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SOME OBSERVATIONS ON THE PRESENT POSITION OF OUR KNOWLEDGE OF THE INTRA-OCULAR FLUID

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THE publication of this paper is premature in that the work upon which it is based is yet unfinished, but since this work has been interrupted by the war it seemed advisable to publish it in its present state. Its general argument is to show that there is no conclusive, or indeed persuasive, evidence that the intra-ocular fluid is a secretion; and the more does its timely publication seem advisable in view of the fact that in the light of several experimental papers which have recently appeared, the old hypothesis of ciliary secretion appears again to be gaining ground. The question is undoubtedly of much more than academic interest, for the importance of a knowledge of the reactions between the aqueous humour and its blood plasma has been enhanced as a result of the exclusion of the vitreous body from the possible causative factors in glaucoma on the basis of swelling pressure measurements (Duke-Elder, Davson and Benham, 1937).

If the theory of secretion is to be accepted the following main points must be investigated. First, a definite glandular activity on the part of a tissue in the eye must be demonstrated, and it must be shown that the intra-ocular pressure is a definite function of the activity of the cells of this tissue. Second, it must be shown that the secretion, if it occurs, is effective in carrying out the objectives, the necessity for which is the basis for the assumption of secretory activity. Third, the negative line of inquiry is to investigate whether the distribution of dissolved constituents in the aqueous humour and plasma is such as to necessitate the assumption of an accumulating or diluting activity on the part of some hypothetical glands.

Advocates of the secretory theory have concentrated almost exclusively on this last negative line of attack; thus the whole secretory theory is enshrouded in a vagueness which makes it difficult for the impartial investigator to tackle experimentally. As opposed to the secretory theory the filtrate-dialysis theory is perfectly clear in its assumptions, and its consequences in respect to the relation of intra-ocular pressure to blood-pressure and composition are well recognised and have been submitted to experimental test. It is possibly for this reason that the filtrate-dialysate theory is so often attacked, the opposing theory being so vague and ill-defined as to make criticism of it on any specific point impossible.

The various arguments which have recently been put forward in support of the secretory hypothesis may be summarised as follows.

1. *That the distribution of electrolytes between the aqueous and the blood is not in accordance with the postulates of the Donnan theorem.*—Thus Hodgson (1938) found that the chloride ion in dogs and humans is more concentrated in the aqueous humour than would be expected on the basis of a simple Donnan equilibrium. These results are at variance with those about to be reported in this paper on cats, and the matter is at present under investigation in other species (Davson and Weld); it has been found so far that the sodium ion in dogs is distributed almost exactly as demanded by the Donnan equilibrium, and further that the individual variations in this ratio are very considerably smaller than those encountered in the cat. Thus even if there is an excess of Cl^- in the eye, and Hodgson's results leave little doubt of that, this excess is not accompanied by an excess of the cation Na^+ , so that it cannot be stated that NaCl is secreted into the eye to maintain a high intra-ocular pressure; it seems more likely that the excess of chloride is compensated by a decrease of bicarbonate in the aqueous humour. It should be realised that the chloride ion must be considered as a metabolite since its

concentration in the blood is changing continuously to meet the acid-base requirements of the body, so that it is unlikely that a true equilibrium will ever be established between the aqueous humour and blood plasma in respect of this ion; it may be that the high concentration of chloride in the aqueous humour is the observable sign of a steady-state produced by the formation of metabolites in the lens or retina which react on the distribution of chloride between the aqueous humour and blood plasma. Consequently it is unsafe to generalise from the distribution of the chloride ion between the aqueous humour and blood plasma in respect to a possible secretory activity on the part of the ciliary body. For this reason it is essential that comparison of two ions at least should be made if such a study is to have real value, and further, the distribution of sodium is more likely to have a real meaning than the distribution of other ions for the reasons given in the second section of this paper. How misleading chemical studies may be if all possible factors are not taken into account is shown by the work of Sary and Winternitz (1932), who found that the concentration of calcium in the aqueous humour is 7.4 mg. per cent. compared with a value of 12.1 mg. per cent. in serum, values which give a distribution-ratio very much larger than that demanded by the theory of dialysation. However, these authors showed that the concentration of calcium in an ultra-filtrate of plasma is 7.4 mg. per cent., *i.e.*, the same as in the aqueous humour, the reason for the discrepancy from theory being due to the fact that some of the calcium in the plasma is associated with protein and consequently does not participate in the Donnan equilibrium.

2. *That the distribution of non-electrolytes between the aqueous and the blood is not in accordance with the postulates of the Donnan theorem.* Thus Adler (1933), in a paper entitled "Is the aqueous humour a dialysate?", concludes quite categorically that it is not so because he found a higher concentration of urea in the plasma of cats than in the aqueous, while a similar conclusion, based on results showing a wide variation, was reached by Walker (1933). A similar argument has been raised with regard to glucose and other substances. In this connection two circumstances must be borne in mind: (a) The possibility that the chemical methods available for the estimation of freely diffusible organic materials are not sufficiently specific, so that comparison between a protein-free and a protein-rich fluid are not warrantable. Thus Benham (1937), using improved chemical methods, found much more consistency in the distribution of urea. (b) Where the non-electrolyte is a metabolite its concentration in the aqueous humour and plasma will vary continuously. It would not be fair to dismiss the results on non-electrolyte

distribution which conflict with the dialysis theory by merely raising these points; the decision as to whether there is an active secretion of urea, glucose, etc., will depend on investigation of the sort about to be described in this paper. Nevertheless, it would be very unsafe to argue that, because some non-electrolytes are not equally distributed between the two fluids, there is therefore an active secretion of these substances, either into or out of the eye. This point is well exemplified by the work of Fischer (1930), who found that the distribution of lactic acid between blood and aqueous humour was different according as the eye was normal or aphakic, the concentration in the aqueous humour in the latter case being smaller owing presumably to the removal of the lens, the glycolytic activity of which uses up glucose and contributes continuously to the amount of lactic acid present in the fluids of the eye. In this light also must the large quantity of hexuronic acid (vitamin C) in the aqueous be viewed. It is undoubtedly present in excess of the concentration in the serum sometimes being increased by 20 times; a circumstance which has suggested a secretory process. It is, however, reduced or absent in cataract and is present only in traces in aphakia (Müller, 1932-3; Monukova and Fradkin, 1935; Bellows, 1936); and in these conditions it is present not in the reduced form as occurs normally, but only in the reversibly oxidized form (Buschke and Goldmann, 1935; Bellows, 1936). On the other hand Bietti (1935) found that the oxidized form is in all circumstances equal to the concentration in the blood as if it had merely filtered through; and the work of Müller and Buschke (1934), Fischer (1934), Müller (1935-7) and Bellows (1936) would seem to show that in the eye in the presence of the lens this substance is continuously converted by dehydrogenation into the reduced form, thus playing a part in the oxidation-reduction system of the lens and incidentally accumulating in the aqueous, a preservative action on the vitamin which is lost on the death or removal of this tissue.

In addition to the lens, the retina is the seat of metabolic activity, and the products of such activity may influence the distribution of ions involved in the acid-base balance of the blood; further, on the whole, the metabolic products themselves will tend to be more highly concentrated in the aqueous humour than in the plasma whereas the initial reactants, *e.g.*, glucose, will be expected to have a lower concentration. It may easily be that the net effect of such an activity will be even to produce an excess of osmotically active material in the eye (Benham, Duke-Elder and Hodgson, 1938).

If this interpretation is correct, then such departures from the expected distribution-ratios on the basis of a static Donnan equilibrium may be due not to a secretory activity of the membrane

separating the aqueous humour from the blood plasma, but to the metabolism occurring within the eye; such an interpretation therefore allows of a reconciliation between the dialysate theory and the two or three outstanding observations in apparent conflict with it. It is quite possible that some substances, such as hyaluronic acid (Meyer and Palmer, 1936; Meyer, Dubos and Smyth, 1937), which is present particularly in the vitreous, may be secreted by the retinal and ciliary epithelium; but this in no way invalidates the general conception.

3. The argument has been advanced that there is an *irre-ciprocal permeability of the ciliary epithelium to salts and water*.—The experiments described in this paper purport to show that this claim is without foundation.

This argument was first propounded by Gaedertz and Wittgenstein (1927) on experiments based essentially upon studies of the penetration of dye-stuffs into the eye, acid dye-stuffs being found to penetrate, whereas basic dyes, *e.g.*, neutral red, did not. Dye-stuffs, in general, are weakly acidic or weakly basic, and it is now generally realised that the mechanism of penetration of salts of weak acids and bases through a membrane is essentially different from that of salts of strong acids and bases (*e.g.*, NaCl), the former being brought about by the penetration of undissociated acid or base produced by hydrolysis, followed later by ionic exchanges alone (see Tyler and Horowitz, 1937; Krahl and Clowes, 1938). Thus salts of the weak base ammonium penetrate rapidly into the red blood corpuscle which is *impermeable to cations*, the mechanism being most probably the initial penetration of NH_3 , followed by an exchange of anions (Jacobs, 1927; Jacobs and Parpart, 1938). The demonstration of the rapid penetration of the salts of weak bases such as ammonium into the erythrocyte does not prove that its membrane is specifically cation permeable but rather supports the opposite and correct view that it is specifically anion permeable; nor has it been accepted as evidence of secretory activity.

There are many other objections to the use of dyes in permeability studies which need not be entered into here; suffice it to say that the failure or otherwise to observe the penetration of a dye into a given cell is not simply due to an impermeability or otherwise of the cell-membrane to the molecules of this dye, but involves the more general problem of vital staining, a much more complicated matter (Gicklhorn, 1931).

An interesting investigation based on the use of dyes is that of Friedenwald and Stiehler (1938), who claim that the epithelium of the ciliary body shows a selective permeability in the sense that water and basic dyes are preferentially transferred in the direction of blood to aqueous humour, and acidic dyes in the

reverse direction; the cause of this selectivity is said to reside in the concentration of Warburg's yellow enzyme in the ciliary epithelial cells. In view of what has been said about the fallacy of arguing from the behaviour of dye-stuffs to the behaviour of ions in general, it is perhaps unfortunate that these striking claims rely essentially on observations of selective staining, the more so as one of the dyes principally used in this investigation, *viz.*, Rose Bengal, is a strong haemolytic agent in concentrations upward of 1×10^{-7} M in the presence of light and upward of about 1×10^{-4} M in the dark (Blum, Pace and Garrett, 1927), *i.e.*, in concentrations considerably less than those used by Friedenwald and Stiehler (M/1000). The action of lysins of this class is not confined to the erythrocyte (Lillie, Hinrichs and Kosman, 1935).

4. A novel argument in favour of a secretory mechanism is that of Robertson (1939). This author has followed the penetration of glucose, urea, etc., into the lymph, the gastric juice, the cerebro-spinal fluid, and aqueous humour after an intra-venous injection of these substances; he argued that, because the course of penetration into the aqueous humour follows that into the gastric juice more closely than that into the lymph, the aqueous humour is a secretion. Reference to the equation* derived to suit the conditions of penetration into the eye will show that the rate of change of concentration of the penetrating substance is a function of A/V , the ratio of the area of the membrane to the volume of solution contained by it. For comparisons between rates of penetration in two different systems this ratio must be known otherwise differences in rate may simply be attributable to differences in this ratio and not to any special characteristic of the membrane. It is quite clear that in two such anatomically divergent systems as the optic and lymphatic, even an approximate equality of A/V will not be found, so that the comparisons are without value. Quite apart from this consideration the argument ignores certain obvious dissimilarities in circulations of the fluids compared.

The arguments put forward by Robertson (1939) that the alteration of the intra-ocular pressure after varying the osmotic pressure of the blood by intra-venous injection shows a time-lag and corresponds in its variations with the time-curve of the gastric juice rather than with the tissue-fluids, and that, in the colloid osmotic disturbance of nephrotic oedema, it shows no tonometrically apparent variation are of great interest but may be susceptible to several explanations. In the first place, not only has the fluid interchange to traverse the capillary walls (a blood-tissue barrier as in other tissues) to reach the chambers of the eye, but there is

* *Vide infra.*

interposed a second more complex membrane, which will be referred to at a later stage, which constitutes a tissue-aqueous barrier. In the second place, it must be admitted that in their reactions the capillaries vary considerably among the organs of the body. There is evidence, for example, that the ocular capillaries are much less permeable than most, a circumstance which accounts for the small protein content in the intra-ocular fluids. Moreover, there is presumably a self-regulating mechanism, probably of central origin and probably acting mainly through vascular reflexes, governing the physiology of the tension of the eye as there is of every physiological function. In the dramatic exigencies of animal experiment this control is broken down. Although experimentally the intra-ocular pressure varies directly with the blood pressure, clinically such variations appear to be buffered by some such control. Similarly it might be argued that although the intra-ocular pressure varies with colloid pressure of the blood experimentally (Duke-Elder, 1927; and again confirmed in this paper), such a variation need not be allowed to become tonometrically evident in the state of clinical nephrotic oedema. It may be of significance that the eye has an exceptionally efficient system of vaso-motor reflexes (Duke-Elder, 1931); and it may also be of significance that in conditions where such control would be expected to break down owing to the local liberation of histamine-like substances, the eye does share in a general oedema with a resulting marked glaucoma involving pressure over 100 mm. Schiötz, as in epidemic dropsy (Kirwan, 1936). The whole of this argument, however, is highly hypothetical and must wait upon further research.

These conditions, however, open up questions which our present knowledge of physiology cannot solve; but in the meantime it may be tentatively suggested that some of the arguments put forward in favour of the view that secretory activity dominates the fluid-traffic and the pressure of the eye are at least inconclusive in so far as they are susceptible to more than one interpretation.

SECTION II.—CHEMICAL ANALYSES.

In this section we shall describe the results of comparative chemical analyses of the aqueous humour and blood serum of cats. In the first series (Davson, Duke-Elder and Benham, 1936) all three of the ions Na, K and Cl were determined simultaneously in the two fluids, and in the second (Davson, 1939) attention was concentrated mainly on the distribution of Na.

Experimental.—Cats were anaesthetised with Et₂O and bled from the carotid; during the bleeding the aqueous humour was removed from both eyes with a syringe. After clotting, the serum was removed and 5 ml were measured into a centrifuge-tube. Proteins were removed with trichloroacetic acid, the

washings evaporated to dryness in a silica flask and the residue ashed with conc. HNO_3 (Kutz, 1931). The ashed residue was made up to 10 ml with distilled water and used for the Na^+ and K^+ determinations. For determinations of Na^+ and K^+ in the aqueous humour, 0.5 ml samples were measured into silica flasks and ashed directly with HNO_3 . The ashed residues were washed out into silica beakers, evaporated to dryness and taken up with 1 ml of water. Na^+ was determined by the method of Barber and Kolthoff (1928), K^+ by Kramer's method (1920), and Cl^- directly on the serum and aqueous humour by a modification of the method of Van Slyke (1923). The modification consisted in filtering off the AgCl precipitate through an asbestos filter before titration with KCNS . All methods gave errors of about 1 per cent.

TABLE I

Exp. No.	Na			K			Cl		
	Aqueous Humour	Serum	R_{Na}	Aqueous Humour	Serum	R_{K}	Aqueous Humour	Serum	R_{Cl}
1	155	161	1.04	—	—	—	130	124	0.96
2	153	157	1.03	6.25	6.50	1.04	128	123	0.96
3	147	161	1.09	—	—	—	122	117	0.96
4	162	170	1.05	6.20	6.05	0.97	135	135	1.00
5	—	—	—	6.30	6.80	1.08	129	122	0.95
6	151	161	1.07	6.15	6.75	1.10	127	123	0.97
7	150	160	1.05	6.00	6.50	1.08	129	123	0.95

Concentrations are expressed in millimols. per kg. of water.

In Table I are shown the results of experiments on six individual cats (Exps. 1-6) and on the pooled aqueous humours and plasmas of four cats (Exp. 7). In this latter case the individual samples of plasma and aqueous humour were pooled in proportion. This rendered possible the use of 5 ml samples of aqueous humour for ashing just as with plasma so that the triplicate determinations of all three ions could be made; although trichloroacetic acid was unnecessary in the case of the aqueous humour, it was added so as to make the conditions identical. In the table the R values indicate the ratios of the concentrations of the ions, expressed as millimols per kg. of H_2O , in the serum and aqueous humour; thus R_{Na} equals $(\text{Na})_{\text{S}}/(\text{Na})_{\text{Aq}}$. Blanks in the table indicate that insufficient material was obtained for all three analyses.

It is evident that in all cases R_{Na} is greater than unity as would be expected on the basis of the Donnan equilibrium; in all cases but one R_{Cl} is less than unity; the values of R_{K} were more variable than the others but similar in trend to those of R_{Na} . The values for Exp. 7 represent the pooled serum and aqueous humour of four cats with a considerably smaller margin of error in the actual determinations.

Van Slyke (1926) has shown on the basis of certain assumptions that human plasma in equilibrium with its dialysate should give values for R_{Na} and R_{K} of 1.04 and for R_{Cl} 0.96; the experimental values obtained show a fairly satisfactory agreement with expectation. Thus Exp. 7, which is the most significant, gave values for R_{Na} and R_{K} of 1.05 and 1.08 respectively and for R_{Cl} 0.95. However, it seems unlikely that the variability of the values of R as seen in Exps. 1-6, especially of R_{K} , can be attributed to experimental errors in the determination of the ions. It must be emphasised that the Donnan ratios should be expressed

in terms of activities, and there seems no reason to suppose that the activity coefficients will be the same in both aqueous humour and serum; furthermore, the procedure for obtaining the fluids, anaesthesia, insertion of a hypodermic needle through the tough cornea, etc., must have temporary influences on the local concentrations of these ions; for example D'Silva (1934) has shown that adrenaline, which is liberated during the anaesthetising process, causes an increased blood K^+ .

Thus, so far as these results go, it would appear that the ions Na, K, Cl, are distributed approximately in accordance with the laws of simple dialysis, or if this is to overstate the matter, it may be said that *the variations from expectation are neither so large nor so consistent as to warrant the supposition of a secretory activity in respect to these ions.*

In this work the aqueous humour was divided into three portions for determinations of the separate ions; owing to the limited quantity of fluid it was not possible to make duplicate determinations, so that the errors were larger than they would have been had triplicate determinations of a single ion been compared. It was thought at the time that single determinations on all three ions would have more value, since, if deviations from the theoretical Donnan ratios occurred, the relative magnitudes and directions of the deviations of the positive and negative ions would give a clue to the nature of the disturbing factor. We concluded, however, that more accurate analyses of a single ion, *i.e.*, Na^+ , would give a more decisive answer to the problem. The reasons for this are as follows (Davson, 1939).

(a) The Na^+ is the main cationic contributor to the osmotic pressure of the blood and aqueous humour; consequently any differences in concentration between these fluids caused by experimental technique or changes in hydration of the animal will be comparatively rapidly levelled out by osmosis of water. This is not the case with K^+ , since changes in the concentration of the fluids with respect to this ion must be levelled out by a process of diffusion and may take hours for completion (Davson and Quilliam, 1940, in press).

(b) So far there is no evidence that the Na concentration of blood is influenced by anaesthesia, but D'Silva (1934) has shown that the K concentration in the plasma of the cat rises during ether anaesthesia.

(c) Changes in the CO_2 tension of the blood of the order of magnitude encountered in the experimental technique used in this type of work are reflected in negligible changes in the Na concentration of the plasma, whereas they may have a considerable

influence on the Cl concentration; thus there is a difference of 1.5 per cent. between the plasma Cl concentrations in arterial and venous blood (Doisy and Beckmann, 1922). Similarly a variation in the temperature at which the blood is allowed to clot will have a minimal effect on the cation concentration of the plasma.

(d) A more careful investigation into the Barber-Kolthoff (1928) method of Na determination reveals that it has greater potentialities as an accurate means of determining small differences of concentration than the methods available for the determination of K and Cl.

In this work the distributions of Na between the aqueous humour and blood-serum of 18 cats are recorded. Blood was drawn under nembutal anaesthesia from six cats, and by heart puncture without general anaesthesia from the remainder. Owing to the improved technique, to variations of 0.5 per cent. or greater in these distribution-ratios from the theoretical ratio of 1.04 may now be ascribed a significance which it would have been unjustifiable to assume in the earlier work. Hence, although the present results bear out the earlier ones in that the mean Na ratio lies fairly close to the theoretical value, they do, nevertheless, leave room for a possible secretory activity on the part of the membrane separating the eye-fluids from the plasma. Owing to the divergencies from theory obtained in these experiments, both Na and Cl were determined in a group of six cats. Duplicate estimations were possible in both instances owing to the use of the AgIO_3 method for Cl determination (Sendroy, 1937), which requires much smaller quantities of fluid than the Volhard method. The variations in the Cl ratios did not correspond in any regular way with those of the Na.

Determination of sodium.—Three 0.5 ml lots of each fluid were measured out into silica flasks with long thin necks; 1 ml of conc. HNO_3 and two drops of conc. H_2SO_4 were added to each and the contents heated gently for 30 minutes on a sand-bath. The temperature of the bath was then raised so that the HNO_3 evaporated. When the contents of the flask had evaporated to apparent dryness the latter was cooled so that the H_2SO_4 and HNO_3 remaining inside condensed; the flask was then replaced on the sand-bath and more organic matter was destroyed. By cooling and heating in this manner three or four times all the organic matter was removed without raising the temperature above about 350° ; further the amount of acid used for ashing was the same for all samples, thereby controlling the blank due to the presence of Na in this reagent. The contents of the flask were washed out into a silica beaker with four successive lots of 2 ml of H_2O , the solution was evaporated to dryness on a sand-bath and after the addition of 1 ml of H_2O the Na was determined by the Barber-Kolthoff (1928) gravimetric technique. The following precautions, in addition to those described by these authors, should be observed. After addition of the reagent the mixture is stirred with a thin glass rod for at least 1 min.; the precipitate adhering to the rod may be washed off into the beaker with a few drops of the reagent. As a washing medium for the precipitate, 95 per cent. EtOH saturated with freshly

prepared sodium-zinc-uranyl acetate is used; it is useless to attempt to store this wash fluid as a fine precipitate settles out continuously.

Of triplicate determinations made in this way, two usually agreed to within 1 in 500, and it was rare to find one of the three deviating from the mean by more than 1 per cent.

Determination of chloride.—This was carried out exactly as recommended by Sendroy (1937), without removal of proteins from the serum, with the exception that the excess of AgIO_3 was removed by filtering through asbestos. The individual results rarely differed from the mean by more than 0.5 per cent.

Determination of total solids in the serum.—About 2 ml of serum were weighed in a bottle and evaporated to dryness on a sand-bath; the bottle was then transferred to an oven at 105° and the contents dried to constant weight.

Errors in the determination of Na.—In work of this kind, in which fluids of very nearly equal compositions are compared, it is essential that a fair statement of the errors should be made. In Table III the results of which the authors wish to stress most, an idea of the accuracy of the figures presented may be derived from the following: In Exps. 1, 5, 6, 9 and 11 two of the three determinations of Na in the serum and two of the three determinations in the aqueous humour gave values differing by less than 1 in 600; in Exps. 2, 4, 7, 8, 10 and 12 two of the determinations on one fluid differed by less than 1 in 600, whilst two of the determinations on the other fluid differed by less than 1 in 300. In Exp. 3 two determinations of the aqueous humour differed by 1 in 600, whilst the discrepancy between the values for the serum was less than 1 in 100.

TABLE II

Na contents of serum, expressed in millimol. per kg., from blood drawn first by heart puncture and then from the carotid artery after ether anaesthesia.

Exp. No.	Heart Puncture	Ether Anaesthesia	Change (%)
1	152.5	152.0	-0.3
2	154.5	154.0	-0.3
3	149.0	149.0	0.0
4	149.0	154.0	3.4
5	144.5	146.0	1.0
6	148.0	152.0	2.7

The effect of ether anaesthesia on the Na content of the serum of the cat is shown in Table II; the blood was drawn first by heart puncture, and about 15 min. later from the carotid under ether anaesthesia. In three instances the influence of ether was negligible, and in the remaining three an increase of 1.3 per cent. was observed. These last results may account for the rather large values of the ratio $(\text{Na})_s/(\text{Na})_{Aq} = (R_{Na})$ observed earlier.

In Table III the Na contents of serum and aqueous humour of twelve cats are shown, together with the percentage solids in the serum. In the fifth and sixth columns the values are expressed as millimol. per kg. of H_2O , assuming the aqueous humour to contain 1 per cent. of solids. In the final column the values of the ratio $(\text{Na})_s/(\text{Na})_{Aq}$ are given, the figures being given to the nearest 5 parts in 1,000. In Exps. 1-6 the blood was withdrawn by heart puncture, and in Exps. 7-12 under nembutal anaesthesia. It is seen that the mean R_{Na} is the same for the two groups. It is clear, however, that individual ratios may differ by as much as 3.5 per cent. from the theoretical value of 1.04 (Van Slyke, 1926), and there seems to be no correlation between the protein content (*i.e.*, the percentage solids) and the magnitude or sign of the deviation.

In Table IV are shown the results of six experiments on the distribution of both Na and Cl between the serum and aqueous humour; blood was drawn by heart puncture and allowed to clot under paraffin. In the final column the

products $R_{Na} \times R_{Cl}$ are shown; on the basis of the Donnan equilibrium and assuming that activities are equal to concentrations, this product should equal unity. It is clear that marked deviations from unity occur, and further that a low R_{Na} value does not always correspond to a low R_{Cl} as would be expected were NaCl, as such, being excreted into the aqueous humour. The mean R_{Na} is equal to that for Exps. 1-12 (Table III).

TABLE III

Sodium contents of serum and aqueous humour of twelve cats. Exps. 1-6, blood withdrawn by heart puncture; Exps. 7-12, under nembutal anaesthesia.

Exp. No.	Serum millimol./kg.	Aqueous Humour millimol./kg.	% solids in serum	Serum millimol./kg. H ₂ O	Aqueous Humour millimol./kg. H ₂ O	R _{Na}
1	152.3	158.0	7.5	164.7	155.9	1.055
2	154.4	157.0	8.9	169.5	158.6	1.070
3	148.7	158.9	7.8	161.3	160.5	1.005
4	149.2	159.8	7.9	162.0	161.4	1.005
5	144.3	153.9	8.5	157.7	155.6	1.015
6	147.9	154.4	9.1	162.7	156.0	1.040
7	162.3	172.0	8.2	176.8	173.9	1.015
8	147.9	152.9	8.1	160.9	154.6	1.040
9	146.4	152.8	8.5	160.0	154.4	1.035
10	152.2	158.2	9.0	167.2	159.8	1.045
11	150.7	155.3	7.0	162.0	156.9	1.030
12	145.7	153.2	8.4	159.0	154.7	1.030
Mean (Exps. 1-6)						1.030
Mean (Exps. 7-12)						1.030
Standard deviation (Exps. 1-12; vide Table IV)						0.018

TABLE IV

Results of simultaneous determinations of sodium and chloride in the serum and aqueous humour of six cats. Blood was withdrawn by heart puncture.

Exp. No.	R _{Na}	R _{Cl}	% solids in serum	R _{Na} × R _{Cl}
13	1.005	0.950	8.4	0.955
14	1.050	0.965	7.7	1.010
15	1.030	0.950	8.6	0.980
16	1.005	0.910	6.8	0.915
17	1.045	0.940	8.4	0.980
18	1.035	0.955	8.3	0.990
Mean	1.030	0.945	—	0.970

The results of the experiments described in this sub-section show that the distribution of Na between the serum and aqueous humour of the cat may vary from the theoretical ratio derived on

the basis of a Donnan equilibrium; the variation is irregular, however, and the means of the successive groups are equal and differ only by 1 per cent. from this theoretical ratio. The product $R_{Na} \times R_{Cl}$ is neither equal to unity in all the cases examined nor is it the same from one individual to another. So far a "theoretical" R_{Na} has been mentioned as though this were an invariable quantity; this, however, is not so, since its value depends on the number of indiffusible anions present in the plasma, and this will depend on the protein concentration, the relative amounts of the constituent serum proteins, and the acidity of the serum. The latter may be taken as constant, since intravital variations in acidity are not large enough to influence these results. Inspection of Tables III and IV shows that the percentage solids, and hence presumably the protein concentration, varies from serum to serum, so that deviations from the "theoretical" value of R_{Na} might be expected to be related to the protein content of the serum; nevertheless, there is no correlation between the protein concentration and the deviation of individual values of R_{Na} from the mean.

Let us now turn to an investigation of *in vitro* ultra-filtrates of plasma. Ingraham, Lombard and Visscher (1933) have calculated that the relative concentrations of diffusible salts in a dialysate of plasma should be equal to those from an ultra-filtrate. They then determined the distribution of ions between dogs' plasma and its ultra-filtrate. The present authors have calculated the results as the ratio $(Na)_p/(Na)_{Aq}$.

The seventeen determinations gave a mean value of 1.08 with a standard deviation of 0.021. Six ratios selected at random gave values of 1.05, 1.11, 1.09, 1.09, 1.02, 1.09.

Two points emerge from these results: first, that the variability of the distribution of Na between plasma and its dialysate *in vitro* is slightly greater than that found for the distribution of Na between serum and aqueous humour (standard deviation, 0.018), and secondly, the mean R_{Na} is greater than the calculated value of 1.04. Ingraham and his co-workers show, however, that this high value may be due to the proteins which depress the activity of the Na in the plasma.

Some results of Greene and Power (1931) on artificial *in vivo* dialysates of plasma may next be considered. The following values of the R_{Na} have been taken at random: 1.65, 1.11, 1.14, 1.11, 1.15, 1.11. The mean of 15 determinations by these authors was 1.10. It is clear that here, also, the deviations from the mean are greater than with the aqueous humour and serum; also the ratios are very much greater than the theoretical value of 1.04. Similar values were found by Greene, Bollman, Keith and

Wakefield (1931) for the distribution of Na between plasma and ascitic fluid.

In the light of these results on *in vitro* systems and artificial *in vivo* dialysates it would be very rash to conclude that deviations from a certain theoretical R_{Na} of 1.04 of the size met with in this work are proof that a secretory process is at work in determining the formation of the aqueous humour. In fact, if a direct comparison of the various systems is permissible, it would appear that *the membrane separating the aqueous humour from the blood-plasma in the cat is a more efficient dialysing apparatus than the collodion sac*. Any proof that a secretory activity occurs in the cat must come from other sources.

SECTION III.—PERMEABILITY STUDIES.

In Section II we have seen that the results of chemical determinations of sodium, potassium, and chloride in the aqueous humour and serum of cats would suggest that these ions are on the average distributed in accordance with the Donnan equilibrium; the most accurate work would indicate a variability in the distribution which may not be due entirely to adventitious changes in the concentrations of these ions in the plasma an hour or two before the fluids were withdrawn although the extent of the variability of the distribution ratios is rather less than determinations of similar ratios in *in vitro* ultra-filtrates and *in vivo* artificial dialysates of serum. Let us therefore assume, as one of us has recently suggested (Duke-Elder, 1938), that the aqueous humour may behave in a general way as a dialysate but that superimposed on this behaviour is the complication of a second multi-layered membrane forming the tissue-aqueous barrier and perhaps also an activity of the epithelial cells of the ciliary body to modify to a small extent the composition and consequently the ionic distribution-ratios of the aqueous humour and plasma. If secretory activity is to be postulated, evidence must be produced that the membrane separating the eye contents from the blood plasma has the power of moving salts against concentration-differences in the same way, for instance, as does the tubular epithelium of the kidney. So far the exponents of the secretory theory have failed to provide any direct evidence on this point although claims have been made respecting the selective activity of the membrane which would at first sight seem to provide some basis for postulating a secretory activity. Thus perhaps the only really convincing argument made by the advocates of secretion is that the membrane of the eye shows uni-directional permeability, crystalloids and water being able to penetrate into the eye but not out (Friedenwald and Pierce, 1931; Friedenwald and Stiehler, 1938). If this were

the case a membrane with this property would, from analogy with other systems, be expected to show secretory functions, although uni-directional permeability *per se* would not necessarily preclude an equilibrium-distribution of crystalloids indistinguishable from that in a dialysate. Gaedertz and Wittgenstein (1927) on the basis of experiments carried out chiefly with dyes claim that the membrane is specifically permeable to anions and impermeable to cations; this fact of itself is not sufficient to justify the postulation of a secretory mechanism since specific ionic permeability is associated with cells which show no secretory activity, *e.g.*, the erythrocyte (Davson and Danielli, 1938), but the demonstration of the existence of a specific anion permeability of the membrane would again provide a useful theoretical basis for secretory activity, whereas a membrane incapable of distinguishing between negative and positive ions would give greater difficulties.

In the present work three main problems have been investigated.

(a) Does the membrane, as claimed by Friedenwald and his co-workers, show uni-directional permeability in respect to crystalloids?

(b) Is the membrane as claimed by Gaedertz and Wittgenstein specifically anion permeable?

(c) Has the membrane any secretory functions in respect to ions?

By using the isolated head preparation and varying the potassium, sodium and chloride in the perfusing fluid, unequivocal answers to the problems (a) and (b) were obtained showing that these claims are without foundation. To test (c) a similar set-up was used, and it was argued that since secretion is associated with an oxidative activity on the part of the cells which is normally poisoned by cyanide (*vide, e.g.*, Hober and collaborators, 1927-1930, in respect of kidney and liver, and Huf, 1936, in respect of frog's skin), then the rate of penetration of an ion, *e.g.*, K^+ , across the membrane should vary according as the eye is alive or poisoned with cyanide. If this argument is correct, then the results shown here would indicate the absence of a secretory activity on the part of the membrane in respect to the potassium ion at least.

The rate of penetration of potassium was measured in the direction from blood to aqueous humour as it is possible to maintain a reasonably constant high level of potassium in the blood. An attempt was made to measure the rate of penetration of potassium from the eye into the blood; to do this necessitated reducing the potassium content of the perfusing blood, this being done by dialysing the blood against isotonic $NaCl - NaHCO_3$;

it was found, however, that the level of potassium in the blood rises again so rapidly to its normal value during the perfusion that it was impossible to maintain a concentration-difference between the inside and outside of the eye long enough to make accurate measurements. Presumably the rise in blood potassium was due to an escape from muscle (Fenn, 1936) and/or nerve cells.

The rates of penetration of sodium and chloride were measured in the direction eye to blood, as it is possible to reduce their concentration in the blood by diluting with isotonic solutions containing a colloid, thereby maintaining the relative osmotic pressures reasonably constant. A single experiment was carried out using a raised sodium content, *i.e.*, sodium penetrating from blood to aqueous humour, but the result was equivocal owing to the large osmotic pressure difference created by the addition of excess sodium chloride which, of course, caused water to pass out of the eye.

Experimental.—The essentials of the procedure were to perfuse an isolated cat's head with cat's blood containing either an excess or deficiency of one of the ions, potassium, sodium or chloride, using the aqueous of one eye to obtain the initial value of the concentration of the ion considered, and after a definite interval of time the aqueous of the other eye was withdrawn for analysis. Determinations of the concentration of the ion in the blood-serum during the perfusion gave the concentration-difference between the serum and the aqueous, and from these values a permeability constant could be calculated.

When it was desired to compare the rates of penetration of a given ion into the living and dead eyes of the same head, a determination of the concentration of the ion in the animal's own blood at the time of severance of the head enabled an approximate calculation of the concentration initially present in the aqueous. Consequently the first withdrawal of the aqueous was made after one hour of perfusion, and the second after a further hour during which the head was poisoned with cyanide. In this way two permeability constants were obtained, one for the penetration into the living eye, and one into the poisoned eye.

The cat was anaesthetised with ether and subsequently with chloralose. The dorsum of the second cervical vertebra was exposed and cleaned and a dissection was then made to expose the common carotids for about one and a half inches to their bifurcation. A portion of the larynx and trachea were removed. The common carotids were clipped and cannulated and connected with the perfusion circuit. Just prior to the occlusion of the vertebral circulation with an ecraseur the clips were removed thereby establishing the perfusion of the head, and consequently the head was at no time during the procedure deprived of a fully oxygenated blood supply. A Dale-Schuster pump was used to propel the blood through the perfusion circuit which included a resistance and an oxygenator, the latter being of the type used by Gregory (1939) and Chute and Smyth (1939),

and a current of 95 per cent. oxygen and 5 per cent. carbon dioxide was passed through it. The temperature of the circulating blood was maintained constant at 37°C. by immersing most of the circuit in a water-bath, the temperature of which was controlled by a thermo-regulator of the type used by Lythgoe and Quilliam (1938). Two cats were usually bled under ether anaesthesia to provide defibrinated blood for the perfusion apparatus before the experiment. For further details of the procedure the reader is referred to a paper by Chute and Smyth (1939). The perfusion pressure was maintained as constant as possible at 180 mm. Hg. The flow varied in different animals, from 40 to 90 ml per min. and invariably increased after poisoning the preparation with cyanide.

The isolated head thus prepared exhibits a blink reflex in response to a puff in the eye, or to movement of the vibrissae, or to direct stimulation of the cornea or the inner canthus of the eye. A tap on the nose elicited a jaw jerk and a blink, and often in raised blood potassium experiments a series of jaw movements. The pupil of the eye was constricted and spontaneous eye, ear, and jaw movements were occasionally seen. When the isolated head is deprived of an oxygenated blood supply, or is poisoned with cyanide, reflexes vanish and the pupils widely dilate. The constriction of the pupil was taken as an indication that the preparation was alive. Eye-fluids for analysis were withdrawn with a clean dry syringe.

Chemical Methods of Analysis.—Davson (1939) has recently described a procedure for the exceptionally accurate determination of sodium in serum and aqueous humour involving the Barber-Kolthoff (1928) gravimetric precipitation. This method was used in this work for both sodium and potassium, the latter being precipitated by the Kramer (1920) sodium cobaltinitrite procedure and estimated volumetrically. The chloride was determined by the method of Sendroy (1937).

The rate of penetration of a substance into the eye will be given by the following equation :

$$\frac{dx}{dt} = k A (C_s - C_{Aq})$$

where x is the amount of the substance penetrating into the aqueous humour, C_s and C_{Aq} are respectively the concentrations of the substance in the serum and aqueous humour.

A is the area of the membrane through which diffusion occurs.

k is a permeability constant.

t is the time in minutes.

This assumes that the rate of passage across the membrane is slow compared with the rate of diffusion in the eye and blood.

Since the concentration of the substance at any moment is given

by $C_{Aq} = \frac{x + I}{V}$ where I is the amount initially present in the

eye and V the volume of the aqueous, which remains virtually constant,

$$\begin{aligned}
 \text{we get } \frac{dC_{Aq}}{dt} &= \frac{kA}{V} (C_s^+ - C_{Aq}) \\
 \text{which gives } \frac{1}{(t_2 - t_1)} \log. \frac{(C_s - C_{Aq})t_1}{(C_s - C_{Aq})t_2} \\
 &= \frac{kA}{V} \\
 &= K
 \end{aligned}$$

assuming that the serum concentration remains constant (actually the serum concentrations did vary slightly so that a mean value for the whole period was used), t_1 is the time at the beginning and t_2 the time at the end of the experimental period. Thus if it is assumed that A/V , the ratio of the area of the blood-aqueous barrier to the volume of the eye is a constant for different eyes, the logarithmic ratio may be considered as a measure for comparison of the rates of penetration of a given substance into the eye. Such a treatment is not strictly correct for ionic permeability, since potential differences will be set up owing to the unequal rates of diffusion of the positive and negative ions; however, in view of the obvious approximateness of many of the assumptions at the basis of the equation, and further since the experiments show that the relative rates of penetration of anions and cations are not greatly different, a more exact treatment would be supererogatory.

Results.—In Table V the initial concentration of the potassium ion in the aqueous humour (A_1) is made equal to 100 and its concentration in the aqueous humour after a definite interval of perfusion (A_2) and its mean value in the serum (S) are scaled up appropriately.

Inspection of the values of K , the measure of the rate of penetration of potassium, shows a variability between the extremes of 16 and 36, with a mean value of 24 for the rate of penetration into the living eye against a mean value of 22 for the poisoned eye. In view of the individual variations a difference between the means of only two units is without significance. Thus so far as these experiments go *there is no great difference in behaviour between a living and dead eye in respect to the rate of penetration of potassium.* Whether or not the variability from one preparation to another is due to different permeabilities of the membrane or merely to variations in A/V of course cannot be decided. The fact that out of 9 experiments on living eyes five of the values of K differ by not more than 4 units from the mean indicates a regularity in behaviour in response to a raised blood potassium that is reconcilable with a purely mechanical diffusion process.

TABLE V

Penetration of potassium into the aqueous humour. Initial value of the concentration of potassium in the aqueous humour (A_1) equals 100. A_2 equals concentration after one hour of perfusion with blood of potassium concentration equal to S . In experiment, January 12, 1939, the time period was 75 min.

Experiment	A_1	A_2	S	$100 \left[K = \frac{1}{t_2 - t_1} \log. \frac{(S - A_1) t_1}{(S - A_2) t_2} \right]$	Remarks
January 3, 1939 - -	100	134	160	36	Alive
December 21, 1938 -	100	163	256	23	Alive
December 14, 1938 -	100	148	253	16	Alive
December 7, 1938 -	100	155	220	27	Alive
November 15, 1938 -	100	115	143	20	Alive
January 18, 1939 -	100	160	227	28	Alive
May 5, 1939 - - -	100	121.5	149.5	25	Alive
May 11, 1939 - - -	100	122.5	160	20	Alive
May 15, 1939 - - -	100	123.5	174	17	Alive
Mean = 24					
January 12, 1939 - -	100	176	258	23	Poisoned
March 24, 1939 - -	100	124.5	166	20	Poisoned
March 31, 1939 - -	100	134	195	19	Poisoned
May 18, 1939 - - -	100	147	206	25	Poisoned
May 25, 1939 - - -	100	138.5	185	26	Poisoned
June 28, 1939 - - -	100	116	146	19	Poisoned
Mean = 22					
Mean of $K \times 100$ for both living and poisoned heads 23.					

In an endeavour to obtain more accurate evidence in respect to a possible difference in behaviour of living and dead eyes, experiments were carried out in which the head was perfused with a blood containing a raised potassium for two successive hours, one hour alive and one hour either alive or poisoned with cyanide. In this way a direct comparison on the same animal could be made, provided a value for the initial concentration in the aqueous humours of the eyes before perfusion began could be obtained without actually withdrawing the aqueous humour. An approximation to this value can be obtained by estimating the potassium content of the serum of the animal whose head was used in the experiment, and multiplying by the factor 1/1.07 which is about the average ratio of the concentrations of potassium in the aqueous humour and serum at the end of the operative procedure, this high value being due to a gradual increase in the potassium content of blood under anaesthesia, the aqueous concentration lagging behind. This, unfortunately, introduces a fairly large possibility of error into the calculation, perhaps in an extreme instance of plus or minus 6 units; not sufficient, however,

to mask any large change in permeability which would be expected supposing that cyanide suppresses a secretory mechanism. Results of this series of experiments are shown in Table VI, where A_1 is the value of the initial concentration of potassium in the aqueous humour calculated in the aforementioned manner, A_2 is the value after one hour of perfusion, and A_3 after a further hour. S_1 and S_2 are the mean values of the serum potassium concentration during the first and second hours respectively. K_1 and K_2 refer

to the values of $\frac{1}{t_2 - t_1} \log \frac{(S - A_1)t_1}{(S - A_2)t_2}$ calculated for penetration during the first and second hours.

TABLE VI

Penetration of potassium into aqueous humour of both eyes. A_1 equals the calculated potassium content of the aqueous before perfusion, A_2 and A_3 the observed concentrations after 1 and 2 hr. of perfusion with blood containing potassium in the mean concentrations S_1 and S_2 respectively.

Experiment	A_1	A_2	A_3	S_1	S_2	100K ₁	100K ₂	Remarks
May 15, 1939 - - -	100	144.5	179	244	252	16	17	Alive
May 11, 1939 - - -	100	161	197	258	252	22	20	Alive
May 5, 1939 - - -	100	151	184	226	226	23	25	Alive
May 25, 1939 - - -	100	171	237	298	317	19	26	Poisoned
March 31, 1939 - - -	100	140	188	248	275	14	19	Poisoned
March 24, 1939 - - -	100	140	174	206	233	21	20	Poisoned
June 28, 1939 - - -	100	134.5	156	187	196.5	22	19	Poisoned

In the first three experiments shown in Table VI, the head was alive for both periods of one hour, and it is seen that the values of K_1 and K_2 are reasonably constant. In the last four experiments the head was poisoned with cyanide at the beginning of the second hour, and it is seen that although there is a slightly greater variation in the relative values of K_1 and K_2 the differences in any given experiment fall within the limits of experimental error; actually a slight increase in the value of K during the second hour might be expected owing to the increased flow that occurs after poisoning with cyanide.

In Table VII are shown some results on the diffusion of sodium and chloride.

In the first five sodium experiments the concentration of sodium in the serum was reduced by the addition of a diluting mixture consisting of isotonic glucose and gelatine or gum arabic; in this way the osmotic pressure was maintained as constant as possible.

TABLE VII

Permeability of the aqueous humour-blood barrier to sodium and chloride. In experiment January 25, 1939 penetration is in the direction blood to aqueous humour, in the remaining experiments the direction is reversed.

SODIUM

Experiment	A ₁	A ₂	S	100 K	Remarks
March 17, 1939 - -	100	94	75.5	10	Alive
February 22, 1939 -	100	90.5	70	17	Alive
May 19, 1939 - - -	100	97.5	74.5	5	Poisoned
May 26, 1939 - - -	100	96.5	80	8	Poisoned
May 31, 1939 - - -	100	94.5	60	6	Poisoned

Mean value of $K \times 100 = 9$

January 25, 1939	100	111	119	38	Alive
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CHLORIDE

June 19, 1939 - - -	100	94.5	80	14	Poisoned
June 14, 1939 - - -	100	94	78	14	Alive

It is very difficult to make viable preparations using such a perfusion fluid, and a number of preparations died soon after the change over. In these instances cyanide was added to ensure complete suppression of any secretory activity and the experimental values of K obtained have been presented. It should be emphasised here that we do not wish to make a close comparison between the behaviour of the living and dead eyes in respect to sodium and chloride permeability owing to the small number of experiments on these ions. It is merely desired primarily to show that sodium and chloride can diffuse out of the eye. If the suppression of a secretory activity could be expected to produce a manifold variation in the permeability to sodium and chloride, then of course a comparison of the figures presented would certainly indicate the absence of a secretory activity. The results, however, show beyond doubt that sodium can penetrate the blood-aqueous humour membrane. In the sixth experiment the sodium content of the blood was raised by addition of molar NaCl, thereby increasing the osmotic pressure, and therefore causing a loss of water from the eye; this was apparent by the marked decrease in intra-ocular pressure as soon as the sodium chloride was added. It is to be noted that the value of K in this instance is 38 as against values of 5-17 for the rate of penetration in the opposite direction, and this difference is doubtless due to the migration of water producing an apparent penetration of sodium. Whether any sodium actually penetrates can, of course, not be definitely proved.

In the same Table two experiments are shown on the rate of loss of chloride from the eye, one on a live head and the other on a poisoned one. In these cases the blood was diluted with isotonic NaNO_3 - NaHCO_3 solution. The results show no obvious difference in behaviour between the living and dead eye. The close agreement between the two values is, of course, accidental. The point we wish to emphasise from the results in Table VII is simply that chloride and sodium are able to migrate across the membrane.

The mean value of K for sodium under the conditions of penetration from aqueous humour to blood is 9 compared with a value of 23 for potassium, penetrating, however, in the reverse direction. This difference is obviously significant and is what one would expect from the comparative sizes of the two ions. Chloride seems to diffuse at about the same rate as sodium, thereby showing that the claim of specific anionic permeability is both qualitatively and quantitatively unfounded.

The results described in this section provide definite proof that potassium and sodium may penetrate into the eye and that sodium and chloride may pass out; consequently any argument in favour of a secretory mechanism of the formation of the aqueous humour, based on an alleged irreciprocal permeability of the membrane or on an alleged specific ionic permeability, is without foundation; we have already pointed out that it is based on the very equivocal results of vital standing.

CONCLUSIONS.

1. The distribution of ions between the aqueous humour and the blood serum is consistent with the conception of dialysation, and shows deviations from theoretical values less than those obtained in dialysates in other *in vivo* systems and in artificial dialysates of serum. The distribution of non-electrolytes can provide no competent basis for argument.

2. The permeability between the serum and the aqueous is comparable in the normal animal, in the surviving perfused head, and in the perfused head poisoned with cyanide; in these circumstances the existence of any secretory activity in transit is impossible. The results of the present experiments show no difference in general behaviour between the living and the dead eye, a finding which argues against the existence of secretory activity.

3. The claims advanced for the existence of irreciprocal permeability between blood and aqueous are qualitatively and quantitatively unfounded.

4. In general terms we consider that it would be unwise to revive the old theory to the effect that the aqueous humour is a secretion elaborated by specialised cells, and that it would be preferable, and more in consonance with the experimental facts, to regard the aqueous humour primarily as an ultra-filtrate of plasma; *but*, as a result of metabolic activity occurring in the eye, and perhaps of the interposition of a second membrane (as opposed to a secretory activity of the membrane), to conclude that the concentrations of certain dissolved constituents in the aqueous humour are modified so that the observed concentrations represent a steady state and not the static Donnan equilibrium which one would expect in the absence of such disturbing activity.

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EXPERIMENTS ON THE EFFECT OF ASCORBIC ACID IN MUSTARD GAS BURNS OF THE EYE

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A STIMULATING article by Livingston and Walker¹ appeared recently on the effects of mustard gas ($\beta\beta'$ di-chloro-diethylsulphide) on the eyes of rabbits and the results of certain forms of treatment. The authors stated that they had not reached any final conclusion as to the value of local applications in treatment, but that their results at present pointed to the probability that local therapy would have little, if any, effect, and that the only procedure likely to prove of value would be the intravenous or parenteral injection of some substance which might prevent or counteract the peculiar chemical and physical effects of mustard gas on the cornea. They made use of ascorbic acid for this purpose and gave an account of their preliminary experiments, which suggested that this substance might be of value. The time at their disposal had been too short to arrive at a final conclusion, but the importance of the subject justified publication of the results so far achieved. The present research has been done in response to an invitation from the authors of the paper mentioned above to repeat their experiments.

Two problems among those in need of further enquiry emerge