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COMMUNICATIONS

STUDIES ON THE INTRA-OCULAR FLUIDS

2.—The Penetration of Certain Ions into the Aqueous Humour and Vitreous Body*

BY

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IN the first of this series of papers (Duke-Elder and Davson, 1949) a detailed study was made of the entry of sugars into the various parts of the eye from the blood; the present paper is a continuation of the same study with regard to salts. Exchanges of water between the blood and intra-ocular fluids occur rapidly, so rapidly as to indicate that these exchanges take place over a wide area of contact between the two systems and are not confined to a limited region such as the ciliary body. The osmosis of water into, or out of, the eye must influence the intra-ocular pressure immediately and effectively; and, since it is the salt content of the two fluids that

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largely determines their osmotic pressures, the conditions under which salts may penetrate from the blood into the intra-ocular fluids are of fundamental importance. It has been shown from this laboratory that the osmotic pressure of the aqueous humour is greater than that of plasma (Benham, Duke-Elder and Hodgson, 1937) and again that the difference in osmotic pressure is due, at least in part, to an excess of sodium and chloride ions in the aqueous humour over that demanded by the Donnan equilibrium (Davson, Duke-Elder and Maurice, 1948). On these grounds, therefore, we may expect that sodium and chloride will be secreted into the eye, but whether or not the only mode of entry of these and other ions is by a secretory process from the ciliary epithelium is a matter that has not yet been decided.

THEORETICAL

It is hoped that kinetic studies on the mode of penetration of certain ions will throw some light on the problem, although in view of the formal similarity of certain equations for diffusion to those for secretion, it seems unlikely that a mere mathematical analysis of the process of penetration of a given ion can decide whether it is entering by a simple process of diffusion or secretion, or by a combination of these. The development of the mathematical formulae required here has been taken up elsewhere (Davson, Duke-Elder, Maurice, Ross and Woodin, 1949); for our purpose we need only indicate that the rate of penetration of a substance into the aqueous humour may be presented in the form of a parameter, K'_A, calculated from the experimental material by substitution in the following formula:—

$$\frac{r}{t} \log \frac{rC_p - C_{Aqo}}{rC_p - C_{Aq}} = K'$$

where t is the time of penetration in hours, C_p and C_{Aq} are the concentrations of the substance in plasma and aqueous humour at time t; C_{Aqo} is the original concentration in the aqueous humour and r is the ratio $\frac{C_{Aq}}{C_p}$ at infinite time. A parameter, K'_V, for penetration into the vitreous body, is computed similarly from the appropriate concentrations in this fluid.

The principal assumption at the basis of the derivation of the formula is that the penetration occurs in accordance with Fick's Law, *i.e.*, that the rate of transfer across the blood-aqueous barrier is proportional to the concentration gradient across the barrier.

METHODS

General. The operative technique was similar in essentials to that described earlier (Duke-Elder and Davson, 1949); a high and relatively constant concentration of a given substance was maintained in the blood of a cat anaesthetised with nembutal. This was achieved, in general, by a single intravenous injection of an isotonic solution followed by repeated smaller injections in accordance with empirically determined schedules which varied with the substance considered. In the case of radio-active potassium (K^{42}), however, the rate of loss from the blood was so rapid that it was impracticable to maintain a constant high level; consequently the solution was injected by a drip-infusion technique. The concentration of K^{42} rose rapidly during the first few minutes, and then more slowly and approximately linearly during the rest of the experiment. K'_A and K'_V were calculated by graphical integration. The eyes and blood samples were removed at appropriate intervals and the aqueous humour, vitreous body and plasma submitted to analysis.

Chemical. Thiocyanate was determined by the colorimetric technique of Aldridge (1945) on trichloracetic acid filtrates.

Radio-active Tracers. With the exceptions described below, all the determinations of radio-active tracers were done with the pipette-type counter described earlier (Maurice, 1948) and figured and described in Fig. 1. It was supplied with its high tension from a Dynatron Type 200 power unit, the counts being recorded on a Type 200 scale-of-ten scaler preceded by a modified Neher-Pickering circuit. The counter was always washed and dried between samples except when there was a reasonable certainty that their difference in activity was small. Sufficient counts were made to give a standard deviation of between one and two per cent. in case of plasma and aqueous humour, and of five per cent. in case of the vitreous body,

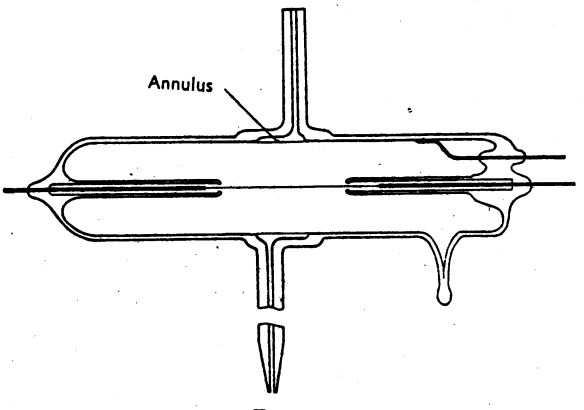

FIG. 1.

PIPETTE-TYPE GEIGER COUNTER FOR SMALL QUANTITIES OF BIOLOGICAL FLUIDS

The sample to be assayed (0·5 ml.) is sucked into the glass annulus which is formed round the cylindrical body of the counter itself and separated from it by a very thin glass wall. A high voltage is applied between the central wire and the wall of the counter, which is then sensitive to radio-active particles passing into it from the sample. These are individually registered on auxiliary apparatus. The number of particles counted in a given time is proportional to the concentration of the radio-active material in the sample.

Corrections were applied for the background count, the radio-active decay of the samples, and the resolution time of the counter. This last correction never rose above two or three per cent. The counter-voltage and the temperature were noted but they never varied sufficiently during an experiment to make a correction necessary.

In the case of the first five measurements of Na^{24} activity, the samples were measured into small glass dishes, which were stood on the window of a bell-type counter and located by means of a wax cast fixed by the edge of the window. Quadruplicate 0·2 ml. samples were used, the agreement being that expected from the number of counts taken.

For the first four phosphate determinations the sample, after removal of proteins if necessary, was treated with sufficient sodium phosphate solution to give about 1 mg. of precipitate, excess of magnesium and ammonium chlorides was added and the mixture made alkaline with ammonia. The precipitate of magnesium ammonium phosphate was collected by suction on a sintered glass filter, 4 mm. in diameter, sealed into the end of a glass tube. It was found necessary to repeat the precipitation on the first filtrate in order to complete the collection of the phosphate. The collected precipitate was dried and the "filter stick" was accurately positioned by a brass frame in proximity with the window of a bell-type counter.

Na^{24} was used in the form of sodium chloride with an activity of 1 mC per g.; K^{42} as potassium chloride of similar activity. P^{32} was received as a solution of phosphoric acid containing less than 10 mg. per ml. of total solids and with an activity greater than 0.3 mC per ml. and this was brought to pH 7·4 with NaOH.

TABLE I

THE RELATIVE RATES OF PENETRATION OF CERTAIN IONS INTO THE INTRA-OCULAR FLUIDS

Substance	No of Expts.	100 K'_A	100 K'_V	K'_A/K'_V
Thiocyanate	12	46·5 ± 2·3	18·3 ± 1	2·5
Sodium	15	37·6 ± 1·2	6·8 ± 0·3	5·5
Potassium	14	42 — 31	12 — 7·7	3·5 — 4
Phosphate	6	5·2 ± 0·9	0·33 ± 0·05	16

RESULTS

The *relative rates of penetration* of thiocyanate, sodium, potassium and phosphate into the intra-ocular fluids are shown in Table I. The differences in the rates of penetration are of considerable significance. In the first place the rate of penetration of thiocyanate is significantly greater than that of either sodium or potassium; and all three of these ions penetrate very much more rapidly than phosphate. The slow rate of penetration of the latter substance agrees with the results of a more exhaustive study by Palm (1948) on the rabbit. That thiocyanate enters more rapidly than sodium was confirmed by injecting both radio-active sodium and thiocyanate into the same animal, and measuring the rates of penetration of both. The mean of two experiments gave the following:—

	K'_A	K'_V
Sodium	34.4	6.8
Thiocyanate	43.7	20.7

A comparison of the rate of entry of thiocyanate and the sugars is also of interest. In the earlier paper of this series it was shown that the value of K'_A for the monosaccharides is, on the average, 32·5 and that for K'_V 11·2; it appears, therefore, that thiocyanate penetrates rather more rapidly than the monosaccharides into the aqueous humour and nearly twice as rapidly into the vitreous body. This is confirmed by simultaneous measurements on the same animal as is seen in Table II.

TABLE II

THE SIMULTANEOUS PENETRATION OF SUGAR AND THIOCYANATE INTO THE EYE OF THE CAT

Animal	Substance	100 K'_A	100 K'_V
No. 1	Thiocyanate	42·5	16·8
	Galactose	26·7	6·5
No. 2	Thiocyanate	40	20
	Glucose	27·1	7·8
No. 3	Thiocyanate	27·5	16
	Galactose	21·2	4·6

The variability of the rate of entry of potassium is noteworthy. In Table I the values of K′ for potassium have been indicated as a range varying between 42 and 31 for the aqueous humour and 12 and 7·7 for the vitreous body. This variability was due, in general, to the fact that the value of K′ determined from the first half-hour of penetration, was consistently greater than that obtained from the second half-hour. There is thus an unmistakable tendency for the rate of penetration of potassium to slow down during the course of the experiment under the conditions employed in this work. This matter is under investigation and will not be discussed further at present: the difference between the constants derived from the first and second half-hours of penetration is brought out by the following:—

K'_{A_1}	K'_{A_2}	K'_{V_1}	K'_{V_2}
40.5	30.3	11.8	7.4

which may be contrasted with the following figures for sodium:—

K'_{A_1}	K'_{A_2}	K'_{V_1}	K'_{V_2}
38.7	37.3	6.8	6.6

The site of penetration. In earlier experiments on the penetration of sugars (Duke-Elder and Davson, 1949) it was concluded that these substances entered the cavity of the eye from all the surrounding vascularized tissues. It will be remembered that this conclusion was based on the separate analysis of different sections of the frozen vitreous body. A similar technique was followed here, the frozen vitreous body being separated into three parts by sections at right angles to the antero-posterior axis, the anterior portion closest to

the ciliary body, the posterior part farthest away. If diffusion from the blood into the vitreous body occurred not only from the ciliary body but also from the whole of the posterior segment of the globe, the middle section would have the lowest concentration of the penetrating substance owing to the unfavourable area-volume relationship pertaining in this region. If, on the other hand, diffusion occurred exclusively from the ciliary body, the posterior portion would have the lowest concentration. As with the sugars

TABLE III

RELATIVE CONCENTRATIONS OF THIOCYANATE IN THE THREE SECTIONS OF THE VITREOUS BODY AFTER THE CONCENTRATION IN THE BLOOD HAD BEEN MAINTAINED AT A HIGH VALUE FOR VARIOUS TIMES

Time (min.)	Aqueous Humour	Concentration		Posterior
		Anterior	Middle	
30	100	46	49	46
30	100	55	64	82.5
31	100	47	34	55
34	100	39	28	33
38	100	53	37	64
60	100	58	42	44
60	100	55	37	57
60	100	56	52	80
60	100	68	68	80

TABLE IV

RELATIVE CONCENTRATIONS OF Na^{24} IN THE THREE SECTIONS OF THE VITREOUS BODY AFTER THE CONCENTRATION IN THE BLOOD HAD BEEN MAINTAINED AT A HIGH VALUE FOR VARIOUS TIMES

Time (min.)	Aqueous Humour	Relative Concentrations		Posterior
		Anterior	Middle	
28	100	44	12	18
60	100	38	29	25
7	100	27	12	6
8	100	24	11	10

it was found that with thiocyanate the middle sections, on the average, had the lowest concentration at the end of a given period of diffusion: the concentrations in the aqueous humour and the different parts of the vitreous body are as shown in Table III. One may tentatively conclude from these results, that thiocyanate diffuses into the vitreous body from all the vascularized tissue surrounding it. With radio-active sodium and potassium, on the other hand, a significantly different picture of distribution in the frozen vitreous body was obtained as is shown in Tables IV and V.

TABLE V

RELATIVE CONCENTRATIONS OF K^{42} IN THE THREE SECTIONS OF THE VITREOUS BODY AFTER THE CONCENTRATION IN THE BLOOD HAD BEEN MAINTAINED AT A HIGH VALUE FOR VARIOUS TIMES

Time (min.)	Aqueous Humour	Relative Concentrations		Posterior
		Anterior	Middle	
30·5	100	43	20	14
31	100	54	20	13
31	100	92	41	16
31	100	49	39	18
38	100	53	23	20
59	100	64	44	23
58	100	71	31	13

From these tables it will be seen that, with both sodium and potassium, by far the highest concentration is that found in the most anterior segment of the vitreous body which is in closest proximity to the ciliary body. The region having the smallest concentration is the most posterior segment which is farthest away from the ciliary body. From these findings it is difficult to escape the conclusion that penetration of sodium and potassium into the posterior segment of the eye is predominantly from the ciliary region.

DISCUSSION

We wish to discuss at present three points relating to the facts described in this paper. First, we have the observation that thiocyanate penetrates from the blood into the aqueous humour and vitreous body more rapidly than either sodium or potassium;

the difference in rate is particularly evident in respect to penetration into the vitreous body. This difference seems to rule out the possibility that the main route of penetration of salts is by water-filled pores as it is throughout the body generally, that is, by way of the inter-cellular spaces of the capillaries and the lining membranes of the eye. If this simple mechanism were responsible, the relative rates of penetration of the different ions would correspond with their ionic mobilities, *i.e.*, the values for sodium, potassium, and thiocyanate should be as 44 is to 65 is to 56. We may therefore conclude, on this count, that the factors of cell membrane permeability (lipoid solubility, adsorbability, etc.) play an important part in determining penetration into the intra-ocular fluids from the blood. It follows that, so far as these substances are concerned, *transference from the blood into the chambers of the eye takes place through cell bodies and not inter-cellular spaces,* a conclusion which is in conformity with the fact that the salt content of the aqueous humour is greater than can be accounted for by a simple process of ultra-filtration.

Secondly we may note that the relative rates of penetration into the aqueous and vitreous differ very considerably between the various substances studied. All the salts studied enter the vitreous more slowly than the aqueous, but while thiocyanate enters the former most easily, phosphates do so only in very small quantities. Thus the ratio K_A/K_V for the thiocyanate ion is 2·5 whilst for sodium and potassium it is in the region of 5 and for phosphate it is 16. It seems probable that these differences depend on the differing sites of penetration of the respective ions. The evidence presented here indicates that thiocyanate penetrates throughout all the vascularized parts of the eye, *i.e.*, from the entire uveal, and presumably the retinal, circulations while sodium and potassium penetrate into the posterior segment of the eye through a restricted area only—the ciliary region. If this is the case it is obvious that the rate of accumulation of the first substance in the vitreous body should be greater, other things being equal, than that of the latter two. It may be recalled here that the monosaccharides, which also appear to penetrate into the vitreous body from all parts of the barrier, gave a ratio of K_A/K_V of 2·9 which is comparable with that for thiocyanate. In discussing this point earlier (Davson and Duke-Elder, 1948), it was pointed out that, if the barrier between blood and vitreous body were more selective than that between blood and aqueous humour, we should expect the ratio of K_A/K_V to become greater, the slower the rate of penetration of a substance; thus with the sugars the ratio was 2·2 for a pentose; 3·3 for hexoses, and 17 for sucrose. The high ratio reported here for phosphate, namely 16, is probably due chiefly to this factor; the amounts

penetrating the vitreous body were so small that it was not feasible to estimate the separate amounts in different parts of the vitreous body and so to find out whether penetration was confined to the ciliary region or not.

A final word will not be out of place on the bearing of these results on the problem of the formation of the intra-ocular fluid. Such mathematical treatment as the results have been given has been on the basis of the assumption that the amount of substance entering the fluid in unit time is proportional to the concentration gradient at that moment; in other words it has been assumed that the penetration is determined by the simple physico-chemical laws of diffusion. With the possible exception of potassium, the experimental results conformed with the equations in the sense that the parameter K′ appeared to be independent of the concentration gradient and the time of penetration. One might be inclined, on the basis of this finding, to suggest that penetration of the salts discussed is predominantly a matter of simple diffusion. Such a conclusion, however, would be unjustified; it is easy, as Kinsey and Grant (1942) have shown, to establish an equation on the basis of a supposed secretory mechanism, which has essentially the same form as the one employed here and derived without any assumptions of secretory activity. Kinsey and Grant concluded that salts entered the eye by a process of secretion largely as a result of this conformity of their results with their arbitrary secretion equation, but it is quite evident that in the present state of our knowledge an appeal to the results of mathematical analysis unsupported by other evidence is not yet warrantable, since the same equation may be derived on the basis of such entirely different postulates (Bárány and Davson, 1948). It would seem, however, that since the concentration of salts into the intra-ocular fluids is higher than can be accounted for by a process of simple ultra-filtration, some cellular activity (that is, a process of secretion) in their transference must be postulated.

SUMMARY

1. The salt content of the intra-ocular fluids is greater than can be accounted for by a process of simple ultra-filtration. Some cellular activity is therefore involved in the transference across the blood-aqueous barrier: a process of secretion must be postulated.

2. The various electrolytes tested (sodium, potassium, thiocyanate) penetrate across the blood-aqueous barrier at varying rates which cannot be accounted for by a simple diffusion through inter-cellular spaces, but only by penetration through cell bodies.

3. While some ions (thiocyanate) penetrate into the intra-ocular fluids throughout the whole of the blood-aqueous barrier, others

(sodium) enter the posterior segment of the cavity of the eye (in large measure, at any rate) by way of the ciliary body.

All the thiocyanate determinations described in this paper were carried out by Mr. A. M. Woodin.

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DISEASES OF THE EYE IN RELATION TO DENTAL SURGERY*

BY

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THE current fashion of labelling diseases "allergic" has to some extent diverted attention from focal sepsis. Nevertheless that controversy is still alive, and it would be safe to predict widely varying estimates if any twelve ophthalmologists were invited to answer the question: "What percentage of the ocular disease in your practice is due to unhealthy teeth?" There are, of course, many other organs and tissues on which attention has been riveted by people seeking to explain inflammatory lesions of the eye. The vermiform appendix, the colon, the prostate gland, the skin and the tonsils have all been singled out for blame, and that list might be greatly extended.

In all the mass of literature dealing with ocular disease of supposedly dental origin, one feature has escaped comment, so far as I have been able to ascertain. I refer to the curious assumption that infective traffic between the teeth and the eyes must be one-way. When will some champion take up the cudgels on behalf of these much-maligned gomphoses? Such a man might begin by

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