EXPERIMENTAL DATA ON THE PROBLEM OF THE PERMEABILITY OF THE CORNEA

II.—The Permeability of the Excised Cornea with Respect to Water and Chloride Ions

BY

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THE data in the literature dealing with the permeability of the cornea give variable and dissimilar results both with regard to the irreciprocity of the permeability and to the magnitude and quality of the necessary osmotic forces and to the rate of diffusion. The purpose of our experiments was to estimate these discrepancies using a different method, and to examine the points brought out above.

Method:—Our experimental apparatus was similar to the chamber system described by Hamburger. The chambers were separated by the excised cornea. Various solutions were placed in the two chambers and the changes in volume, and in the chloride ion concentration were determined. The volume change was determined by capillary measurement, the chloride ion by a micro-method. (Diameter of capillary 0.8mm. 10 units of the capillary scale equalled 5c.mm.) The volume of each chamber was approximately 4c.cm. The solutions used were pig's blood diluted with Ringer's solution to various degrees. The air bubbles in each section were carefully displaced and liberated. The corneae were obtained with the aid of a slice made through the equator of the

FIG. 1.

Moulded rings of soft rubber used to clamp the cornea.

globe and they were fixed between rubber rings at the scleral margin. (See Fig. 1.) Usually three chambers and two corneae were used in such a manner that each experiment gave two parallel determinations.

Experimental Results

In the first instance we determined whether or not, with the aid of osmotic pressure, fluid would pass through the cornea. A photograph of the apparatus is given below.

FIG 2.

The complete apparatus employing two corneae (epithelial or endothelial surfaces opposed).

In the determination of the permeability in the endothelial-epithelial direction, pig's blood or serum, or a dilution of these with Ringer's solution was placed in the outside chambers, and in the middle chamber there was Ringer's solution.

TABLE I

Flow of liquid effected by osmotic forces. The convexity of the sign □ or D corresponds to the epithelial surface.

	Mins.	Blood	□	Ringer	D	Blood
Feb. 23, 1937	0-30	13		- 7		10
	30-60	33		0		36
Feb. 23, 1937	0-30			- 3		11
	30-60			0		28

PERMEABILITY OF THE CORNEA

411

		Doubly diluted serum	□	Ringer		
Feb. 26, 1937	0-30	- 3		- 17		
	30-60	15		3		
	60-90	13		0		
		Doubly diluted serum	□	Ringer	D	Doubly diluted serum
March 8, 1937	0-30	25		0		5
	30-60	5		0		5
	60-90	20		0		15
	90-120	25		0		15
	120-150	20		10		15
	150-180	10		5		10
		Doubly diluted serum	□	Ringer		
March 5, 1937	0-30	- 2		- 15		
	30-60	17		- 12		
		Doubly diluted serum	□	Ringer	D	Doubly diluted serum
March 8, 1937	0-60	- 10		0		0
	60-90	10		0		15
	90-120	15		0		30
	120-150	20		5		25
		Doubly diluted serum	□	† Ringer	D	Doubly diluted serum
March 18, 1937	0-20	- 20		0		15
	20-40	- 2		0		15
	40-60	0		0		15
	60-80	- 5		0		5

† Represents an impermeable wall.

Our results all showed that the volume of the solution containing a colloid was increased. The volume of the Ringer's solution either decreases or remains unchanged. In fact, in a few cases, in the later stages the volume of Ringer's solution increases, but this increase does not compare with the volume increase of the colloidal solution. This may be explained on the supposition that an outside factor (temperature change) influences the meniscus in all cases to the same extent. This possibility was, however, excluded by our experiments in which we determined the effect of this factor in a closed chamber of solution connected with a capillary, and even after taking this factor into consideration we discovered incongruous volume changes (Table I. III/18, 1937).

The explanation may be that the solution did not pass through the cornea, but the cornea itself exuded or absorbed fluid from

its surroundings. For instance a gel may take up or give off a measureable quantity of water without appreciable volume change. If this is the explanation the volume of the colloidal solution due to the effect of the cornea placed in it, as it comes in contact with both surfaces of the cornea, undoubtedly and measurably should increase. According to our experiments this does not occur, for a volume change is not appreciable.

TABLE II
The volume-changes of the cornea under the influence of various solutions.

		Doubly diluted serum		Doubly diluted serum	
		+ cornea	† Ringer	† + cornea	
March 10, 1937	*Mins.				
	0-60	5	0	0	
	60-120	0	0	5	
		Ringer		Doubly diluted serum	
		+ cornea	† serum	† + cornea	
April 12, 1937	* *				
	0-30	-10	-5	0	
	30-60	0	5	5	
	60-90	5	0	0	

* The experiment was begun one hour late due to technical difficulties (*i.e.*, three hours after death).

** The experiment was begun half-hour late due to technical difficulties.

| † Represents an impermeable wall.

We must therefore investigate a further supposition, that the changes noticed are brought about by the liquid passing through the cornea. The absence of a quantitative connection may be explained by the fact that, besides the diffusion, swelling of the cornea takes place under the influence of the various solutions, in such a manner that the results obtained are the resultant of the two changes occurring simultaneously. As to the acceptability of this explanation our experiments with chloride ions served as a basis which as we shall later show gave similar results.

We examined the diffusion in the epithelial-endothelial direction by placing the solutions in the appropriate chambers. The results (Table III) while coinciding with the former were not as constant, although it was to be expected from the data in the literature that a diffusion in this direction (epi-endothelial) could more easily be demonstrated. These experiments showed in most cases that in the first half of the experimental time the volume of the colloid solution increased, while the volume of the neighbouring chambers showed a variety of changes. In the second half of the experimental time in most cases the volume of the Ringer's solution increased, in some cases this was noticeable from the very beginning.

PERMEABILITY OF THE CORNEA

413

TABLE III
Flow of liquid through the cornea.

	*Min.	Ringer	Doubly diluted serum		Ringer
			□	D	
March 10, 1937	0-30	- 5		0	0
	30-60	0		10	0
	60-120	0		10	0
March 12, 1937	* *	Ringer	Doubly diluted serum		Ringer
			□	D	
				45	0
				20	5
				10	15
				0	5
March 16, 1937			Doubly diluted serum		Ringer
			□	D	
				- 5	- 10
				0	5
				0	5
				0	10
April 6, 1937	***		Doubly diluted serum		Ringer
			□	D	
				10	2
				5	0
April 8, 1937			Doubly diluted serum		Ringer
			□	D	
				5	20
				0	25
April 8, 1937			Doubly diluted serum		Ringer
			□	D	
				5	30
				0	10
April 8, 1937			Doubly diluted serum		Ringer
			□	D	
				0	0
				0	5
				10	2
				5	3
				15	40
April 12, 1937	****	Ringer	Doubly diluted serum		Ringer
			□	D	
				0	20
				0	10
				0	10
				0	10
				0	10

* The readings were begun 1½ hour late.

** The readings were begun 1 hour late.

*** The readings were begun ½ hour late.

**** The readings were begun 1 hour late.

† Represents an impermeable wall.

As this usually happened at a later stage in the experiments we could not regard it as a sign of diffusion but as a result of the destruction of the cornea, possibly autolysis. This time, approximately the third hour of the experiments, *i.e.*, the fifth-sixth hour after excision is, according to other workers, about the limit of viability. We accordingly limited the time of our experiments. Our results show that under the influence of osmotic pressure the diffusion of liquid is not only possible in the epithelial-endothelial direction but also in the opposite manner.

We followed the same procedure in our experiments with chloride ions with the difference that we did not use a colloidal solution, but used variously diluted Ringer's solutions in contact with the two surfaces of the cornea and made our continuous chloride ion determinations with these solutions. For each determination we took 0.2c.cm. solution, added 4c.cm. distilled water and 2c.cm. of dilute nitric acid and by further adding 4c.cm. N/100 silver nitrate solution titrated the remaining Ag ions with N/100 NH₄SCN solution. The indicator ferri alum was added in larger quantities for with a greater indicator concentration the point of colour change is much more clearly defined.

In these experiments various concentrations of chloride ions were brought into contact with the epithelial and endothelial surfaces. The data show the appreciable chloride ion changes which resulted.

TABLE IV
Diffusion of NaCl through the cornea. The convex surface of the sign corresponds to the epithelial surface.

	Time H. M.	per cent.		per cent.		per cent.
July 27, 1937	10 00	0.87	□	0.49	▷	0.89
	11 30	0.89		0.50		0.88
	13 10	0.86		0.51		0.87
August 3, 1937	9 20	0.88	□	0.63	▷	0.88
	11 00	0.87		0.65		0.88
	12 15	0.86		0.64		0.88
	12 50	0.88		0.64		0.88
August 3, 1937	9 20	0.88	□	0.70	▷	0.89
	11 00	0.86		0.70		0.87
	12 15	0.88		0.68		0.89
August 5, 1937	10 00	0.92	□	0.33	▷	0.93
	11 15	0.89		0.33		0.91
	12 20	0.90		0.36		0.87
	12 50	0.88		0.35		0.85

PERMEABILITY OF THE CORNEA

415

August 5, 1937	10 00	0.93	□	0.32	□	0.92
	11 15	0.92		0.34		0.92
	12 20	0.91		0.34		0.92
	12 50	0.89		0.34		0.90
August 9, 1937	9 00	0.89	□	0.66	□	0.88
	10 15	0.89		0.65		0.89
	13 15	0.88		0.68		0.86
August 9, 1937	9 00	0.89	□	0.70	□	0.89
	10 15	0.90		0.72		0.90
	13 15	0.89		0.72		0.88
August 13, 1937	10 10	0.89	□	0.88	□	0.89
	11 15	0.89		0.87		0.88
	12 20	0.88		0.87		0.88
August 13, 1937	10 10	0.87	□	0.87	□	0.88
	11 15	0.88		0.88		0.89
	12 20	0.89		0.88		0.88

That this is not a change as a result of evaporation (the chambers were not covered) is apparent from the fact that there were also decreases in concentration. Our results were undoubtedly influenced by the water diffusion due to the osmotic pressure difference, but we are unable to believe that along with this chloride ion diffusion did not also occur; for the reason that to induce the concentration changes that we noticed, 150-300c.mm. of water must have diffused, but this figure is far larger than the results we obtained for liquid diffusion in the above mentioned experiments. It is reasonable therefore to attribute the chloride ion concentration changes to an actual chloride ion diffusion.

We used in our experiments solutions in which the ion proportion corresponded to that of the Ringer's solutions. The chloride ion concentration in contact with the epithelial surface expressed in NaCl was 0.88-0.90 per cent. Various diluted Ringer's solutions were brought into contact with the endothelial surface.

When the NaCl concentration of the solution in contact with the endothelium is between 0.88 to 0.60 (the epithelial concentration being 0.88-0.9 per cent.) diffusion can hardly be shown; but if the concentration is decreased below this figure, the changes become gradually much more noticeable, and the dilute solution becomes concentrated, the concentrated solution becomes dilute. This result expresses a great deal. It shows for instance that increase in the concentration of the endothelial solution runs parallel to the chloride ion decrease in the epithelial solution.

This fact would not have occurred had this diffusion taken place between the solution and the corneal tissue, for in that case the 0.88 per cent. solution in contact with the epithelium would behave in the same manner independent of whether a 0.90 per cent. or a 0.30 per cent. solution was in contact with the endothelium. The fact, therefore, that the concentration of the solution in contact with the epithelium can be influenced by the concentration of the solution in contact with the endothelium, of itself is proof that the forces responsible for the diffusion are not located in the cornea, in other words the living cornea behaves as an inactive permeable membrane. These results agree completely with the results obtained in our experiments with the diffusion of water.

These results are not only applicable to the "outside-in" direction, but also for the endothelial-epithelial direction. This was shown by our experiments in which the solution in contact with the endothelium was the 0.88 per cent. and various concentrations were used in contact with the epithelium.

There is evidence that a much smaller concentration difference will induce a measureable diffusion in this direction.

TABLE V
Diffusion of NaCl through the cornea. The convex surface
of the sign corresponds to the epithelial surface.

	Time						
	H.	M.					
July 26, 1937	10	15	0.57	□	0.88	□	0.54
	11	15	0.55		0.84		0.53
	17	10	0.55		0.84		0.55
July 26, 1937	10	15	0.53	□	0.88	□	0.52
	11	15	0.53		0.86		0.52
	14	10	0.54		0.82		0.53
July 30, 1937	9	45	0.88	□	0.34	□	0.90
			0.89		0.34		0.88
	11	45	0.86		0.35		0.86
			0.85		0.36		0.88
	12	45	0.85		0.36		0.85
			0.85		0.37		0.86
August 11, 1937	10	15	0.87	□	0.63	□	0.86
	12	10	0.87		0.65		0.87
	13	00	0.85		0.66		0.86
August 11, 1937	10	15	0.87	□	0.63	□	0.86
	12	10	0.86		0.66		0.86
	13	00	0.85		0.66		0.86

Summary

Our experiments dealing with diffusion through the excised cornea gave the following results :

1. Under the influence of colloidal osmotic forces a flow of water through the cornea is possible.
2. This permeability exists not only in the epithelial-endothelial direction, but also in the reverse direction.
3. Chloride ions under the influence of osmotic pressure can diffuse to the opposite surface in both directions.

NEW DEVELOPMENTS IN THE PHARMACOLOGICAL TREATMENT OF PRIMARY GLAUCOMA

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Introduction

It is surprising that so little emphasis has been placed upon the significance of the fact that the pharmacological treatment of glaucoma consists, almost exclusively, of local applications of drugs which act principally upon the two subdivisions of the autonomic nervous system *in the eye*, the parasympathetic and sympathetic. This is probably one of the two reasons why the understanding of the mode of action of sympathomimetics (Sy-Mi) and parasympathomimetics (Pa-Sy-Mi)* upon the eyes has made little progress during past decades.

The other reason for this stagnation is that certain physiological data were missing. Without the knowledge of these data, no advance could be made. Recently, thanks to the theory of the chemical mediation of nerve impulses, the much-needed information has finally begun to become available. It has taken thirty

* Local applications of pilocarpine, eserine and of the new synthetic products, such as acetylcholine, mecholyl, prostigmin and doryl, have a stimulating action upon the ocular parasympathetic system and miosis is only one of several effects produced by these drugs. Atropine has an inhibitory or paralyzing effect upon the ocular parasympathetic system and mydriasis is only one of several reactions of the eye to this drug. It is, therefore, preferable to use the term, *para-sympathomimetics* (abbreviated: Pa-Sy-Mi), instead of miotics or mydriatics, when speaking of these substances.

The same reasoning is applicable to drugs which stimulate, inhibit or paralyze the ocular sympathetic. They should be called *sympatho-mimetics* (abbreviated: Sy-Mi).