EXPERIMENTAL DATA ON THE PROBLEM OF THE PERMEABILITY OF THE CORNEA

I.—Experiments on Excised Pigs' Eyes and Living Rabbits' Eyes with Iodide and Nitrite

BY

DR. M. KLEIN

BUDAPEST

TOWARDS the end of the past century evidence had already been brought forward showing that the cornea plays an important rôle in the liquid exchange in the eye. Laqueur performed experiments which showed that potassium cyanide is capable of diffusion from the aqueous humour outwards through the cornea; he accorded the cornea an important rôle in the fluid exchange of the eye. Since that time this fact has been the subject for a good deal of discussion. The question of the permeability of the cornea is very important when it is considered that the greater part of all drugs applied to the conjunctiva enters the interior of the eyeball through the cornea. Nakamura, who investigated the question more thoroughly, showed that hyper- and hypotonic solutions diffuse rapidly through the cornea, while isotonic solutions do not do so. His opinion was that hypotonic solutions are more effective, for in this case the flow is from the outer surface to the inner which aids the diffusion of the substances dissolved in the liquid in question. According to the determinations made by Gallenga the hypertonic solutions diffuse relatively more rapidly. Antonibon considered the osmotic pressure differences a decisive factor in the permeability of the cornea and at the same time called attention to the fact that certain chemicals such as KCl, LiCl, increase the effectiveness of eye drops (cocaine, atropine, eserine, pilocarpine). In the presence of rubidium and caesium chlorides this effect is not noticeable. These examinations were made on living or excised animal eyes.

According to Fischer the cornea may be regarded as a membrane which hardly allows water to diffuse, while chloride ions diffuse in the epithelial-endothelial direction with difficulty and not at all in the reverse direction. Some of these results were obtained with eyes in contact with, or after being rinsed with sodium citrate, and some after the eye had been exposed to the influence of a concentrated saccharose solution. He determined the diffused chloride ions and analysed the aqueous humour. He found that epi- and endothelial lesions increased the original permeability at least ten times. It is apparent, therefore, that the permeability

is regulated by the presence and condition of these two layers. While these layers are anatomically and physically (as far as the swelling "Quellung" is concerned) in a physiological condition, diffusion takes place away from the layer more inclined to swell in the direction of the layer less able to swell, *i.e.*, in the epi-endothelial direction. Similar irreciprocal permeability was noticed by Wertheimer in the frog's lungs and skin, and by Cohnheim in the intestinal wall of the frog. Following this Fischer's experiments dealing with the swelling ability of the epi-and endothelial walls were of great importance. These experiments in which the ultramicroscope was employed proved that the epithelium is able to withstand less diluted solutions, retaining its original degree of swelling, than is the endothelium. His experiments with stains also showed that permeability is dependent on the condition of swelling of the corneal layers. A further observation was that acid stains, if finely dispersed, pass through the cornea, while basic stains do not.

Ridley (1930) described a series of experiments in the living eye which show that the cornea is permeable in both directions and that this permeability is most marked at the periphery at the site of the filtration angle. His experiments led to the conclusion that Descemet's membrane is relatively impermeable, but that the area described as the filtration angle is freely permeable to dissolved substances which spread rapidly by a process of diffusion throughout the substantia propria. He found that the epithelium is much less permeable than the substantia propria but demonstrated that it is permeable in both directions.

Author's Experiments

The purpose of these experiments was to examine the permeability of the cornea. The experiments were done with potassium and sodium iodide and sodium nitrite. The iodide experiments were done in two groups, in the first experimental series the determinations were made on freshly enucleated pigs' eyes, the second on living rabbits' eyes.

The pigs' eyes were placed on wooden slats which had a slight indentation in them, so that the cornea faced upward and a 1cm. diameter glass tube was placed on the cornea and held in place by a spring with slight pressure. The end of the glass tube was first rounded in a flame to prevent injury to the epithelium of the cornea. The edge of the glass tube was covered with vaseline to prevent the escape of the solution in the tube. In the "inside-out" determinations the experimental solution was placed in the anterior chamber through the optic nerve with a long syringe

needle, the needle was then withdrawn and the optic nerve tied off with a ligature.

In the experiments on living rabbits' eyes, the rabbits were under urethane anaesthesia and, with the head properly fixed, a glass tube 1/2cm. in diameter was placed on the eye. In the "inside-out" experiments a needle was used in the filling of the anterior chamber. To avoid loss of aqueous humour the needle was forced through the opposite side and was left in place for the duration of the experiment.

The iodide was determined by Scheffer's method, which fundamentally consists of the following procedure: in the presence of organic substance after the addition of KOH and oxidation in a sand bath, the oxidised product is extracted with alcohol, the iodine shaken out with carbon tetrachloride, iodated with chlorine water and determined titrimetrically.

The determination of the nitrite was made with the colorimetric micromethod. In the first series of experiments made on pigs' eyes, potassium iodide was used. The outer solution placed in the tube was 2ccm. of 0.9 per cent. NaCl.

TABLE I

Pigs' eyes, in-out. Fluid in the tube 2 c.cm. 0.9 per cent. NaCl, tube diameter 10 mm.

No.	Into the A.C.	Time	Permeated
		minutes	
1	0.5 c.cm. 5 per cent. KI	60	202 γ
2	0.5 " 5 " "	60	210 γ
3	0.5 " 2 " "	60	100 γ
4	0.5 " 2 " "	60	74 γ
5	0.5 " 2 " "	60	56 γ
6	0.5 " 2 " "	75	104 γ
7	0.5 " 2 " "	75	144 γ
8	0.5 " 2 " "	120	204 γ
9	0.5 " 2 " "	120	240 γ
10	0.5 " 2 " "	120	182 γ
11	0.5 " 2 " "	120	99 γ

As the table shows iodine was demonstrable in quite large quantities in the outer solution.

TABLE II

Pigs' eyes, out-in. Into the A.C. 0.9 per cent. NaCl.
In the tube 2 c.cm. 2 per cent. KI.

No.	Time	Aqueous	Permeated
	minutes		
1	60	0.3 c.cm.	130 γ
2	60	0.3 "	139 γ
3	60	0.3 "	147 γ
4	120	0.5 "	240 γ
5	120	0.5 "	345 γ
6	120	0.5 "	260 γ

In the second experimental group "outside-in" iodine diffusion was examined. The anterior chamber was filled with 0.9 per cent. NaCl solution and 2cm. 2 per cent. KI was placed in the tube.

As the table shows iodine was demonstrable in large quantities in the aqueous humour. As in the first group of experiments the permeated iodine increased proportionally with the time of the experiment.

In further experiments it was investigated whether or not the addition of 10 per cent. of blood-serum to the sodium chloride solution, acting as a colloid osmotic factor, increased the amount of diffused iodine.

TABLE III

Pigs' eyes, in-out. Into the A.C. 0.5 c.cm. 2 per cent. KI.
Tube 0.9 per cent. saline + 10 per cent. serum.

No.	Time	Permeated
	minutes	
1	60	92 γ
2	60	138 γ
3	60	77 γ
4	60	131 γ
		control 33 γ
		" 107 γ

As control serve also the experiments, Table I, Nos. 3, 4, 5.

As is readily noticed, in the serum experiments the quantity of diffused iodine is increased.

The question was also investigated as to what extent the time elapsing since death affects the cornea with regard to its permeability.

TABLE IV

Pigs' eyes, in-out. Into the A.C. 0.5 c.cm. 2 per cent. KI.
Tube 2 c.cm. 0.9 per cent. NaCl.

No.	Time since death	Time of experiment minutes	Permeated
1	8 hours	150	460 γ
2	2 „	150	150 γ
3	2 „	60	223 γ
4	2 „	60	184 γ
3 and 4 the cornea was dipped in boiling water for 10 secs.			
5	2 hours	60	268 γ
6	2 „	60	307 γ
7	2 „	60	294 γ
5, 6 and 7 the cornea was dipped in boiling water for 30 secs.			
8	Control cornea not dipped in boiling water		
		60 minutes	115 γ

While very little iodine diffused through the freshly excised cornea, after standing in the refrigerator for 8 hours the diffused iodine increased almost 3 times the original amount. In a further experiment the eyeball was held with tweezers and only the cornea was dipped into boiling water. As is seen the adverse effect of 10 seconds doubly increased the quantity of diffused iodine, and after 30 seconds the quantity of demonstrable iodine was even greater.

Rabbit Eye

The experiments were performed in such a manner as to place the chemical to be examined in the aqueous humour without injury to the eye itself. This was done by giving the rabbit intravenous injections of 5 per cent. sodium iodide and allowing time for sufficient concentration of the iodine in the aqueous humour in order for it to diffuse to the outside surface. The animals received 6-10c.cm. according to body weight. During the experiment 34 γ of iodine was found in 1/10c.cm. of the aqueous humour. After an experimental time of 40 minutes

permeated iodine was not demonstrable in the outer solution. This is to be expected if we take into consideration the results of the former experiments where the diffused ion quantity is approximately 1.5-2 per cent. In this instance the amount of diffused iodine to be expected is so small as to be at or below the limit of demonstrability. Therefore in order that the small iodide concentration might diffuse through the cornea in sufficient quantity to be demonstrated, 6 hour experiments were done. The rabbit was under urethane anaesthesia during the experiment. During the experiment 2 intravenous injections were given, each 7ccm. of 5 per cent. NaI. The outer liquid was 0.9 NaCl solution. The second injection was given for the purpose of ensuring a proper blood iodine mirror. The liquid in the ring on the cornea was changed hourly. Iodine was demonstrable in the outer solution collected over the six hour period. The pink colour of the carbon tetrachloride corresponded, according to approximate estimations, to 5-7 γ of iodine.

In the case of intra-ocular injections of NaI, KI, the results were as follows:—

TABLE V
Rabbit eyes. In-out.

No.	In the a.c.	Time	Permeated iodine
		minutes	
1	0.3 c.cm. 5 per cent. NaI	15	202 γ
2	0.3 " 5 " "	15	93 γ
3	0.3 " 5 " "	15	284 γ
4	0.3 " 5 " "	15	214 γ
5	0.3 " 5 " "	15	216 γ
6	0.3 " 2 " KI	60	58 γ
7	0.3 " 2 " "	15	18 γ
8	0.3 " 2 " NaI	15	51 γ
9	0.3 " 2 " "	15	34 γ

As the table shows the diffused iodine was demonstrable after 15 minutes. After the intra-ocular injection of sodium iodide the cornea became a little hazy after 3 or 4 hours, but became clear again after a longer period of time. In many of the rabbits after the disappearance of the haziness a very fine nebula could be found.

It can be seen that iodine diffusion in living rabbits' and excised

pigs' eyes is quite possible in both directions. In these experiments not only the qualitative but the quantitative determinations of iodine were done.

Nitrite Experiments

On the pigs' eyes: the aqueous humour was replaced with 1/10 equimolar nitrite—Locke-solution /0.5c.cm./. The outer fluid was 0.9 per cent. NaCl solution.

TABLE VI
Pigs' eyes. Nitrite. In out.

No.	Time	Permeated iodine
	minutes	
1	60	5.18 γ
2	60	4.66 γ
3	60	6.78 γ

The experiments all show that nitrite is capable of diffusion through the cornea. The question of nitrite diffusion through the living rabbit cornea was also investigated. After the drainage of the aqueous humour 0.3c.cm.—Locke solution—was injected, of which 1/10th part was the above-mentioned nitrite solution. During the 80 minute experimental period, using a 5mm. diameter external collecting tube, 3.45 γ permeated. After a 15 minute experimental period the diffused nitrite amounted to 0.87 γ . In the aqueous humour, after the end of the 80 minute experimental period, only 4.9 γ of nitrite was demonstrable. These experiments show that the cornea is also permeable to nitrites.

Summary

In two groups of experiments it was shown that freshly enucleated pigs' eyes are equally permeable from "outside-in" and "inside-out" to KINaI and NaNO₂. The amount of salt recovered after passing through the cornea is closely proportional to the concentration of the salt and to the time elapsed in each case. Further experiments show that the permeability increases three times by the end of the 8 hour period after death, but is not materially increased during the first 2 hours from which the above observations were obtained. The addition of 10 per cent. of serum to the fluid applied to the epithelium increased the amount of salt permeating in one hour as compared with the control. By dipping the cornea into boiling

water for 10 and 30 seconds, injuring the epithelial cells and repeating these experiments, it was shown that destruction of the epithelium increases the amount of salt permeating.

A series of experiments on living rabbits under urethane anaesthesia using 5 per cent. and 2 per cent. concentrations of sodium iodide and 1/10 equimolar nitrite gave similar results. The amount permeating being approximately proportional to the concentration of the solution and the duration of the experiment. It is important to point out that if a known quantity of a dissolved salt is introduced into the anterior chamber of a living animal and the salt diffusing through the cornea is collected, it is found that only a small proportion, about 1.5-2 per cent. is recovered. This is due to the fact that the salt diffuses by other ways into the capillaries of the iris and ciliary processes from whence it is at once carried away by the blood stream, and to a less extent into the lens and vitreous humour. In control experiments it was found that at the end of 80 minutes 2 per cent. of the salt introduced had permeated but that only 3 per cent. could be recovered from the anterior chamber. It seems that for every 100 units by weight introduced into the anterior chamber 2 pass out through the cornea, 3 remain in the anterior chamber and 95 units are eliminated by diffusion in other directions in 80 minutes.

The objection may be raised against these experiments that both the iodide and nitrite are not found under normal conditions in the system and the possibility remains that the cornea is permeable to them as foreign substances, and that the cornea shows an entirely different behaviour to the ions normally found in the system. The question might also be raised whether concentration of the chemicals used was not abnormally high, and it might be held that the experimental results since they were obtained with hypertonic solutions, cannot be generalised.

It was necessary therefore to repeat the experiments with a special apparatus with ions normally found in the system in a physiological concentration or one close to that and further, to investigate the diffusion of water. These results obtained in collaboration with Dr. Sárkány are given in a separate paper.

REFERENCES

- ANTONIBON.—*Zentralb. f. Ophthalm.*, Vol. XX, p. 328.
 ——— *Zentralb. f. Ophthalm.*, Vol. XXI, p. 657.
 ——— *Zentralb. f. Ophthalm.*, Vol. XXII, p. 660.
 FISCHER, F. P.—*Arch. f. Augenheilk.*, Vol. XCVIII, p. 41.
 ——— *Arch. f. Augenheilk.*, Vol. C, CI, p. 481.
 GALLENGA.—*Zentralb. f. Ophthalm.*, Vol. XVIII, p. 216.
 NAKAMURA.—*Klin. Monatsbl. f. Augenheilk.*, Vol. LXX, p. 195.
 RIDLEY, F.—*Trans. Ophthalm. Soc. U.K.*, Vol. L, p. 268.