in case of ultrafiltration. However, the broken line on the set of curves representing secretion again represents this equilibrium ratio.

It is clear from Fig. 3 that the best fit for the filtration hypothesis is given when little or no flow is present, a result similar to that found for sodium. In this instance k_2 has an average value of 4.5. On the basis of the secretory hypothesis the best fit results when k_2 is 3.9 and k' is 3.85.

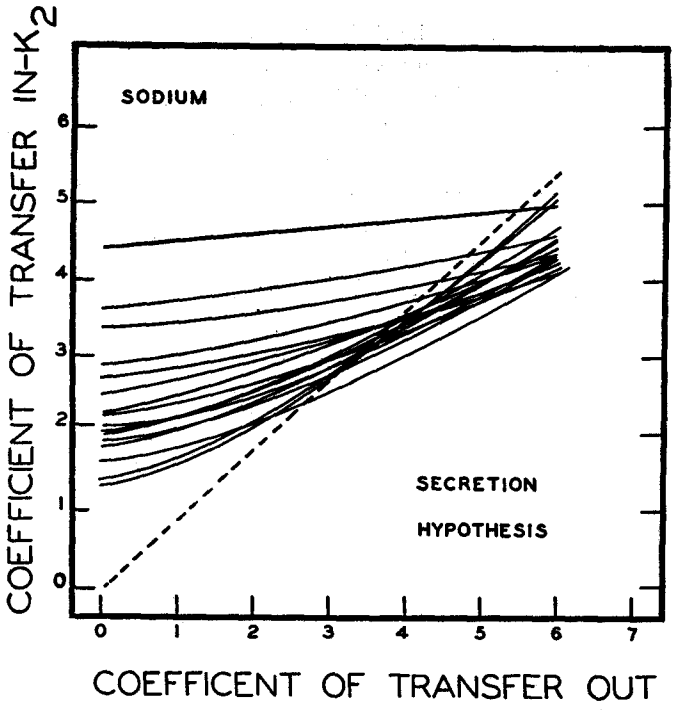
COEFFICIENT OF TRANSFER IN-K2
SODIUM
SECRETION
HYPOTHESIS
0 1 2 3 4 5 6
0 1 2 3 4 5 6 7
COEFFICENT OF TRANSFER OUT

FIG. 2

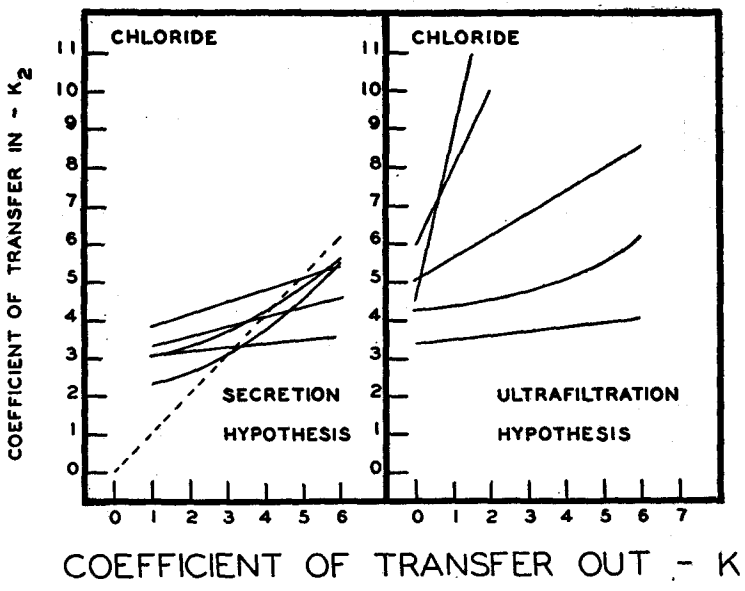
COEFFICIENT OF TRANSFER IN - K2
CHLORIDE
SECRETION
HYPOTHESIS
CHLORIDE
ULTRAFILTRATION
HYPOTHESIS
0 1 2 3 4 5 6
0 1 2 3 4 5 6 7
COEFFICIENT OF TRANSFER OUT - K

FIG. 3

Similarly, the results from the SCN experiments are plotted and shown in Fig. 4. On the basis of the ultrafiltration hypothesis it would appear that again the best fit is when k' is equal to zero, and the corresponding value of k_2 is 3.8. The curves based on the secretory hypothesis are most similar at $k_2 = 4.0$ and $k' =$ approximately the same value.

In the case of lithium only two experimental points were available to compute the rate of transfer into the anterior chamber. Nevertheless it may be seen from Fig. 5 that on the basis of ultrafiltration a fit is given only when k' is about zero. If the lithium enters by secretion, however, k' again is found to be equal

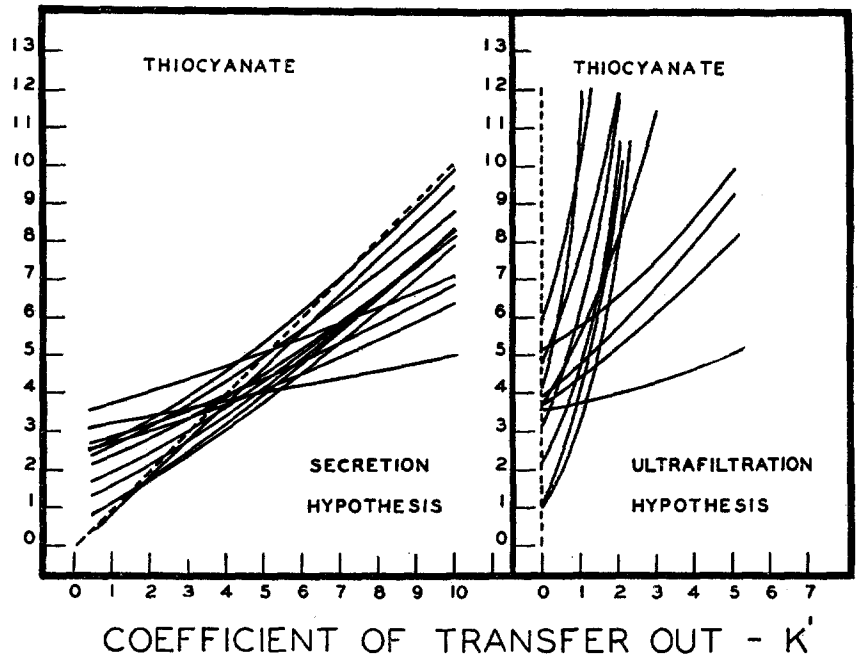

FIG. 4

to approximately 4, and k_2 in this instance appears to be about 3.5. The broken line is plotted on the assumption that the final equilibrium ratio of lithium in the aqueous to that in the plasma is 1.

From Fig. 6 it may be seen that the best fit on the basis of ultrafiltration for bromide occurs when $k_2 = 4.0$ and $k' =$ zero, while $k_2 = 4.0$ and $k' = ca.$ 4 if it be assumed that bromide enters the anterior chamber by a secretory process. Again the broken lines are based on an assumed equilibrium ratio of 1.

It will be recalled that except for minor differences two consistently different values (zero and 4) are obtained for the coefficient of outflow (k'), depending upon whether one assumes that these electrolytes enter the anterior chamber by filtration or secretion. Since either set of values for the coefficients of transfer

k_2 and k' will account for the determined concentrations there is no way of distinguishing which hypothesis is the correct one. Moreover, while the rel-

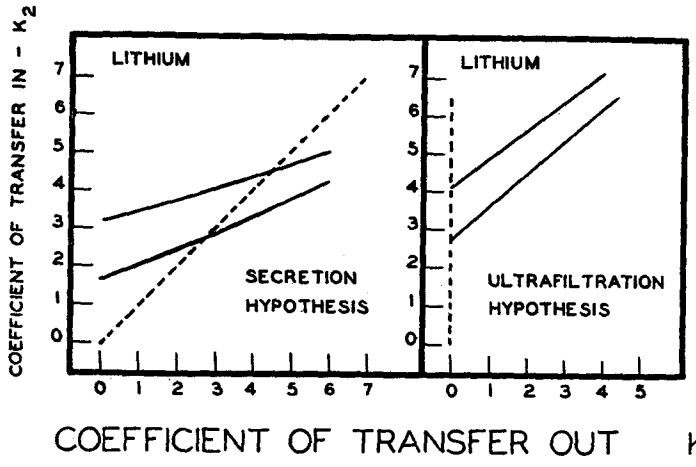

FIG. 5

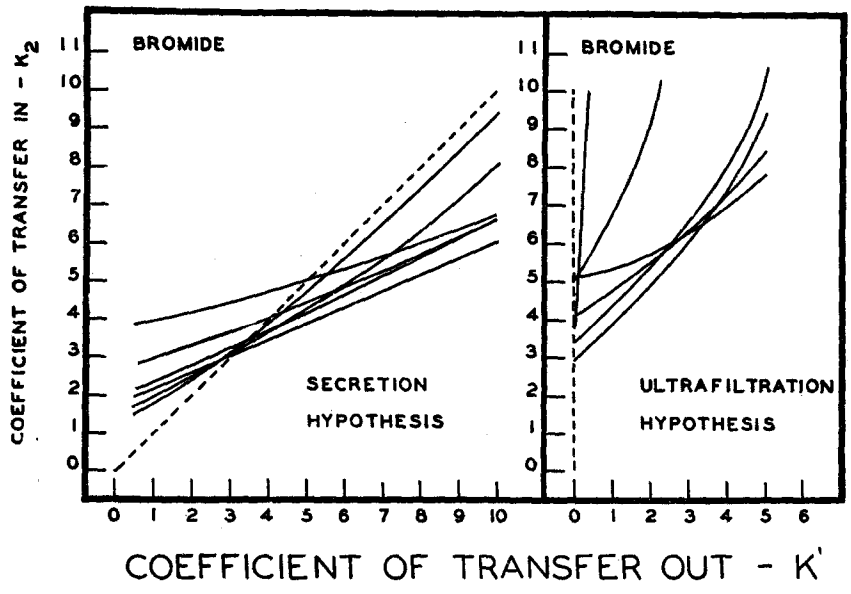

FIG. 6

ative rates of transfer into the anterior chamber have now been determined the absolute rate too, depends to some extent upon one's assumption of the processes involved.

If, for the present, we do not examine the evidence from other sources for a through and through circulation of aqueous, but first consider the general relationship between k_2 and the equilibrium ratios required by the two hypotheses for various values of k', and secondly, consider the case of substances which give rise to equilibrium ratios significantly less than 1, it will be seen that additional information is obtained which is helpful in deciding which mechanism is operative for transferring the substances tested into the anterior chamber.

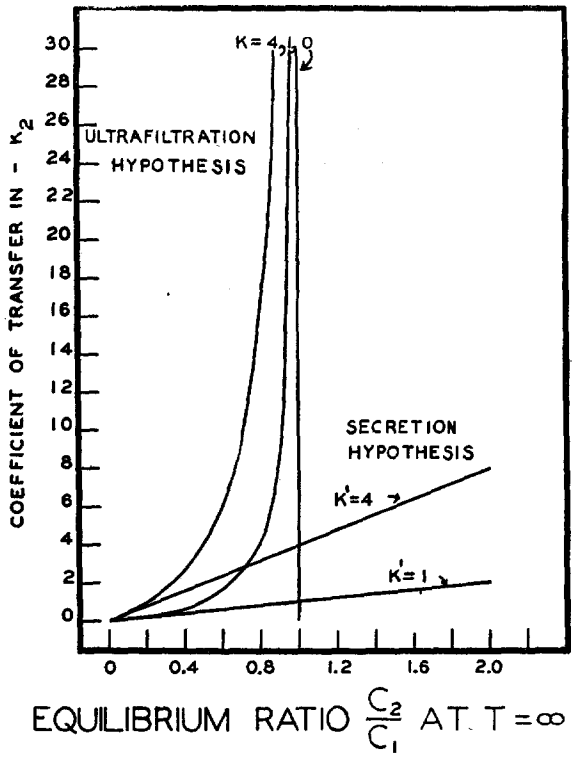

FIG. 7

The relation between the equilibrium ratio and the coefficient of transfer out of the anterior chamber by means of a flow (k') on the basis of the ultrafiltration and secretory hypotheses may be seen from Fig. 7. Here three values, 0, 1, and 4, have been assumed for k' and the equilibrium ratios have been calculated for various values of k_2 from equations 3 and 6, where t equals infinity. The solid perpendicular line shows the expected ratio on the basis of the ultrafiltration hypothesis at k' equals 0. This calculation has not taken the Gibbs-Donnan equilibrium into account, but had this been done the line would still be perpendicular but would be shifted to the right or left slightly depending upon whether one were dealing with an anion or cation. On the basis of the

secretion hypothesis there would be no equilibrium for k' equal to 0, hence no line is shown. As was to be expected, equilibrium ratios significantly below 1 cannot be accounted for by either hypothesis under the assumed condition of $k' = 0$.

The two curved lines show the expected equilibrium ratios when k' equals 1 and 4 for the ultrafiltration, and the two sloping straight lines show the analogous ratios for the secretory hypothesis.

The great increase in value of k_2 required to produce an equilibrium ratio above 80 per cent on the basis of the ultrafiltration hypothesis, compared to that required by the secretion hypothesis suggests that on the knowledge of the rate of transfer of water and urea few substances would be found in the anterior

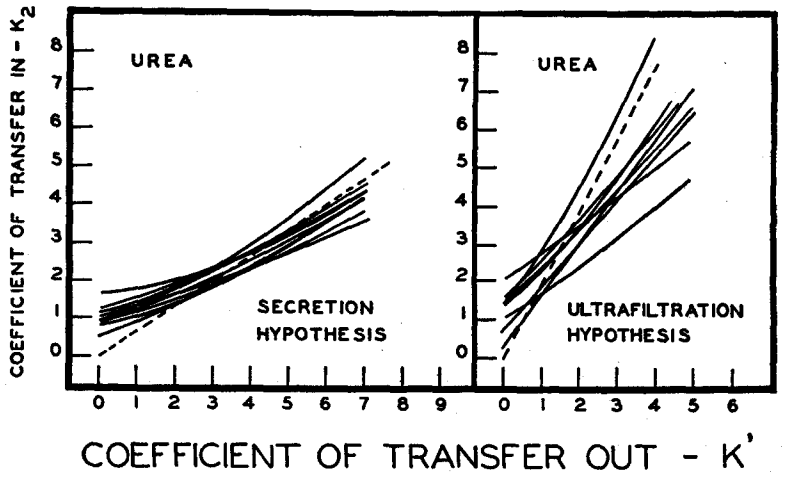

FIG. 8

chamber having equilibrium ratios above this amount if they entered by means of filtration and any substantial loss occurred through flow.

Urea was one substance which gave an equilibrium ratio of less than 1. The coefficients of transfer in and out of the anterior chamber for this compound are shown plotted against each other in Fig. 8. Again the broken line represents the equilibrium ratio (0.66). Unlike previous results on the basis of the ultrafiltration hypothesis the best fit is obtained when k' is more than zero—in this instance it would appear to equal approximately 1.7 and $k_2 = 2.8$. The corresponding values given by the secretion hypothesis are $k' = 4.0$ and $k_2 = 2.7$. Thus, as was anticipated for all substances which show a deficiency in concentration in the anterior chamber, the experimental data give a reasonable fit only when it is assumed that there is some leakage regardless of which of the two hypotheses is presumed to be operative.

Since it has been shown that concentrations of electrolytes found experi-

mentally would not be attained by an ultrafiltration process if there is any substantial leakage by flow, but could be attained by a secretory process under such conditions, it would appear that all of the data are compatible only with the idea that some leakage does occur.

If we assume that the k' value for urea is 4 and calculate the final equilibrium ratio which gives a good fit for the k_2-k' plots on the basis of the ultrafiltration hypothesis it is found to be 58 per cent. While this value was closely approximated by 4 out of 5[4] of the last experimental points shown on Fig. 5 of the preceding paper it will be recalled that the average equilibrium value found in control animals was 66 ± 5 per cent.

Considering the variability of urea ratios in both treated and untreated animals the authors are inclined to believe that the possibility of urea entering the anterior chamber by ultrafiltration cannot be discarded.

It will be recalled from Fig. 6 of the preceding paper that the levulose concentrations in the blood of different rabbits was too variable to permit representing them all with a single curve. For this reason it is not possible to analyze quantitatively the results in the manner used for the other test substances. Nevertheless, the fact that the maximum concentration reached in the anterior chamber is only about 35 per cent of that in the plasma seems indicative that either the excess levulose is quite rapidly metabolized[5] or that it too, may be lost from the anterior chamber as a result of a flow process.

If the coefficient of flow (k') is set equal to 4 and one solves for k_2 on the bases of both hypotheses when the equilibrium ratio is 35 per cent, k_2 is found to equal 2.15 for ultrafiltration and 1.4 for secretion. From a plot of the results obtained by substituting these values in Equations 14 and 15 and solving for the concentration ratios at various times, curves are obtained which produce a reasonably good fit for the aqueous curve of Fig. 6 of the previous paper.

Out of interest a similar calculation was made letting $k' = 1.7$ (the result obtained in the case of urea-ultrafiltration hypothesis). The resulting curve in this instance was much flatter at the beginning than the others and does not produce as good a fit.

The phosphate results too (Fig. 4 in the previous paper) were so variable that

[4] The ratios were 60, 59.4, 68, 57.5, and 58.5 per cent for t = 81, 91, 115, 140, and 176 mins. respectively.

[5] The question of utilization has frequently been invoked to account for deficiencies in the anterior chamber without proof that it actually could quantitatively do so. Several years ago one of the authors (V. E. K.) in an *in vitro* experiment increased the amount of glucose available for rabbit lens utilization from 90 mg. per cent to twenty times this concentration and found that the oxygen uptakes were identical. While the glycolysis was not measured in these experiments it would appear that the oxygen uptake of the lens, at least, does not fluctuate significantly with concentrations of carbohydrate in excess of normal.

only a qualitative analysis seems warranted. It is certain, however, that the ratio when equilibrium is obtained is definitely below 1; again the conclusion seems inevitable on the basis of present knowledge at least, that some leakage must occur to account for the findings. With regard to the actual rates of transfer into the anterior chamber, if k' is assumed to be 4, a value of k_2 equal to 7.4 for the ultrafiltration and 2.6 for the secretion hypothesis would account for the observed equilibrium ratio of about 65 per cent.

From a consideration of the results obtained with urea, levulose, and phosphate it would appear that the ratios observed may be accounted for only on the assumption that there is a flow out of the anterior chamber. The exact magnitude of this flow may be open to some question but from the fact that a reasonably good fit to the experimental curves results if one substitutes a value of 4, as obtained from the other test materials, it seems justifiable to conclude that this value is substantially correct. One implication of such a conclusion is, as was suggested before, that the rate of leakage out by flow is the same for all of the compounds used. Obviously this may not be the case, but the fact that ions of sizes as different as Na and SCN appear to leave the anterior chamber at essentially the same rate suggests that for ions of this range of size at least, it is true. If, as is usually assumed, the flow occurs through Schlemm's canal, the walls, being composed of endothelium similar to that found in capillaries, would not be expected to differentiate between compounds no larger than levulose.

In any case so long as k' is substantially above zero the data for all of the electrolytes tested, except PO_4, give a good fit only on the assumption that they are secreted. Phosphate too, gives a fit on such an assumption, but since an equally good fit is obtained assuming ultrafiltration, the choice in this instance cannot be made from the data at hand. Since the data for the compounds having equilibrium ratios less than one are not differentiable by any such conspicuous differences in k' values as was found for the electrolytes on the basis of the two hypotheses, the choice of which one best accounts for their passage from the blood to the anterior chamber must come from other sources.

DISCUSSION

That the units for the constants k_2 and k' are in c. mm. per minute may readily be seen from Equation 1. It should be emphasized that the k_2 values show the relative rates at which different substances can enter the anterior chamber under identical concentration conditions, in the plasma only, when the transfer is the result of a secretory process, and in the plasma and in the anterior chamber when the transfer is the result of ultrafiltration.

Since the concentration is different for each substance in the plasma the actual rate of transfer must likewise be different and is equal to k_2C_1 on the basis of secretion and $k_2(C_1 - C_2)$ on the basis of ultrafiltration. Thus each

substance enters the anterior chamber from the blood at a characteristic rate due both to differences in the coefficient of transfer k_2 and to differences in concentration.

On the other hand k' appears to be approximately the same for all of the electrolytes tested and probably for the non-electrolytes too, so that the loss of aqueous humor constituents from the anterior chamber as a result of flow is proportional only to the concentration of a given substance in the aqueous humor. This flow in the case of rabbits amounts to about 4 c. mm. per minute. Incidentally this measurement of rate of flow appears to be the only one available for normal eyes under rigourously physiologic conditions.

It should be pointed out that for any substance which may be entering the anterior chamber by an ultrafiltration process, an exchange of molecules between aqueous humor and plasma would be simultaneously operative, and no *net* loss would result from such a process. The coefficient k_2, of course, is a measure of net rate of transfer and is valid no matter what the total exchange is.

Considerable additional data have appeared in the literature which support the idea that constituents of the aqueous humor may leave the anterior chamber by means of a through and through circulation. See review by Robertson (4). Probably the most direct proof that the aqueous humor is not stagnant may be had from the experiments of Friedenwald and Pierce (5) who, among others, have made measurements of the rate of flow of aqueous humor. After presumably blocking the normal exit channels of the anterior chamber of dogs by means of preliminary injections of serum proteins, the latter workers then attached a manometer to a needle inserted into the anterior chamber and, maintaining the intraocular pressure at a normal level, found that about 1 c. mm. of aqueous humor left the anterior chamber per minute. From the rate at which aqueous humor left the anterior chamber of a child who had a lens so dislocated that it formed a ball-valve with the iris they calculated the rate of flow in this instance to be from 1.5–2.5 c. mm. per minute.

Evidence in favor of the secretory hypothesis, as previously mentioned, has been largely negative, in that it has been based on the erroneous assumption that equilibrium ratios below 1 could not be accounted for except on the assumption of a secretory hypothesis. On the other hand Friedenwald and Stiehler (6) have found that the epithelium of the ciliary body exhibits a selective permeability to basic and acid dyes. Friedenwald and coworkers have found that the mechanism for ion transport is one of oxidation and reduction.

Other experimental findings which suggest that the aqueous humor is formed by secretion rather than by filtration are those of Benham, Duke-Elder, and Hodgson (7) who found the aqueous humor of dogs to be slightly hyperosmotic to blood serum. Similar results were obtained by Roepke and Hetherington (8) on rabbits and dogs; these workers also found that the reinjection of aqueous humor containing $HgCl_2$ into the anterior chamber posterior to the iris abolished

the difference in osmotic activity. This difference amounted to an average of 5.4 mM of NaCl per kilo of water.

While our studies have shown that the rates of transfer into the anterior chamber of both anions and cations are compatible with a mathematical formulation based on a secretory process, it should be pointed out that active secretion of ions bearing only positive or only negative charges may occur. In such an instance the oppositely charged ion would of necessity be transferred into the anterior chamber in order to maintain electrical neutrality. This latter process would lead to concentrations indistinguishable from those which would be obtained had the active secretion applied to the other ion or for that matter to both ions.

If we are to accept the mechanism for ion transfer by secretion as propounded by Friedenwald and coworkers, and we are aware of no others, it would appear that it is the cation which is actively secreted into the anterior chamber and the anion is transferred as a result of electrical attraction. The transfer of non-charged substances would presumably occur, in the absence of experimental evidence to the contrary, by means of simple filtration. Suggestive of this is the penetration of neutral dyes through the stromal-epithelium barrier of the ciliary body, as shown by Friedenwald and Stiehler.

With the object of determining the effect of valence on the rates of transfer the substances chosen for study included mono-, bi-, and trivalent ions. For reasons already cited the results for the bivalent ion (Mg) and the trivalent one (Fe) could not be used, and while the phosphate lead to an equilibrium ratio significantly less than one the contribution of charge in establishing such an equilibrium ratio is complicated by the several ionic forms of phosphate having different valences.

It is interesting to note that the rates of transfer into the anterior chamber on the basis of the secretory hypothesis for all of the monovalent electrolytes tested were approximately the same irrespective of the size of the test substance. By contrast, the proportionately lower transfer rates found by us for urea and levulose and the progressively lower equilibrium ratios found by Weld (9) for glucose, sucrose, and raffinose are suggestive of the passive nature (ultrafiltration) of the process of transfer for non-charged molecules.

From the fact that a flow out of the anterior chamber produces concentrations in the aqueous humor lower than in the blood for all substances which enter by means of ultrafiltration and for any which enter by secretion having k_2 values less than 4 (*i.e.* less than the rate of flow), one would anticipate that some other substances would have k_2 values in excess of 4, and thus be present (as a result of secretion) in higher concentrations than in the blood in order to maintain the osmotic equilibrium. Chloride appears to be one such substance. Moreover the osmotic equilibrium, hence the intraocular pressure, is continually dependent upon both the magnitude of the rate of flow and the rate of transfer

into the anterior chamber, particularly that part of the latter due to secretion. Any decrease in the rate of flow or increase in rate of secretion (or rate of ultrafiltration) would be expected to increase the intraocular pressure and *vice versa*. It is probable that in a normal eye an increase in pressure would augment the rate of flow, or perhaps lower the transfer rate. These compensatory changes would tend to minimize the rise in pressure.

The application of the mechanisms outlined in this paper to the problem of intraocular pressure and its relation to glaucoma will be discussed in a later paper.

SUMMARY

The importance of considering the effect of a possible flow out of the anterior chamber before inferring any mechanism of aqueous humor formation from the relative concentration of a substance in the aqueous humor and plasma under equilibrium conditions has been stressed.

Several processes to account for the chemical equilibria between aqueous humor and blood based on the ultrafiltration and secretion hypotheses with a possible simultaneous loss of aqueous humor by flow have been outlined. On the basis of these processes, equations were formulated which would relate the rates of transfer into and out of the anterior chamber to the ratio of concentration of a substance in the aqueous to that in the blood at various intervals after its introduction into the blood. The explanation of equilibrium ratios above and below one for aqueous constituents is made apparent from the mathematical formulations. For each substance tested a determination was made of the best fit when the concentration in the aqueous humor is plotted against time. This fit was obtained by plotting the rate of transfer in against the rate of transfer out of the anterior chamber for all of the experimentally found concentration ratios on the basis of both the ultrafiltration and secretory hypotheses. Two sets of values were obtained from these calculations, one set for each hypothesis.

The substantial agreement of all the experimental data with an assumed rate of leakage out of the anterior chamber of approximately 4 c. mm. per minute was shown to be compatible only with the idea that all the monovalent electrolytes tested entered the anterior chamber as a result of secretory process. It could not be decided from these chemical studies whether the non-electrolytes and the one multivalent electrolyte tested enter the anterior chamber by ultrafiltration or secretion.

Experimental findings from other sources were cited which would suggest that non-electrolytes enter the anterior chamber as a result of ultrafiltration.

The implications of the mechanism outlined in the paper with respect to intraocular pressure have been discussed.

Supplementary evidence from the literature has been given in support of the conclusions presented here.

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