STUDIES ON THE FILTRATION RATE OF THE AQUEOUS HUMOR - I. THE EFFECT OF ALTERATIONS IN THE H-ION CONCENTRATION - IN THE EXCISED SHEEPS EYE.

by

CARLETON P. PYNN, A.B., M.D.

- 1926 -

CONTENTS

STUDIES ON THE FILTRATION RATE OF THE AQUEOUS HUMOR - I. THE EFFECT OF ALTERATIONS IN THE H-ION CONCENTRATION IN THE EXCISED SHEEP'S EYE.

The present problem was undertaken as a preliminary study of the possible factors which might influence intraocular filtration and pressure. In the great mass of material bearing upon the production and excretion of intraocular lymph, very little mention is made, outside of the discarded theories of Martin Fischer(I), of the possible effect on these much discussed physiological considerations, of changes of the p H of the aqueous humor. Especially is this true in studies of the filtration rate in which a perfusion fluid is used in the excised eye.

Hertel(2), who undertook a study of the aqueous fluids of eyes showing various clinical manifestations of disordered physiology, could show no apparent relation between the tension exhibited by the eye and the concentration of the H-ions present in the aqueous of the respective eye. In his researches he used an indicator method for p H determination, as described by Michaelis(3), in which estimations were made on dilutions of the aqueous fluid obtained from the eyes by paracentesis. The ophthalmological lesions which he studied included cataract and post-cataract cases, iritis, glaucoma

and diabetic coma eyes. He was unable to demonstrate to his own satisfaction that there was any significant departure from the normal range, which he placed at p H 7.7 - 7.8, in any of his cases, save in diabetic coma where the p H reached 7.0. Altho this latter reading was obtained in ocular hypotony of marded degree, he was rather disposed to discount the effect of the associated H-ion increase, attributing the conditions rather to abnormal osmotic pressure relations which had been stablished between the blood and the intra-ocular fluid.

Meesman(4), considered that the subject bore reinvestigation inasmuch as he considered the small increase in osmotic pressure found in diabetic coma, as causing the marked ocular hypotony not feasible. He therefore covered much the same field, using the same methods of approach for his clinical cases, and supplementing them with some observations on experimental animals, concluded very definitely that there was an indication of an inter-relation between the tension of the eyes examined and the p H of the aqueous contained within them. More detailed reference will be made to this later.

With the difference in results apparent, the problem of the real relation between the factors mentioned is still an open question. Proceedures upon the eye in situ are so complicated by extraneous factors that it is not surprising that vastly different results may frequently be had with the same mater-

ial. It was early noted by C. Weber(5), that when a suitable manometer was allowed to record the intra-ocular pressure by means of a cannula which was introduced into the anterior chamber of the eye of a living animal, various rhythmic fluctuations were observed in the resulting graphic record. These fluctuations could be attributed to the pulse beat, the respiratory movements, and even, with a delicate type of apparatus, waves which might correspond to the Traube Hering waves of vascular tonicity in a graphic blood pressure record, could be demonstrated.

EXPERIMENTS ON THE EXCISED EYE.

The preceeding is mentioned to emphasize the very close relationship existing between the blood pressure and the intra-ocular pressure, and to point out, with this in view, how difficult it would be, with all the continuous variations in the former mirrored as they are in a manometric record of the latter, to judge in what manner any given experimental prodeedure had affected directly and primarily the intra-ocular pressure. This complexity of factors operating upon the eye in situ has proved discouraging to many observers who have sought to simplify the problem confronting them by the use of the excised eye.

It is true that the use of such material invites other sources of error which, in the minds of some are quite as ob-

jectionable as those which it was hoped to obviate. Not the least serious of these objections to the use of excised eye are the trauma inflicted upon the organ in the course of its removal from the animal, the degenerative changes which are initiated from the moment at which the natural circulation is suspended, and, when the anterior chamber is cannualized, to the injury to the delicate eye structures, which the resultant sudden pressure changes incur. The latter source of error,is not pecular, however to the use of excised material but is induced whenever the anterior chamber is opened and anterior fluid is lost. To be sure, any method which attempts to handle or subject to scrutiny in an experimental way, the delicate structures of the eye, is bound by contrast to be gross and slumsy.

It is evident that before the use of excised material were allowed, and before one would be permitted to apply any results obtained by its use to the living eye, it would be necessary to show that no new factors had been introduced and that the principles apparent in the former case applied equally well to the latter. Henderson and Starling have shown by numerous careful experiments that such a transition is in truth permissible. These transitional experiments were begun on the eye of a living animal with a cannula leading from a pressure manometer communicating with the naterior chambery and indicating the variations in intra-ocular pressure already referred to. When suitable

data had been obtained the animal was bled to death and saline allowed to enter the eye sufficient in quantity to permit a maintenance of the pressure previously obtained. This rate of inflow was shown to be fairly uniform for a given eye and was considered to represent nearly the true rate of aqueous inflow and outflow. It is rather easy to advance a step further, actually removing the eye from the body of the dead animal and to show that this uniform rate of inflow occurs under the given conditions.

RESULTS OBTAINED BY OTHER WORKERS

Several workers have subjected excised material to somewhat the same procedures already enumerated, notably Bentzen and Leber(6), Niesnamoff(7), and Priestly Smith(8). The first named workers, using human eyes which had been removed a number of hours before, showed that it was possible to introduce 5.0 cmm per minute under the normal intra-ocular pressure of 25mm. of mercury. The other experimenters determined the filtration rate for the eyes of various animals, that for the sheep's eye they agree averages 27.0 cmm. per minute.

HYDROGEN-ION CONCENTRATION

In the early work on filtration rate determination the H-ion concentration value of the perfusion fluids was not con -

trolled, as also was it not in our preliminary work. Martin Fischer(I) first stressed the factor of local tissue acidity in the production of swelling and similar states. He applied some test-tube experiments more or less directly to the problem of glaucoma, assuming this condition to be analagous to the marked swelling of the globe of the eye (which amounted in some cases to actual rupture of these structures) in response to the presence of acid in solution. Altho the H-ion range in his experiments were no comparable to any that might exist in the body, this explanation of the direct cause of glaucoma gained some support and methods of treatment were based upon this conception of the etiology.

With the above results and others, especially those of Hertel(2), in mind, Meesman(4) undertook to test his findings of increased p H associated with lowered intra-ocular tension which seemed to obtain in his clinical series, upon experimental animals. After the removal of small amounts of aqueous by paracentesis, replacing this quantity of fluid by saline of a known p H, the tension of the eye was taken at intervals. His entire results were in agreement, namely that in the clinical cases elevation of intra-ocular tension was associated with decreased p H values, and cases showing reduced pressures gave fluids which were high in p H value, while experimentally these pressure variations could be produced by the introduction of a saline solu-

tion into the anterior chamber which showed a p H value tending in the same direction as that found spontaneously in the clinical cases showing such an abnormal pressure.

Having indicated several methods of approach as well as the results of certain workers in their attempts to clarify the mechanism of aqueous fluid formation and excretion, it might be well at this point to show the bearing which these experiments have upon the present problem. The possibility of the p H value of the aqueous influencing its own rate of outflow has been mentioned. The manner of action is uncertain. In the intact eye, as has been emphasized, it is difficult to separate local effects from general effects. In the excised eye, on the other hand, the blood supply and the nerve supply may be eliminated from consideration, the artificial aqueous is introduced at a certain rate sufficient to insure constancy of intra-ocular pressure (thus eliminating another variable) and procedures may be accomplished in their simplest form.

This latter feature permits us to test the effect of varying the acidity of the aqueous upon the rate of outflow from the anterior chamber. It also permits us to refer more or less exclusively the causes for such variations, not to the blood or nerve supply which have been eliminated but rather to the actual structures, filtering membranes, etc. still present in the excised preparation. Our procedure and results therefore have

been interpreted more or less upon this basis and our interpretation must necessarily carry with it the limitations universally applicable to excised material in general.

AQUEOUS FLUID - ITS NORMAL SITE OF FORMATION AND EXCRETION - ITS PATHS.

It might be considered advisable before going on to the actual conduct of these experiments to mention the generally accepted fundamentals of the physiology of aqueous formation and excretion. According to the prevalent but perhaps not proved conception, the aqueous is formed from the blood by processes akin to diffusion, transudation and filtration by the ciliary process of the ciliary body and to a much smaller extant in man at least by the irir. The fluid passes forward from the posterior chamber where it is formed, on between the iris and the lens where it meets with some resistance due to the pressure of the sphincter of the iris against the lens. Then passing forward thru the pupil it reaches the anterior chamber.

The exact paths of exit of the aqueous have been demonstrated experimentally to be as follows:

1. Thru the spaces of Fontanna and the canal of Schlemm
2. Thru the anterior surface of the iris
3. Thru the small portion of the ciliary body which forms one of the boundaries of the anterior chamber.

After the death of an animal the intra-ocular pressure rather promptly falls to 10 mm. of mercury, after which it more slowly descends to zero. The fall of pressure under these circumstances is of course due to the cessation of aqueous production thru the walls of the blood vessels which show no blood pressure or blood flow. The pressure within the eye more gradually falls because the aqueous outflow is more or less proportional to the intra-ocular pressure which is being represented by a progressively lower level. The experiments herein described are based on the principle that if the intra-ocular pressure is maintained from an outside source, even though the physiological source of intra-ocular pressure, namely the blood pressure, is suspended, excretion will continue at the same rate as it would normally under that same intra-ocular pressure.

EXPERIMENTAL PROCEEDURES

APPARATUS EMPLOYED

A modification of the apparatus of Henderson and Starling(9) was employed, consisting of a horizontal graduated capillary tube, one end of which is connected to a reservoir for the perfusion fluid, with a valve and bulb to introduce a bubble of air into the capillary, intervening. At the other end of the capillary tube (nearest the eye) is a bubble trap for the purpose of catching the air before it has had a chance to enter

the eye under examination. The trap consists merely of a vertical limb of the capillary tube, and is filled with fluid at the start of the experiment after which the air gradually displaces the fluid. A three-way stop-cock, connected to the capillary beyond the bubble trap and between it and the needle, permits one to close off the apparatus from all other connections. Another position of the cock provides for a path for the fluid to the exterior, thus allowing a change of fluid in the apparatus without necessitating its passage thru the needle which pierces the anterior chamber of the eye. The needle is of the aspiration type, of small calibre and filed along the shaft in two places, about 1 cm. apart, with the nearest opening 2.5 cm. from the point. The portion of the lumen intervening between the two holes may be obliterated by flowing solder into it, either thru one of the holes mentioned or thru a third which is filed midway between the other two and which is obliterated in the process.

MATERIAL EMPLOYED.

The material used in these experiments was the freshed excised eyes of sheep. These were removed by a technician directly after the animal was slaughtered. The eyes after enucleation were dropped promptly into buffered saline solution maintained at 38 degrees Centigrade, in a vacuum jar. The eyes so secured

were never more than four hours old when they were worked upon, while the majority of them were much fresher. No attempt was made to record the size of the pupil inasmuch as it had been shown that this has little effect upon the filtration rate of the aqueous in the excised eye. The eye to be used in the apparatus was taken from the container, the contents of which usually showed some increase in H-ion concentration (a fact which will be commented upon later) and placed in an 8 ounce beaker containing buffered saline of p H 7.5 which was maintained at body temperature by circulating hot water, and which had practically the same composition as the perfusion fluid.

TECHNIQUE FOR DETERMINING THE NORMAL RATE OF EXIT OF THE AQUEOUS.

The needle, which has been described, is made to pierce the cornea in the region of the limbus, It is then pushed thru the anterior chamber, and the cornea at a point diametrically opposite its entrance. The eye is thus transfixed by the needle-cannula, the point which is pushed into a piece of cork protruding about 2 cm. from the eye. The two holes in the shaft both lie within the anterior chamber, just in front of the iris, each several millimeters from the internal surface of the cornea. When the eye is so transfixed with the needle and the cork is placed over the point of the latter, it serves only to conduct

fluid into the anterior chamber thru the hole in the shaft which lies nearest the capillary tube. The other hole, obivously does not function when the cork is on the point of the needle as it is during the actual conduct of an experiment. When, however, it is desired to replace the fluid first used by some other, then it becomes necessary thoroly to wash the chambers of the eye and this is accomplished by removing the cork from the tip of the needle. When this is done the pressure within the chambers rapidly falls and the contained fluid leaves by way of the second hole, thru the distal part of the shaft and out of the point. Here, by proper manipulation of the stop-cocks the new fluid may be allowed to enter the capillary tube, whence it passes into the eye thru the first hole, thus displacing the fluid already within the eye, which leaves by way of the second hole. The latter situated on the opposite side of the anterior chamber facilitates a thorough washing of the cavity.

After connection with the apparatus thus has been established, buffered saline of a temperature of 38 degrees C. is allowed to flow upon the anterior surface of the cornea, thus avoiding dessication of this structure which seriously would interfere with normal filtration. If now, with the apparatus and material in their proper relation, a bubble of air is admitted into the proximal end of the capillary (the end nearest the reservoir) by pressing the bulb which is at this situation, the

bubble will be seen to move gradually, with a horizontal column of fluid in front and behind it, towards the needle. At first the speed of the bubble is quite variable in the same and in different preparations. Soon, however, presumably after a sufficiently large number of filtering spaces has been opened up, and after a sufficient pressure has been attained within the eye, the rate becomes fairly constant. In other words after the bubble is admitted into the capillary tube it passes with a uniform rate of speed along the capillary to the other end where it rises up into the bubble trap and out of the way. As we have noted earlier in this paper if the experiment is unduly prolonged the rate of bubble progression again becomes irregular. By reference to the graduations which war mounted in bake of the capillar ahy exact number of millimeters traversed by the bubble per minute may be determined.

It is possible fhus from the above fesults, namely the number of millimeters traversed by the bubble per minute, knowing the bore, or cross-section of the tube, to calculate the amount of fluid which must enter the eye in the same space of time. This procedure and calculation were satisfactorily applied to 35 eges to determine, if possilbe, the normal rate of aqueous production in the sheep eye. Priestly Smith gaives a rate of 26 cmm. per minute for the filtration rate of this type of eye and Mhesnamoff reports averages around 28 cmm. The impression

which one obtains from these authors is that these rates together with other data along similar lines approaches mathematical exactness. Our early work, therefore, was to obtain our own normals and to perfect the technique of this rather delicate manipulation.

PRESENTATION OF RESULTS

A tabulation of the results obtained in this preliminary series of test follows closely. (Table I) Reference to this table shows that an average of 54 cmm. per minute was obtained which represents the rate of outflow of lymph from the anterior chamber with the following restrictions: Artificial lymph used having approximately the same ions and proportioned about as they are in aqueous. This solution kept at a temperature of 37.5 degrees Centigrade and delivered under a pressure of 27 mm. Hg. (36.0 cm. water), The p H of the fluid was 7.7 thruout.

The extreme variability in the filtration rate is plainly evident in this table. It is this factor which makes it obviously impossible to assume any standard of comparison for different eyes because any reasonable change in the rate which might be the result of changes in the p H values could hardly be expected to exceed the variation which is shown by different normal eyes using the same perfursion fluid. For this reason the single reservoir for perfusion fluid, which has been described elsewhere is supplemented by another which is mainitained at exactly the

same level, 36.0 cm. above the eye, and which contains solution 'B'. Solution 'B' is of the same composition as solution 'A' but is variable in respect to its H-ion concentration, being in some instances higher and in some lower than the latter solution which has the standard p H of 7.7.

These two reservoirs, then, are connected by means of short rubber tubing to the upper limbs of a 'Y' tube, the lower limb of which is connected to the apparatus as before. Stop-cocks are so placed so as to connect or disconnect each or both of the reservoirs to the capillary tube. This change in the design of the apparatus now permits us to determine the rate of flow of solution 'A' thru the chambers of the eye, then to switch off this solution, allowing solution 'B' to run in, instead.

In the actual conduct of this type of an experiment the eye is placed in the bath of warmed salt solution of controlled pH, the apparatus, including the needle is allowed to fill with salt solution, which is then turned off, and the eye is pierced in the manner indicated by the needle, the point of which is then run into soft cork. The salt solution is allowed to flow upon the cornea and the perfusion fluid is admitted to the eye, thus raising the pressure to 27 mm. Hg.. After this pressure has been allowed to act upon the eye for about 10 minutes the actual readings are begun. The distance traversed by the bubble which has been admitted to the capillary is recorded for a one minute

period. Subsequent readings are made every ten minutes, with notations of any changes in the p H values of the solutions coming into contact with the eye. The temperatures of the solutions are also recorded at these same intervals.

The stop-cock of the reservoir containing the solution ' A' is now turned to the 'off' position, simultaneously the bubble stops in the capillary. The new solution 'B' is then allowed to communicate with the apparatus and filtration again begins, but the stop-cock near the needle is so adjusted as to divert the stream of fluid to the exterior, thus flushing out the whole apparatus. In about 30 seconds this flushing is completed so the stop-cock is turned back to its original position, and the cork is taken off the tip of the needle, allowing the enclosed fluid to be replaced in a manner already indicated. In one minute the eye should contain only the second solution, at this time, then the p H of the emerging fluid is ascertained and the cork is replaced on the point of the needle. Again the eye is subjected to the normal pressure, that is, after the level of the fluid in the reservoir has been readjusted to compensate for the fluid which has been allowed to run away. After a ten minute interval again one may start to take readings. This list of readings should approximate in magnitude, the first list if the solutions are of the p H. Any difference in the readings apparently would be occasioned by the change of the fluid. The

only variable factor in the actual conduct of the experiment was that of the p H of the solutions used. Of course there is the factor of greater degenerative change occasioned in the tissues of the eye because of the longer interval after removal from the body. In the latter regard, it is stated in various works on the eye (Parsons Pathology of the Eye, vol. III) that accurate and uniform results may be obtained a number of hours after death. Cold storage eyes have shown consistent results following a 36 hour sojourn in the reduced temperatures. In fact the results of Bentzen and Leber (12) to which a previous reference has been made, were obtained upon a human eye, 13 hours after death.

The foregoing method, then, was employed in a series of 50 eyes. The first solution, or control solution 'A' was a modified Ringer-Tyrode solution Buffered by a suitable apportionment of the primary and secondary phosphates to a p H of 7.7, kept at body temperature and with a constitution as follows:

Composition of solution 'A'.

Sodium chloride	0.80%
Potassium Chloride	0.02
Calcium Chloride, anhyd.	0.02
Magnesium sulphate	0.03
Sodium bicarbonate	0.03
Potassium primary phos.	
Sodium sec. phosphate	0.02

This solution was freshly prepared and the p H determinations made upon it at the beginning of the experimental series. Sol-

ution 'B' was of the same basic composition but varied in the p H value because of changes in the relative amounts of the two phosphates which imparted a buffer quality to the solutions. An attempt was made in formulating the compositionsof the fluids to approximate that of the aqueous as given in various analyses. The highest H-ion concentration represented in solution 'B' was p H 6.8 and the lowest p H 8.6. These figures indicate the actual values of the solution, the figures which appear in the tabulations represent the actual value which was obtained from the solution coming from the eye, which, obviously is the important thing to consider.

METHOD EMPLOYED FOR P H DETERMINATIONS.

In as much as this study was considered a preliminary one, it was deemed accurate enough for the purposes at hand to use an indicator method for making the frequent p H estimations that were required. The apparatus furnished by the Hynson, Wescott and Dunning Co. was employed. It is designed for blood p H determinations, and consists of a set of sealed standard buffered color solutions of phenolsulphonephthalein which represent a range of p H from 6.6 to 8.6 in 0.2 graduations. This permits an approximation to .1, which is more accurate than several other factors involved in the experiment. It is in the main the same method used by the German investigators whose results have been given at length.

In tables II and III are given the results obtained when the perfusion fluid was low in H-ions and of greater H-ion concentration, respectively. The fact that the Solution 'A' appears as a variable value is due to the fact as stated, that the figure represents the p H value of the solution as it came out of the eye, the solution always had the value of pH 7.7 as it entered. The same is true of the second solution used.

There is a feature which should be mentioned at this point, however, that might serve to account for the wide range of rates obtained, but more especially, for the dissenting minority of eyes which could not be classified with the rest. This was the fact that the saline solutions which were used to receive the eyes and to keep them warm while they were being transported from the slaughter-house to the laboratory frequently showed a rather definite increase in the p H value. This occured in spite of the buffering of the solution. The change in p H frequently approached 7.0 a factor which could by no means be disregarded in a study of this kind, but a factor which in the past has been disregarded as has the entire subject of the H-ion value as solutions similarly employed. In subsequent work it is intended to test the aqueous fluid by micro-method to see if the change in the p H of aqueous actually follows the change spontaneously brought about in the fluid in which the eyes are immersed. At any rate it would be highly desirable to maintain the p H of the immersion fluid constant. This may be more nearly

approached by using a more highly buffered solution in larger quantity for the same number of eyes. In the majority of these studies about 3 quarts of buffered saline solution served in which to carry 20 eyes. A reduction in the temperature, of course, would serve to reduce the amount of degenerative change and the production of acids, but it was desirable in these studies to use eyes which had nvere completely lost their body heat.

Assuming then that the majority of eyes which showed a reduction in rate with the perfusion of a more alkaline solution did so because of the operation of an actual principle, it still remains to fathom the modus operandi. If it is true that in cases in which the p H is lowered, the outflow is re duced, and if this same observation could be made to apply to the filtration of eyes in situ, then if the lymph porduction were constant, an elevation of the intra-ocular pressure would result! This is in keeping with the findings of Meesman uponhis experimental animals and also bear out the results of his clinical cases. In the latter it would be hard to determine whether the increased alkalinity of the aqueous were a cause or an effect of the reduced rate of outflow, or whether bothof these factors were secondary to some other. At least it indicates a cause, or a possible explanation for the frequent failure to relieve in cases of glaucoma, where subconjunctival injections of alkaline solutions are therapeutically employed. Many instances

are recorded in which such injections were employed to combat the intra-ocular pressure increases with a resulting aggravation of all the symptoms including pressure elevation. In the light of the experimental work here reported and the work of others mentioned at some length, these disastrous results are explained. From the meager information available there is no indication for the use of an alkaline solution in the treatment of elevated pressure within the eye. On the other hand, one would, hesitate to recommend the use of injections of solutions with elevated p H values with no more support of a practical character than is now offered. In this connection however, Meesman secured reductions in the normal tension by the injection of slightly acidulated solutions, a proceedure which the results of this paper show to be not without foundation. In enperimental animals it should be re membered that the pressures dealt with were in the beginning normal and it remains to be shown whether the elevated tensions due to conditions which are analogous to glaucoma can be so reduced by injections of solutions of increased p H. The short duration of the reduction in pressure which is apparent when these solutions are injected into the normal experimental eye would not encourage one to expect any but a transient benefit in glaucoma. In this respect, if it were therapeutically applicable it would be little better than some of the methods now in general use and which are attended with

much less risk in their applications

In table IV, are included the experiments upon excised eyes where as a second filtration fluid, one of elevated p H used. In the main an increased rate of flow is apparent from the chambers of the eye. 70% of the cases show a rise in the rate of filtration amounting to any average increase of 10 cmm. If this observation were applicable to the filtration of an eye in situ, it might account for the depressed tension found in the eye in diabetic coma where, as several have observed, the intra-ocular tension may be reduced to almost nothing and the p H of the aqueous may be as high 7.0 according to Hertel.

The phyotony of diabetic coma is an interesting clinical manifestation. It cannot be produced in the experimental animal by the injection of acid into the blood, even when these acids are injected which are intimately associated with diabetes. The hypotony is not due to severe wasting for it is not found in diseases in which marasmic states are prominent. It is not an agonal or ante mortal condition, it is seldom found separate from the type of coma mentioned. It can be produced in dogs in which an experimental diabetes has been induced. This occurs, however, only after the onset of coma, a fact which one would think rather removed it from a close association with some of the features peculiar to diabetes. Parker(IO) reports a case of glaucoma occuring in a diabetic individual which proved very resistant to treatment and which improved only with a marked

improvement of the constitutional ailment. Hertel considers that the increase in the osmotic pressure in diabetes is sufficient to account for the hypotony of the globe.

The last author has called to our attention that there is also a hypotony in cases of carbon monoxide poisoning. This condition is associated with reduced oxidation in which the accumulation of acid products follows as a natural sequence. Czoniczer (11) recently has reported 5 cases of carbon monoxide poisoning in which he demonstrated a hyperuricacidaemia a fact which may or may not be related in some way. It is interesting to note,however, that in other asphyxial states reduction of ocular tonue is not a prominent feature. These conditions which are seen clinically and which are more or less uniformly associated with intra-ocular pressure changes bear additional study, and we are of the same opinion as Hertel that with a completer knowledge of these conditions will come the unraveling of the mysteries of the factors which control the intra-ocular filtrations.

Conclusions

1. A modified apparatus for the measurement of the rate of the rate of filtration in the excised eye has been described.

2. A much wider range of variability in the so-called normal rate of filtration in the excised eye was obtained.

3. The necessity for determining a control rate for each eye is apparent. Certain results obtained by increasing and lowering the H-ion concentration of the perfusion fluids used, are presented.

4. About 70% of the eyes examined show some decrease in rate (16 cmm.) with decreases in the p H of perfusion fluids.

5. Essentially the same averages are obtained in the opposite direction when the p H of the solutions are increased.

TABLE I.

Number of Eye	Cmm. outflow per minute	Number of Eye	Cmm. Outflow per minute
1.	74.	19.0	64.0
2.	79.	20.	70.
3.	132.	21.	73.
4.	43.	22.	31.0
5.	57.	23.	28.
6.	46.	24.	43.
7.	52.	25.	50.
8.	50.	26.	37.
9.	48.	27.	63.
10.	54.	28.	100.
11.	33.	29.	27.
12.	17.	30.	61.
13.	76.	31.	46.
14.	55.	32.	69.
15.	48.	33.	51.
16.	55.	34.	33.
17.	50.	35.	27.
18.	50.		

35 Eyes - Average rate 54.0 cmm. per minute.

TABLE I.; representing the results obtained in terms of cubic millimeters per minute flow, when the saline was maintained at body temperature, admitted under a pressure of 25-27 mm. of mercury, (36.0 cm. water) . The results represent a preliminary series performed for perfecting technique and to determine, if possible a normal rate of outflow that might be applicable to all sheep eyes.

It is evident, however from the wide range of variation that no such average rate would be applicable. It is therefore apparent that the normal flow must be obtained for each eye examined, and any other rate occasioned by differences of proceedure contrasted with this first determination.

TABLE IX.

Showing the Effect of Decreasing the p H of filtration fluid to be used as artificial aqueous in Excised Sheep Eyes.

No. of Eye	pH of 1st Fluid	pH of 2nd Fluid	Decrease in Rate per minute	Increase in Rate per minute
37.	7.4	8.2	9.0 c mm.	
39.	7.4	8.1		1.0 cmm.
40.	7.4	8.3	24.0	
41.	7.4	8.3	12.0	
47.	7.7	8.6	00.0	0.0
48.	7.7	8.6	10.0	
56.	7.4	8.2		24.0
57.	7.8	8.5	51.0	
61.	7.5	8.3	16.0	
62.	7.7	8.2	3.0	
64.	7.8	8.5	6.0	
65.	7.4	8.2		3.0
66.	7.5	8.1	3.0	
68.	7.7	8.6	15.0	
70.	7.5	8.2	8.0	
71.	7.7	8.3	0.0	0.0
73.	7.6	8.3		2.0
74.	7.4	8.2	8.0	
75.	7.5	8.1	2.0	2.0
79.	7.6	8.4		
81.	7.7	8.4	2.0	
82.	7.8	8.6	4.0	
84.	7.4	8.2	4.0	
85.	7.4	8.3	8.0	

1.0 cmm.

9.0 c mm. 0.0

24.0 24.0
12.0
00.0
10.0

51.0 3.0

TABLE III.

No. of Eye	pH of 1st Fluid	pH of 2nd Fluid	Increase in Rate per min.	Decrease in rater per Min.
36.	7.7	6.8		6.0
38.	7.8	7.0		4.0
42.	7.8	7.0	5.0 c mm	
43.	7.8	6.8	7.0	
44.	7.8	7.1	3.0	
45.	7.8	6.8	12.0	
46.	7.7	6.8	29.0	
49,	7.6	6.9	9.0	
50.	7.4	6.8	14.0	
51.	7.4	6.8	21.0	
52.	7.5	6.8	0.	12.0
53.	7.5	7.3	0.0	0.0
54.	7.5	7.1		28.0
55.	7.7	7.2	7.0	
58.	7.6	7.1	2.0	
59.	7.7	7.2	0.0	0.0
60.	7.6	7.1		26.0
63.	7.7	7.0	8.0	
67.	7.7	6.8	18.0	
69.	7.5	6.9		2.0
72.	7.7	7.0	9.0	
76.	7.7	7.0	4.0	
77.	7.6	6.8	9.0	
78.	7.5	6.8	6.0	
80.	7.7	7.0	4.0	
83.	7.6	7.0	13.0	

TABLE III. Showing in the column headed " Increase in Rate per Minute " numerically the cubic millimeters increase which the corresponding increase in the p H of the perfusion fluid produced. Altho the original rate is not included in the table, it will be remembered that the average rate was around 54. cm per minute. 70 % of the eyes showed an average increase in rate of 10 % when the p H of the perfusion rate was increased.

BIBLIOGRAPHY

1. Martin Fischer — Edema and Nephritis. John Wiley and Son 3rd Edition 1921.

2. Hertel, E. — Studies of Blood and Aqueous in Glaucoma Heidelburg Ophthal. Congress 1920 pp23-76

3. Michaelis, L. — Vereinfachung der Indicatorenmethode. Deut. Med. Wochschr. 47: 465

4. Meesman — Dependence of the Intra-ocular Pressure on the H-ion Concentration of the Aqueous Humor. Arch. f. Augenh 1924 v 94 p 185

5. Weber, C. — Pathology of the Eye. Herbert Parsons. vol. 3 p 1055. 1906 Putnam.

6. Bentzen and Leber — A. f. O. xlii, 3, 1895

7. Niesnamoff — A. f. O. xlii, 4, 1896

8. Priestly Smith — Ohthal. Rev. vii, 1888

9. Henderson and Sterling — Parson's Path. Eye. vol. iii. (modification of method used by Niesnamoff under Leber.)

10. Parker, W. H. — Ocular Manifestations of Diabetes., Am. J.O. 8: 284. April 1925.

11. Czoniczer — Uricacidemia in Carbon Monoxide Poisoning. Muchner '20.