AN INVESTIGATION OF THE SUGAR CONTENT OF THE OCULAR FLUIDS UNDER NORMAL AND ABNORMAL CONDITIONS, AND THE GLYCOLYTIC ACTIVITY OF THE TISSUES OF THE EYE*

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I. Introduction

A study of the amounts of sugar present in the various fluids of the eye is of importance for a number of reasons. Our theories of the formation of any bodily fluid will depend in a large measure upon its chemical characteristics. Any fluid having a composition compatible with the known laws of dialysis may be assumed to be a dialysate from the blood; whereas, if the chemical composition is such that it cannot be produced by dialysis from its parent fluid, the blood, then we must explain its formation by some other process, or cloak our ignorance by saying that the fluid is formed as the result of vital activities on the part of certain body cells. Since sugar, like urea and certain other substances, is known to be freely diffusible through inert semipermeable membranes and through the normal walls of the capillaries, it should occur in all bodily fluids, and in a concentration equivalent to, or at least not greater than, that of the parent blood. Since it is a non-ionizable substance in solution its passage across the capillary wall is not subject to those electrical constraints which play a large part in the partitioning of ionizable salts between blood and various body fluids, and hence the exact analysis of sugar affords an easy

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first step toward confirming or destroying our belief in the formation of such a fluid by dialysis. If the aqueous humor is a dialysate from blood serum, it should contain an equivalent concentration of sugar.

Further interest is attached to such a study in that sugar is the primary fuel for most of the activities of the body cells. It is of considerable physiologic importance to know to what degree various tissues of the body have the power of utilizing sugar, *i. e.*, what their carbohydrate metabolism amounts to. One would not expect tissues such as the cornea or lens, whose functions are apparently entirely optical, and hence served by their anatomic peculiarities, to have as high a metabolism as the retina.

Finally, it is well known to what extent the eye suffers when the carbohydrate metabolism of the body as a whole is disorganized. There is practically no tissue in the eye which cannot be affected during severe diabetes, and our knowledge of these pathologic changes must be based on known alterations of physiologic function. To this end it is of importance to know how the various fluids in the eye react to changes in concentration of sugar in the circulating blood.

II. The Concentration of Sugar in the Normal Aqueous and Vitreous

a. One finds in the literature many determinations of the sugar content of aqueous. The values given agree on the whole, but the majority are of little value in that no simultaneous blood sugars are given. Osborne[1] gives a mean of 90 mg. per cent. Cohen, Killian, and Metzgar[2] give as low a sugar concentration for the aqueous of ox eyes as 36 mg. per cent. In horses' eyes the average was 79 mg. per cent. No blood sugar figures are given.

Yudkin, Krause, Goldstein, and Berman[3] note the differences obtained in the aqueous sugar of the dog with different anesthetics, and give a mean value for aqueous aspirated

under cocain anesthesia of 77 mg. per cent. No blood sugars are given.

Duke-Elder[4] has compared the sugar content of aqueous and blood more carefully by taking arterial and venous plasma from the same animal (rabbit) at the time the aqueous was withdrawn. His average results are as follows:

Aqueous	= 151 mg. per cent.
Arterial plasma	= 158 mg. per cent.
Venous plasma	= 133 mg. per cent.

These figures show that the sugar concentration of aqueous lies between that of arterial and venous plasma, and suggest that it corresponds closely to that of capillary plasma.

The figures given by de Haan and Creveld,[5] however, do not bear out such an exact similarity:

Sugar Concentration of Blood Plasma. Ear Vein of Rabbit	Sugar Concentration of Aqueous withdrawn Simultaneously
200 mg. per cent.	190 mg. per cent.
280	190
200	150
200	190
280	160
270	210
240	220
220	190
250	170
200	190
222	190
210	190
260	240
320	250
220	180
190	80
120	50
260	280
190	150

These investigators point out that the sugar concentration in the aqueous is considerably lower than that of venous blood plasma. They raise the question as to the possibility of some of the sugar in blood plasma being so bound by the protein fraction that it cannot diffuse through the walls of the capillaries into the aqueous. The whole question of "bound sugar" has been disputed.

Abel, Rountree, and Turner,[6] Hess and McGuigan,[7] and Rona and Michaelis[8] believe that the total amount of sugar in blood plasma is free and diffusible.

Van Creveld[9] and Rusznyak,[10] on the other hand, were able to show that a considerable portion of sugar in blood serum would not pass through an ultra-filter. From this van Creveld considered that from one-quarter to one-third of the total sugar in blood plasma was bound. The results of the experiments of de Haan and Creveld above cited confirm this, for, as they point out, the lower sugar value of aqueous compared with blood can be explained only by assuming that either some of the sugar fails to get into the anterior chamber or that it is very rapidly metabolized there. This latter hypothesis is extremely unlikely in view of the probable low metabolic rate of the tissues composing the anterior chamber. (For confirmation of this see the experiments to follow on the metabolism of sugar by the various ocular tissues.)

Ask[11] also has found the aqueous sugar lower than that of whole blood in man, and gives an average of 100 mg. per cent. in aqueous and 120 mg. per cent. in blood.

There are fewer references in the literature to the normal level of sugar in the vitreous humor, and of these only a few give simultaneous blood sugars. Ask[12] has repeatedly found the sugar content of the vitreous lower than that of the aqueous or of the blood.

On the other hand, Duke-Elder[13] gives an average figure of 98.3 mg. per cent. for the vitreous of the horse with a simultaneous blood sugar of 91.0 mg. per cent.

b. Own experiments.

1. Methods Employed.—There are several methods of estimating the sugar concentration in small quantities of fluid. The work reported in this paper was begun in 1927, and during the three years three different methods have been employed in succession. These were in the order they were used:

1. Folin-Wu.[14]
2. Wright's Modification of the Folin-Wu.[15]
3. Hagedorn-Jensen.[16]

Several other methods were tried but abandoned because of failure to give accurate checks. The Wright[15] modification of the Folin-Wu method gave good checks on 0.1 c.c. of known sugar solutions, but has all the disadvantages of any colorimetric method, and was finally given up for the Hagedorn-Jensen method.

This latter is a simple, extremely accurate method, and can yield checks on 0.1 c.c. known-unknown sugar solutions with a 2 per cent. error.

The following protocol is an example of check-ups made from time to time during the course of these experiments:

Solution of dextrose = 137.4 mg. per cent.
Seven samples taken, each with a different 0.1 c.c. pipette.

Sample		Titration Value	Per cent. Dextrose found
Blank		1.908	..
Unknown	1	1.139	136.1
	2	1.145	135.1
	3	1.133	137.2
	4	1.133	137.2
	5	1.120	138.4
	6	1.140	135.9
	7	1.142	135.9

This does not imply that the method is without difficulties or drawbacks. The following is the method slightly modified by Cole:[17]

Principle.—The blood is coagulated by heading with soda and zinc sulphate. The filtrate is heated in alkaline solution with a measured amount of potassium ferricyanid, some of which is reduced by the glucose to ferrocyanid. The fluid is treated with potassium iodid and zinc sulphate and acidified. The excess of ferricyanid liberates iodin, while the ferrocyanid is precipitated as the double potassium zinc salt.

$$2H_3Fe(CN)_6 + 2HI = 2H_4Fe(CN)_6 + I_2$$
$$2K_4Fe(CN)_6 + 3ZnSO_4 = K_2Zn_3[Fe(CN)_6]_2 + 3K_2SO_4.$$

The iodin is titrated with standard thiosulphate, using starch as indicator. A blank determination, using all the reagents, is performed. The difference in the amounts of thiosulphate required is a measure of the amount of glucose.

Solutions: A. Sodium hydroxid, 0.1 N. This is conveniently stored in a small bottle with a rubber stopper, carrying a 1 c.c. pipette.

B. Zinc sulphate. 0.45 per cent. solution of the crystals. Stored in a bottle carrying a 5 c.c. pipette.

C. Potassium ferricyanid. 3.3 gm. of the purest salt to 1 liter of distilled water. Stored in a dark bottle and kept in a dark cupboard, it is stable for at least two months.

D. Sodium carbonate. 21.2 gm. of pure anhydrose sodium carbonate dissolved in water and the volume made up to 1 liter. Small bottles carrying a 1 c.c. pipette are convenient.

E. Potassium iodid. 25 gm. of pure sodium chlorid are dissolved in distilled water and the solution made up to 100 c.c. 7.5 gm. of potassium iodid are dissolved in this. Fit bottle with a 2 c.c. pipette.

F. Zinc sulphate in sodium chlorid. Dissolve 7.5 gm. pure crystalline zinc sulphate and 25 gm. pure sodium chlorid in water and make volume up to 100 c.c. Fit bottle with a 2 c.c. pipette.

G. Acetic acid. 3 per cent. made from pure glacial acetic acid (free from iron). Store in a bottle carrying a 2 c.c. pipette.

H. Soluble starch. 1 per cent. in saturated sodium chlorid solution.

I. Sodium thiosulphate solution, 0.005 N. This must be freshly prepared each day from a stock solution about 0.1 N. The solutions are prepared as follows:

Dissolve 54 gm. of pure crystalline sodium thiosulphate in recently boiled-out, cold distilled water and make the volume up to 2 liters. It is advisable to store this in a cool, dark cupboard for some days before standardization. To standardize the stock solution, dissolve about 2 gm. of pure potassium iodid in about 5 c.c. of water contained in an Erlenmeyer flask. Add about 10 c.c. of a 1.5 per cent. solution of pure potassium iodate and then measure 20 c.c. of accurately standardized sulphuric or hydrochloric acid, between 0.05 and 0.15 N. Titrate with the thiosulphate from a burette until the yellow color has nearly disappeared. Then add a few drops of the solution of soluble starch and complete the titration. If x c.c. of thiosulphate be required and the normality of the

acid is a x N, the thiosulphate is $\frac{20 \times a}{x}$ N. This should be between 0.106 and 0.108 N. Call it S x N.

Preparation of 0.005 N. thiosulphate from the stock. Measure 100 c.c. of recently boiled-out, cold distilled water into a dry flask by means of a 100 c.c. pipette. Add a further amount of water to make the solution exactly 0.005 N. The total volume of the solution should now be 1000 x S c.c. Thus, suppose the stock solution is 0.1074 N., the total volume is 107.4, consisting of 100 c.c. of water, 5 c.c. of the thiosulphate, and a further 2.4 c.c. of water.

Method: (1) Have ready a can of boiling water in which are immersed two test tubes, each containing about 10 c.c. of distilled water, into one of which is placed a 3 c.c. pipette.

(2) Into each of two small test tubes measure 1 c.c. of A and 5 c.c. of B, so that each tube contains 6 c.c. of the coagulant.

(3) Draw the blood as described above.

(4) Using a pipette, calibrated to contain 0.1 c.c. of blood, suck up the blood exactly to the mark. Remove the blood from the exterior of the pipette by means of a piece of filter paper. (A considerable error is introduced if the blood is sucked up much beyond the mark or if the exterior of the pipette is not wiped.) Discharge the blood into one of the tubes, gently blowing out the last portion. Carefully suck the fluid up into the pipette and blow it out again, so as to wash out the blood that adheres to the inner walls of the pipette. Using another clean pipette, measure 0.1 c.c. of the blood into the other tube as before. (The blood pipettes should now be washed out with a little dilute soda, thoroughly with water, then alcohol and ether, and dried by attaching to a suction pump. If the blood be allowed to dry in the pipette, it is sometimes very difficult to remove it.)

(5) Mix the contents of the tubes by smart rotation in the palms of the hands and immerse them in a can of boiling water for three minutes. The proteins are coagulated and collect at the surface or bottom of the fluid.

(6) Filter into a boiling tube (6 by 1 in.). This is best done by means of a small wad of absorbent cotton wool inserted into the stem of a funnel (5 cm. diameter). The wool is lightly pressed into the stem and washed two or three times with some of the hot water. Any surplus water left in the stem is removed by shaking. Filter the coagulated blood through this into the boiling tube. The filtrate should be crystal clear. If it is not, it must be re-filtered. When all the fluid has passed through, wash the test tube with

3 c.c. of the hot distilled water and pour this on to the coagulum on the wool. When this has drained through, repeat with another 3 c.c. of the hot water. When this has drained through, lift the edge of the wool by means of a needle, so that the fluid in the stem of the funnel can run out into the boiling tube. The other portion of coagulated blood can be similarly filtered into another boiling tube, using a fresh piece of wool. The blank (see 10) should now be prepared, so that the two tubes can be simultaneously heated and cooled.

(7) Add 1 c.c. of the ferricyanid (C), measuring this very carefully by means of an Ostwald pipette. Then add 1 c.c. of sodium carbonate (D).

(8) Mix carefully, immerse tube in the can of boiling water and leave it for exactly fifteen minutes. Remove the tube and immerse it in cold water for three minutes.

(9) Add 1 c.c. of E and 2 c.c. of F and mix. Add 2 c.c. of G and mix. Add 2 drops of the soluble starch and titrate with the 0.005 N thiosulphate from a 2 c.c. microburette. The end-point is obtained with a single drop of the thiosulphate, and is marked by the complete disappearance of the last trace of blue.

(10) Blank. Into a boiling tube measure 6 c.c. of distilled water, 1 c.c. of A, 5 c.c. of B, 1 c.c. of C, 1 c.c. of D, and heat for fifteen minutes in the boiling water bath. Cool for three minutes in cold water. Add 1 c.c. of E, 2 c.c. of F, and mix. Add 2 c.c. of G and mix. Add 2 drops of soluble starch and titrate with 0.005 N thiosulphate as directed above. This blank *must* be done with every fresh set of solutions. It is advisable to repeat it every day an estimation is made.

Calculation: Hagedorn and Jensen give a table showing the milligrams of glucose corresponding to the amounts of thiosulphate required. The author prefers the following: Let B c.c. = volume of thiosulphate required for the blank, and E c.c. the volume for the blood filtrate. Then $(B - E) \times 0.177$ = gm. of glucose per cent.

Example: Blank required 1.87 c.c. Estimation required 1.30 c.c. Then $B - E = 0.57$ c.c. Sugar in blood $= 0.57 \times 0.177 = 0.101$ gm. per cent.

Notes: The ferricyanid should be checked against the thiosulphate by taking 1 c.c. of it and treating with the reagents as when taking the blank, except that there is no heating. 1 c.c. should require 2 c.c. of the 0.005 N thiosulphate.

Should the sugar in the blood be over 0.3 gm. per cent., the sec-

ond portion of the filtrate should be treated with a double quantity of C and D. A fresh blank for this double quantity must also be made. The ordinary amounts of the other reagents are used, not double quantities.

Among the many advantages of the method not the least is the fact that extreme accuracy is needed in only three steps:

1. Measuring the unknown to be tested.
2. Adding the ferricyanid.
3. Titration.

Of the disadvantages it was found that a satisfactory starch solution was very difficult to make.

The use of starch solutions as an indicator for the titration of iodin is due to the formation of a colloidal sol made up of the soluble portion of the starch (B-amylose), ioide ion, and iodin. All these factors must be present for the blue color to develop. Unfortunately, when starch is boiled besides B-amylose, which is soluble, another product is formed called a-amylose. This is insoluble and gives a reddish color with iodin which is not discharged as readily as the blue color of the B-amylose. The fraction of a-amylose is relatively high in corn starch (15 per cent.), whereas potato starch contains only about 2 per cent.

The best starch solution was found to be the following:

a. Bring 100 c.c. of distilled water to which has been added 25 gm. NaCl to a boil.

b. Weigh out 1 gm. pure arrow-root starch, and make a thin paste with a little distilled water.

c. Add the paste to the boiling salt solution, washing the paste in with a wash bottle.

d. Boil until perfectly clear. This may require up to an hour's boiling, during which the volume must be kept to 100 c.c. by adding distilled water.

e. Allow to cool before using. This solution will keep at least a month if kept corked.

This gives a good sharp end-point, but, as is usually advised in most analytic chemistries, it is best to titrate the ferricyanid almost to the point of disappearance of the yellow color before adding the starch and finishing the titration.

The reason for this is that both a-amylose and B-amylose "are rapidly hydrolysed in aqueous solutions into their degredation products—amylodextrin, erythrodextrin, achroodextrin, etc.—if the concentration of hydrogen ion is greater than 10^{-2}. All these degredation products give with iodin red colors, which are not discharged at the stoichiometrical point" (Fales).[18] Hence the starch should not be added until this point is just about to be reached. The titration of the ferricyanid is conducted in an acid medium which makes this particularly apropos. The same number of drops of starch solution should be added to each of the tubes being titrated.

Cats were used in all experiments. Whenever possible sodium barbital was used as an anesthetic. This was given as an intraperitoneal injection, using 0.4 gm. per kilo body weight dissolved in as small a quantity of hot water as possible. Complete anesthesia is usually obtained in half an hour after the injection. It is important not to frighten the animal during the injection, which can be made apparently painlessly. An assistant holds the cat by the nape of the neck and the front paws in the air. A large bore needle fitted on a large barrel syringe containing the solution (except in very large cats the total volume should not exceed 8 c.c.) is thrust into the peritoneal cavity in the midline near the navel, and the solution quickly injected. Any excitement will result in a rapid increase in blood sugar. This is the anesthetic of choice, as Tychowski and Crowell[19] have shown that it has no effect on the blood sugar level over a long period of time. If ether or chloroform be used, one must expect abnormally high blood sugar levels.

Method of Obtaining and Running Through All Fluids

For the estimation of sugar in the aqueous and vitreous the following methods were employed:

The eyeball is enucleated, washed free of blood, and dried. The aqueous is aspirated by inserting a needle connected to a 2 c.c. syringe through the cornea. The cornea is then excised at the limbus and the remaining fluid dried rapidly with filter paper. The whole posterior segment is then removed by an incision made just back of the ciliary body. This is easily marked in the cat's eye by the line of junction of white firm sclera with the dark posterior portion where the choroid shows through. This incision is enlarged and completed with scissors until the whole posterior segment can be lifted off as a cap. The remaining ciliary body and sclera are grasped with forceps and the whole vitreous body pulled away intact from its base of attachment to the ciliary processes and posterior capsule of the lens. It is well known that this is the firmest attachment of the vitreous in the eye. (See Heesch.[20])

The aqueous contains such a minimal quantity of protein, i. e., about 0.03 per cent. (Adler and Landis[21]), that it is unnecessary to carry out any precipitation before the actual analysis. Serologic 0.1 c.c. pipettes are used, and all analyses are run in duplicates. Great care must be taken in filling these pipettes to the mark. It is hardly necessary to remark that extreme cleanliness must be observed with all glassware. All pipettes are washed in tap water, distilled water, alcohol, ether, and dried by air through a suction pump. Whenever cleaning fluid is used, the pipettes must be left with water running through them for at least five minutes.

Since vitreous contains a considerable quantity of protein (Duke-Elder[13]), it is necessary to precipitate this as in the case with blood before proceeding with the analysis. One cannot take up the vitreous in a 0.1 c.c. pipette as it is too viscous, so that some way of precipitating the protein had to be devised before the measured 0.1 c.c. was taken. It was found best to do this by grinding the vitreous in a glass mortar with sand, using a glass pestle. The sand used was fine seashore sand which had been washed for twenty-four hours with running distilled water to remove any soluble impurities. The grinding with sand completely disorganizes the structure of the vitreous and precipitates the protein as a flocculent mass. The exact nature of the change which occurs is unknown, but it is in keeping with the recognized fact that the vitreous is a

gel in a state of unstable equilibrium, and even very minor insults, physical or chemical, suffice to destroy this equilibrium and precipitate the protein; *e. g.*, the production of vitreous opacities in inflammatory diseases or the result of contusion, perforating wounds, etc., of the posterior segment. After the grinding with sand the mixture is placed in a small glass tube and centrifuged at high speed for ten minutes. The clear supernatant liquid is poured into a watch-glass and the 0.1 c.c. required measured and treated like the aqueous.

For proof that this method of treating vitreous gives valid results of its sugar concentration see Section VI.

TABLE 1.—SHOWING COMPARISON OF SUGAR IN TWO EYES OF SAME ANIMAL

Blood	Aqueous		Vitreous	
	R	L	R.	L.
Mg. per cent.	Mg. per cent.	Mg. per cent.	Mg. per cent.	Mg. per cent.
..	83	87	..	..
..	113	113	..	..
130	85	93	54	50
188	206	187	82	83
94	93	97	60	61
119	84	107	53	55
111	84	109	52	54
136	123	124	72	67
168	123	121	74	71
Average:				
135 mg. per cent.	110 mg. per cent.	115 mg. per cent.	64 mg. per cent.	64 mg. per cent.

Table 1 gives the results of the concentration of reducing substances calculated as sugar in the two eyes of nine cats.

The average blood sugar = 135 mg. per cent.
" " aqueous = 113 " " "
" " vitreous = 64 " " "

Occasionally the variations in the aqueous sugar of the two eyes are considerable, but in six out of nine experiments the differences are of the order of experimental error. The difference in the vitreous sugar of the two eyes is well within the experimental error in all nine.

It can be seen from the table that the aqueous sugar level parallels to a certain extent that of the blood. The actual concentration of aqueous sugar averages considerably less than that of blood, and were a correction factor applied, as Duke-Elder has done, to allow for the difference in non-sugar-containing substances in the two fluids, the aqueous sugar level would be still lower than that of the blood. These figures are in accord with those of de Haan and Creveld,[5] and suggest that a portion of the blood sugar is held back in the blood-vessels and fails to come into the anterior chamber. Whether this is due to some of the sugar being bound to the proteins in the blood, or to some other unknown factor, need not concern us here.

Do these figures indicate that aqueous is or is not a dialysate from blood? It is certainly correct to say that at least they are in harmony with a dialysate. If the sugar concentration in aqueous were higher than in blood, it is obvious that either the aqueous is formed by dialysis and later concentrated in the anterior chamber by evaporation or by the taking up of water by certain cells lining the anterior chamber (compare the formation of urine; papers of Richards and Wearn[22]), or that the aqueous is secreted by the cells of the ciliary processes, which turn out sugar in a higher concentration than occurs in the capillary blood. It is probable that the aqueous is a true dialysate, but either some of the sugar is bound to the proteins in the blood or it is utilized by the cells lining the capillaries where its formation takes place, *i. e.*, the ciliary epithelium.

Table 1 also shows that the concentration of sugar in the vitreous is considerably lower than that of the aqueous, usually being about one-half that of the blood sugar. The vitreous sugar level also shows changes paralleling those of the blood. The possible cause for this low concentration of sugar in the vitreous is discussed under Section VI.

III. CHANGES IN THE SUGAR CONTENT OF AQUEOUS AND VITREOUS WITH CHANGES IN THE BLOOD SUGAR LEVEL

a. Observations in the Literature.

Takahashi[23] found that the sugar content of the aqueous followed changes in blood sugar in dogs whose pancreas had been extirpated.

Ask[12] showed a similar parallelism between changes in blood sugar and aqueous sugar following an alimentary hyperglycemia. The changes in aqueous sugar were not so great as those of the blood when the hyperglycemia was brought about by the injection of adrenalin. This would be expected as the result of the constriction of the capillaries in the eye.

Cohen, Kammer, and Killian[24] studied the changes in sugar of the ocular fluids with those of blood. The aqueous and vitreous filtrate were analyzed as one fluid, however. The hyperglycemia was produced either by a parenteral or intravenous injection of glucose. They found the ocular fluids rising parallel with rises in blood sugar, but noted that during recovery from the hyperglycemia there was a lag in the decrease of the ocular fluid sugar.

b. Own Experiments.

(1) Methods Used.—A cat was anesthetized with sodium barbital. One eye was then enucleated. A cannula was inserted in a femoral artery, from which blood could be withdrawn for analysis, and a similar cannula was inserted in a femoral vein and this was connected to a burette containing a 10 per cent. solution of dextrose made up in distilled water. A sample of blood was taken from the artery, and then 10 c.c. of the 10 per cent. dextrose solution was allowed to run into the vein at the rate of 1 c.c. per minute. Immediately after the injection of the sugar a second blood sample was taken. An interval of time was now allowed to elapse, different for each cat used, and ranging from five minutes to seven hours. At the end of this predetermined interval the remaining eye was enucleated and another blood sample taken from the artery. Care was used in taking the blood samples to let the stagnant blood re-

maining in the closed artery run off, before the actual sample for analysis was selected.

The three blood samples, the aqueous, and the vitreous were then analyzed in the usual manner. When the interval elapsing between the enucleation of the two eyes was more than half an hour, the animal was kept warm on a heated table, as the animal's temperature control is considerably affected by sodium barbital.

(2) Results.

TABLE 2

Time Elapsed	Blood			Aqueous Eye Enucleated		Vitreous Eye Enucleated	
	Before	Immed.	Later	Before	After	Before	After
5 min.	111	..	280	120	164	66	76
15 min.	130	316	320	113	198	71	91
30 min.	132	310	..	119	178	62	94
1 hr.	130	320	214	128	212	49	118
1 hr. 30 min.	164	290	234	147	224	58	143
2 hrs.	154	302	162	135	156	73	148
3 hrs.	112	..	114	120	188	50	110
1 hr. 45 min.	122	382	228	102	218	63	103
2 hrs. 45 min.	112	244	136	97	183	61	108
4 hrs.	138	284	130	92	164	64	96
5 hrs.	116	270	188	142	142	69	110
7 hrs.	148	296	190	134	150	78	99

Table 2 gives the results of 12 experiments ranging from five minutes to seven hours. The first column gives the blood sugar level after the enucleation of the first eye. It naturally varies widely in the different cats used. The second column gives the blood sugar immediately after the injection of the 10 c.c. of 10 per cent. sugar, carried out identically in all the animals. These figures prove that in each case the sugar injected in the vein actually reached the general circulation, *i. e.*, the vein was not thrombosed higher up, etc. The third column gives the blood sugar at the end of the time indicated at the left of the table, corresponding to the elapsed time between the enucleation of the two eyes. The next two columns give the sugar concentration of the aqueous in the eye enucleated before the intravenous injec-

tion of dextrose and in the second eye enucleated later. The last two columns give the same figures for the vitreous sugar.

TABLE 3.—ABSOLUTE CHANGES

Time	Blood	Aqueous	Vitreous
5 min.	169 mg.	44 mg.	10 mg.
15 min.	190	85	20
30 min.	..	59	32
1 hr.	84	84	69
1 hr. 30 min.	70	77	85
2 hrs.	8	21	75
3 hrs.	2	68	60
4 hrs.	−8	72	32
5 hrs.	72	0	41
7 hrs.	42	16	21

Table 3 gives the absolute changes in sugar concentration expressed in milligrams for blood, aqueous, and vitreous. As would be expected, the blood sugar rises immediately, the maximum increase being within the first fifteen minutes after the injection. After an hour and a half some of the sugar has disappeared, being consumed by the tissues or stored as glycogen in the liver and muscles. The increase found after five hours may be a secondary rise in the animal's blood sugar due to the prolongation of the anesthesia. The injection of sugar intravenously probably brings into play a number of physiologic reactions, complex and varying in response in different animals, *i. e.*, the production of insulin, so that it is not surprising that unusual results are sometimes obtained, and the figures do not follow a uniform curve.

The increase of sugar in the aqueous parallels fairly well the changes in blood sugar. This is what one would expect were the aqueous a dialysate from blood. Any change in blood sugar is reflected almost immediately in the aqueous sugar. The highest value for the aqueous sugar was found fifteen minutes after the injection. It is clear that there is very little, if any, lag in the equilibration of aqueous with blood.

The changes in the vitreous, on the other hand, show a marked lag. The maximum level of vitreous sugar is not reached for one hour and thirty minutes after the injection. One would hardly expect a substance like vitreous to equilibrate itself with changes in blood sugar as rapidly as aqueous. Certainly the diffusion of the sugar molecules through the vitreous would be slower than through the aqueous, assuming that the increased blood sugar immediately presents more sugar to the vitreous. At the end of five hours the aqueous sugar had returned to its normal level, whereas the vitreous sugar was still 41 mg. higher than its original level. The lag then is effective in both directions. If the blood sugar is suddenly increased, the aqueous will also show a rapid increase. The vitreous, however, will come into equilibrium much more slowly. Once the blood sugar is elevated and aqueous and vitreous have come into equilibrium with it, a sudden drop in the blood sugar level will immediately be reflected in the aqueous, whereas the vitreous sugar will decrease much more slowly.

This lag in the equilibration of the vitreous sugar with changing blood sugar concentration may have some significance clinically.

Among the many changes which the eye may undergo as the result of diabetes are the interesting changes in refraction. Not every diabetic is subject to these marked changes in the static refraction of the eye, but, as Duke-Elder[25] has pointed out, with the increasing use of insulin we may expect to see them more frequently. The change in refraction is apt to be an increase in myopia when the blood sugar is rising, and an increase of hyperopia when the blood sugar level is falling. Hagen[26] laid stress on the observation that the development of hyperopia always occurred when the diabetic was first put on a rigid carbohydrate-free diet. Changes in the amount and axis of astigmatism have also been reported, and lend strong support to the theory that

the underlying cause of the change is to be found in the lens. This is further corroborated by a case reported by Elschnig,[27] in which one eye was aphakic and showed no change in refraction, while the opposite eye containing the lens showed a marked increase of hyperopia. On the other hand, the transitory character of the changes makes it difficult to attribute them to any actual deformation of the lens or to changes in the density of its various layers.

Of all the theories which have been proposed to account for these changes, the most attractive is that they are due to changes in the refractive index of the various ocular media with variations in the concentrations of sugar or salts. Unfortunately for the simplicity of this theory it has been pointed out by several investigators, chiefly Hagen,[26] that an impossible change in sugar concentration would have to occur in the aqueous and vitreous to give a sufficient alteration of the refractive index to account for the observed ametropia. The theory as it has been presented calls for a simultaneous change in the refractive index of both aqueous and vitreous.

Hagen points out that the blood sugar in diabetes could seldom exceed 1 per cent. The very slight increase in the refractive index caused by such an increase in the percentage of sugar in the aqueous and vitreous could not account for the observed ametropia.

Material	Index of Refraction
Normal aqueous	= 1.3353
0.5 per cent. sugar solution (in 0.9 per cent. NaCl)	= 1.3354
1.3 " " " " " " " " "	= 1.3363

Any higher concentration of sugar would cause a cataractous change in the lens.

According to Hess, the refractive index of the aqueous would have to reach 1.42 to create a hyperopia of 6 D.

The various theories which have been presented to account for these refractive changes are admirably reviewed by Duke-Elder.[25]

One theoretical possibility, however, has never been considered. In discussing a case of sudden development of hyperopia in a diabetic presbyope with Dr. Alfred Cowan, he made the suggestion that if the refractive index of either the aqueous or the vitreous alone changed, a much smaller change would account for the development of a considerable ametropia. Thus, if the sugar concentration of the aqueous increased while that of the vitreous remained constant, the resulting difference of the refractive indexes in the two media would account for a considerable myopia. On the other hand, if the vitreous sugar were high and the aqueous alone dropped, a considerable hyperopia would be developed.

The experiments cited above were done with a view to testing out the validity of Dr. Cowan's hypothesis under physiologic conditions, and the full credit for this idea is due solely to him. The results show that such a change in the refractive indexes of aqueous and vitreous is possible. Whenever the blood sugar in an animal is altered, the concentration of sugar in the vitreous lags considerably in both directions behind that of the aqueous. This does not prove that the refractive changes in diabetes are due to this factor alone, but the hypothesis accounts for some of the observed clinical facts, *i. e.*, the development of myopia when the blood sugar is rising, and the development of hyperopia when the patients are first placed on a rigid diabetic diet. It also explains the transient character of these changes.

IV. The Utilization of Sugar by the Various Tissues of the Eye

a. Observations in the literature.

The utilization of sugar by animal cells has long been recognized, but the actual chemistry of the process is not thoroughly understood. The works of Meyerhoff[28] and of Hill[29] have thrown much light on the carbohydrate metab-

olism of muscle cells, and the application of the methods devised by Warburg[30] has opened up many new and interesting problems.

According to Levene and Embden, the glycolytic activity of the animal cells results in the breaking down of one molecule of dextrose into two molecules of lactic acid, as follows:

$$C_6H_{12}O_6 = 2C_3H_6O_3.$$

This process is not an oxidation, but a splitting up of the molecules and may consequently take place in the absence of oxygen. When oxygen is present, however, the process is considerably affected, so that one must draw a distinction between glycolysis under aerobic and anaerobic conditions. There are a number of other factors which should be carefully controlled if the glycolytic activities of various tissues are to be compared. These are chiefly the temperature, the hydrogen-ion concentration, the concentration of bicarbonate and of sugar in the solution.

Meyerhoff has shown that in the muscle cell two processes occur: 1. The splitting of sugar into lactic acid. This occurs spontaneously and in the absence of oxygen. 2. The reconversion of lactic acid into sugar. This requires energy for the transformation and is accomplished by the necessary presence of oxygen.

In the course of some experiments on the metabolism of carcinoma cells, Warburg[30] was led to investigate the rate of metabolism of the retina. He found that the retina of the rat, separated from the choroid and placed in an oxygen-free Ringer's solution kept at body temperature, split glucose into lactic acid at such an enormous rate that lactic acid to the extent of 35 per cent. of its weight appeared per hour. He regarded this extremely rapid metabolism of the retina as serving some function peculiar to this tissue. It is significant that brain tissue also has a very high rate of glycolysis.

Cohen[31] has recently referred to the results of some unpublished experiments in which some glycolysis was observed in the lenses of oxen. Sugar was broken down into lactic acid if the lenses were kept in Ringer's solution, and in the aqueous from the same eyes, but none occurred when they were immersed in the vitreous humor.

Kronfeld[32] has determined the glycolysis of lenses by using the method of Warburg. He could show a definite utilization of sugar—1 gm. of lens utilized 0.15 mg. of dextrose per hour.

b. Own Experiments.

The question of the relative rates of glycolysis of the various ocular tissues arose early in the course of these experiments. An answer to the question was sought by the following experiments. We were ignorant of Warburg's work when these were done, and now realize that many important factors should have been controlled before drawing conclusions from our results. They are offered, however, as a crude answer to some of the problems, and since they are in accord with Warburg's results, which were so carefully done, it is believed they are valid. Further work, more carefully controlled, is in progress along these lines.

(1) A series of cats were anesthetized by an amount of chloroform just sufficient to produce unconsciousness. The chest was then opened and the aorta cut. In this way a minimal amount of anesthetic was used which could affect the function of such delicate tissues as the retina. The eyes were immediately enucleated. The posterior segments were then cut away, the remaining vitreous cleaned out, and the whole posterior segments, consisting of retina, choroid, and sclera, placed in a small glass test tube containing exactly 2 c.c. of Ringer's solution which contained 100 mg. per cent. dextrose. A blank tube containing 2 c.c. of the same solution was run with each experiment. The tubes were corked and kept in a water bath at 38° F. for two hours. At the end of this time an analysis of the sugar remaining in each tube was made. From this the amount of sugar utilized by each posterior segment could be calculated.

TABLE 4.—UTILIZATION OF SUGAR BY VARIOUS TISSUES IN THE EYE

Whole posterior segment No.	1	utilized	38	per cent.	in	2	hours
	2	"	32	"	"	"	"
	3	"	45	"	"	"	"
	4	"	27	"	"	"	"
	5	"	24	"	"	"	"
	6	"	43	"	"	"	"
	7	"	27	"	"	"	"
	8	"	24	"	"	"	"

Table 4 gives the results of eight whole posterior segments. The utilization of sugar ranged from 24 to 48 per cent. of the initial sugar present, the average being about 32 per cent.

An attempt was then made to determine grossly what tissues of the posterior segment used the most sugar. One whole posterior segment was placed in a tube, and the retina from the other posterior segment stripped off. This was placed in a second tube and the remaining choroid with the sclera placed in a third tube. All three tubes together with a blank were incubated for two and a half hours. The utilization of sugar was as follows:

Whole posterior segment utilized	34 per cent.
Retina alone utilized	29 " "
Choroid and sclera	14 " "

The retina is responsible for the greater portion of the glycolysis. In order to compare the relative glycolysis of various tissues of the eye, a series of experiments were run in which each tissue was carefully weighed. The utilization of sugar was then calculated per gram tissue per hour.

TABLE 5.—UTILIZATION OF SUGAR BY VARIOUS TISSUES PER GRAM TISSUE PER HOUR

Retina	Iris	Cornea	Muscle
0.490 mg. 0.527 " 1.760 "	0.207 mg. 0.286 "	0.23 mg.	0.21 mg.

Table 5 shows the relative rates of glycolysis. The iris, cornea, and external ocular muscle have about the same rate, whereas the retina is at least double that rate. This illus-

trates the enormous power which the retina has for splitting the sugar molecule.

V. The Influence of Light on the Utilization of Sugar by the Retina

Dr. Jonas S. Friedenwald[33] made the valuable suggestion that light might have some influence on the rate at which the retina split sugar. Following his suggestion a number of experiments were devised to test this hypothesis.

Series 1: A cat was anesthetized with sodium barbital. The lids of one eye were sutured together, so that all light could be excluded. This was the more easily accomplished by the presence of the nictitating membrane, which could be drawn entirely over the eye and sutured before the lids were fastened together. A canthotomy was done on the opposite eye, the lids and nictitating membrane drawn apart, and the pupil dilated with atropin. A saline drip kept the cornea from drying. A blood sample was taken from an artery, and then an arc lamp was turned on the opened eye and an exposure given ranging in different cats from thirty minutes to two hours. The rays of the arc were first passed through a solution of copper sulphate to absorb all heat rays. A thermometer recording in tenths of a degree was hung by the eye in the path of the rays and showed practically no change in a two-hour run. At the end of the exposure a second blood sample was taken, and both eyes enucleated. The aqueous and vitreous were analyzed in the usual way. In one experiment the light was intermittent, obtained by a revolving disc in front of the rays, making the light reaching the eye of one second duration, and one second dark.

Table 6 gives the result of seven such experiments. No effect can be seen from the light. It was thought that perhaps the retina would use more sugar under the influence of light, and that the vitreous sugars in the eyes exposed to the light might, therefore, be lower than the eyes kept in the dark.

Series 2: A series of eyes were enucleated, and the posterior segments of choroid and retina placed in tubes containing 2 c.c. of a 100 mg. per cent. sugar solution (made up in Ringer's). The experiments were carried out like those reported in Section IV. The

TABLE 6

Blood		Control Eye	Exposed Eye	Time
	Mg. per cent.	Mg. per cent.	Mg. per cent.	
Before.....	139	Aqueous = 160	Aqueous = 200	30 min.
After.....	173	Vitreous = 79	Vitreous = 83	
Before.....	145	Aqueous = 143	Aqueous = 145	30 min.
After.....	207	Vitreous = 73	Vitreous = 77	
Before.....	145	Aqueous = 119	Aqueous = 119	30 min.
After.....	219	Vitreous = 65	Vitreous = 67	
Before.....	..	Aqueous = 131	Aqueous = 115	60 min.
After.....	106	Vitreous = 72	Vitreous = 65	
Before.....	..	Aqueous = 78	Aqueous = 83	2 hrs.
After.....	88	Vitreous = 56	Vitreous = 47	
Before.....	..	Aqueous = 94	Aqueous = 107	2 hrs.
After.....	88	Vitreous = 51	Vitreous = 58	Inter-mittent
Before.....	..	Aqueous = 113	Aqueous = 113	2 hrs.
After.....	124	Vitreous = 89	Vitreous = 91	

tissue from the right eye was placed in a tube which was blackened so that all light was excluded. The left eye was placed in a regular glass tube (quartz tubes were not used, no attempt being made to insure the presence of the ultraviolet) and on this tube the rays from an arc lamp were allowed to fall. Care was taken to prevent any change in temperature from the effect of the arc.

At the end of the exposure the sugar remaining in the tubes was analyzed, and the utilization of sugar by the tissue in the dark compared to that kept in the light.

TABLE 7.—EFFECT OF LIGHT ON SUGAR METABOLISM. EYES ENUCLEATED. EXPERIMENTS *IN VITRO*. WHOLE POSTERIOR SEGMENTS UTILIZED

Time	Tissue	In Light	In Dark	Change
2 hrs.....	Whole segment	28 per cent.	10 per cent.	18 per cent.
2 hrs.....	Whole segment	28 " "	32 " "	−4 " "
1 hr. 30 min.	Choroid and retina	24 " "	27 " "	−3 " "
1 hr. 30 min.	Choroid and retina	32 " "	19 " "	12 " "

Table 7 gives the result in four experiments. In two of these no change occurred. In the other two there was a definite increase in the amount of sugar split in the light.

The same experiments were repeated, and this time the tissues were carefully weighed.

TABLE 8.—UTILIZATION OF SUGAR PER GRAM TISSUE PER HOUR

	In Light	In Dark
Retina and choroid	0.309 mg.	0.179 mg.
" " "	0.253 mg.	0.221 mg.
" " "	0.502 mg.	0.458 mg.
Whole posterior segment	0.306 mg.	0.540 mg.

Table 8 gives the utilization in milligrams of sugar per gram tissue per hour. In three of the four experiments there was some increase in the amount split in the light. In one the amount split in the dark was greater.

These experiments are too few and too inconsistent to prove any influence of light on the splitting of glucose by the retina.

It is known that glycolysis is influenced by small changes in the hydrogen ion concentration of the solution, and under the influence of light the bleaching of the visual purple may change the P_H of the solution. Other variable factors would have to be excluded before reaching a final conclusion. Experiments conducted along these lines are in progress to show whether this effect of light on glycolysis is a real physiologic one, or is due to some chemical factor the result of the method of experimentation.

VI. The Cause for the Low Concentration of Sugar in the Normal Vitreous

1. The concentration of sugar in the vitreous is consistently much lower than that of the aqueous. Ask also found this low vitreous sugar and made the hypothesis that the vitreous contained some substances which bound water in a colloidal form without sugar. He gave no experimental proof, however, of this hypothesis.

The following tests were run to make sure that the method of analyzing vitreous introduced no factor to account for the low results:

(a) A solution of sugar equalling 100 mg. per cent. was made. One sample was analyzed as usual. A second sample was ground with sand, centrifuged, and the supernatant fluid analyzed. The values obtained were as follows:

Sample untreated	=	103 mg. per cent.
Sample ground with sand	=	102 mg. " "

The use of sand introduces no factor, therefore, which influences the determination of sugar in aqueous solution containing no protein.

(b) The vitreous from two eyes was pooled, ground with sand, centrifuged, and the supernatant fluid divided into two portions. One was analyzed directly. The other was subjected to the protein coagulants used for whole blood, and the analysis carried out exactly as in the case with whole blood.

The results were as follows:

Experiment 1.	Aqueous	=	143 mg. per cent.
	Vitreous treated in usual way	=	95 mg. " "
	Vitreous similarly treated plus treatment for blood	=	94 mg. " "
Experiment 2.	Aqueous	=	79 mg. " "
	Vitreous treated in usual way	=	53 mg. " "
	Vitreous similarly treated plus treatment for blood	=	51 mg. " "

These tests prove that the sand treatment is sufficient in the precipitation of protein. Further treatment to this end does not alter the sugar concentration.

(c) The vitreous from two eyes was pooled, cut with scissors, and 1.0 c.c. measured in a standard 1.0 c.c. large bore pipette, specially made to permit drawing up such a viscous fluid. This was treated similarly to whole blood. The remaining vitreous was ground with sand and analyzed by the routine method. Aqueous and blood from the same animal were also analyzed. The aqueous was divided into two portions: 1. Usual manner. 2. Ground with sand.

Blood sugar	=	132 mg. per cent.
Aqueous untreated	=	94 mg. " "
Aqueous ground with sand	=	94 mg. " "
Vitreous treated like whole blood	=	60 mg. " "
Vitreous treated in usual manner	=	60 mg. " "

This last experiment proves that the sand method of treating vitreous gives results exactly comparable to the method used for analyzing whole blood.

2. Proof of the Separation of Anterior from Posterior Chamber. If the sugar content of the vitreous is constantly lower than the aqueous, there must be a barrier to the diffusion of sugar from the anterior to the posterior chamber. Ever since Hamburger first suggested that the anterior chamber was entirely shut off from the posterior chamber by a watertight apposition of the iris against the anterior surface of the lens the idea has been contested. Kahn[34] in 1918 reported some experiments which he believed proved the existence of this water-tight junction. Seidel[35] and Adler[36] have objected to the conclusions drawn by Kahn, since no simultaneous blood-pressure readings accompanied his intraocular pressure curves.

The anatomic relations of the iris and lens make it extremely likely that the posterior chamber is closed off from the anterior chamber in the sense that the iris-lens contact forms a valve preventing substance passing from the anterior into the posterior chamber. It is equally likely that this same contact offers no barrier to the passage of substances from the posterior into the anterior chamber, however. Clinical evidence proves that cells and fibrin easily gain access into the anterior chamber and are plastered on the posterior surface of the cornea in inflammation of the choroid. (See H. Friedenwald.[37]) The following experiments were done to find out if sugar could diffuse easily from the anterior into the posterior chamber:

Experiment 1: Cat anesthetized with sodium barbital. Both eyes enucleated and a blood sample taken from an artery. One eye used as a control, aqueous and vitreous sugar being determined in the usual manner. The aqueous of the second eye was withdrawn by a needle with a two-way outlet. The needle was then con-

nected with a burette containing a solution of 260 mg. per cent. dextrose in Ringer's solution. The anterior chamber was irrigated with this solution to get rid of any normal aqueous, and then the burette set so that the pressure of the solution in the anterior chamber equalled 30 mm. Hg. A small amount of the solution kept leaking out through the cut veins draining the anterior chamber, as described by Seidel[38] in his dye experiments. The burette was raised from time to time to keep the pressure constant, however. At the end of an hour an analysis was made of the solution in the anterior chamber and of the vitreous.

Blood sugar	= 146 mg. per cent.
Control eye—aqueous	= 94 mg. " "
vitreous	= 51 mg. " "
Experimental eye—fluid in anterior chamber	= 258 mg. " "
vitreous	= 54 mg. " "

Although the fluid in the anterior chamber of the experimental eye had a sugar concentration of 258 mg. per cent. for a period of one hour, the vitreous of this eye was only 3 mg. higher than the control.

Experiment 2. Similar to No. 1. No blood sugar taken:

Control eye—aqueous	= 42 mg. per cent.
vitreous	= 28 mg. " "
Experimental eye—fluid in anterior chamber at end of one hour	= 240 mg. " "
vitreous	= 33 mg. " "

These experiments show that the diffusion of sugar in solution in the anterior chamber, and at a pressure slightly higher than normal, is almost negligible into the posterior chamber. This is probably due to the normal iris-lens contact. This could be proved by repeating these experiments on eyes which had previously had broad iridectomies done on them.

3. It was shown in Section IV that the retina had the highest rate of utilization of sugar of any of the ocular tissues. Comparing the anatomic structures surrounding the aqueous humor with those surrounding the vitreous, it is apparent that the utilization of sugar by the various structures differs enormously. The cornea, iris, and a small

portion of the lens capsule form the anterior chamber. The metabolism of sugar by all these tissues is not very great. The vitreous body, on the other hand, is enveloped and in intimate contact with the retina throughout at least two-thirds of its surface. Anteriorly it is bordered by the ciliary processes and the posterior surface of the lens. The vitreous, therefore, is exposed to a tissue having a high affinity for sugar.

Further, the exchange of substances from the blood stream into the aqueous and vitreous takes place at different places. Experiment and conjecture lead us to believe that the aqueous is probably formed from the blood-vessels in the ciliary processes, and perhaps from the blood-vessels of the iris. The main body of the vitreous receives substances which come either from the retinal capillaries lying in the anterior retinal layers or from the choriocapillaris. In either case the substances as they pass out of the blood-vessels will be exposed to the action of the cells of the retina.

It would seem likely that the reason for the low concentration of sugar in the vitreous is its rapid utilization by the surrounding retina. In its passage out of the blood-vessels the dialysate from the blood comes in contact with the retinal cells and part of the sugar is taken up. A low concentration of sugar is then presented to the vitreous and diffuses throughout its structure.

4. If this hypothesis is tenable, the concentration of sugar in the vitreous should not be uniform throughout, but there should be a gradient with the highest concentration in the most anterior part, and the lowest percentage in the outer layers in contact with the retina. The anterior layers should receive part of the aqueous sugar as it comes from the ciliary processes, whereas the posterior layers in contact with the retina should constantly be drawn on for sugar by the retina, as all the fluid they receive has to pass through this latter structure.

In order to determine this point the following series of experiments were performed:

A cat was anesthetized with sodium barbital and both eyes were enucleated. A blood sample was taken from an artery. The eyes were carefully washed free of blood, dried, all remaining muscle cut away, and then placed intact in a beaker filled with carbon dioxid ice, so-called dry ice. In five minutes each eyeball was frozen solid. By carefully warming the outer coats with the heat of the hand the tissues could be dissected off. The aqueous was removed intact like a thick watch crystal. The lens was removed from its bed together with its capsule, and the frozen vitreous body sectioned, dividing the anterior from the posterior layers. Various methods were used in doing this latter, the end-result in all being the same. Both eyes were treated alike, and the blood, aqueous, anterior and posterior layers of the vitreous analyzed for sugar.

TABLE 9

Eye No.	Blood	Aqueous	Anterior Vitreous	Posterior Vitreous	Difference
1[1]	284	86	63	53	−10
2[1]	284	86	81	64	−17
3	108	99	71	44	−27
4	104	73	63	70	+ 7
5	104	83	63	52	−11
6	118	96	65	67	+ 2
7	118	127	64	47	−17
8[1]	281	158	129	106	−23
9	127	65	42	35	− 7
10	150	..	68	57	−11
11	150	114	88	51	−37

[1] These animals were anesthetized with ether or chloroform, hence the high sugar values in blood and the discrepancies.

Table 9 gives the results for eleven eyes. In nine eyes the posterior portions of the vitreous contained considerably less sugar than the anterior portions. In one the difference was within the limits of experimental error, and in one other the posterior portion was higher than the anterior.

This bears out the hypothesis that the low sugar content of the vitreous is due to the utilization of sugar by the retina.

SUMMARY AND CONCLUSIONS

1. Determinations of the amount of sugar present in the aqueous humor of cats show an average of 113 mg. per cent. The average concentration of sugar in the whole blood drawn simultaneously was 135 mg. per cent. The concentration of sugar in the aqueous of each eye of the same animal is practically the same in the majority of instances. Where discrepancies occur, they can probably be accounted for by the rapidity with which aqueous equilibrates itself with changes in the blood sugar level.

2. In almost every instance where the aqueous is compared with the blood drawn at the same time the concentration of sugar is considerably lower in the aqueous. This is contrary to the findings of Duke-Elder, but in accord with the results of de Haan and Creveld and of Ask. If the aqueous sugar be compared with that of blood serum instead of whole blood, and if a correction factor be applied for the differences in protein content of aqueous and blood serum, as Duke-Elder has done, the difference in the normal sugar levels of these two fluids is even more marked. This suggests that in the formation of aqueous some of the sugar fails to get into the anterior chamber. Whether this is due to some of the sugar being bound by the protein of the blood, as de Haan and Creveld suggest, or whether it is utilized by the ciliary epithelium cannot be said. In any case the low concentration of aqueous sugar is not incompatible with the hypothesis that aqueous is a dialysate from the blood.

3. The concentration of sugar in the vitreous is very much lower than that of either the aqueous or whole blood drawn at the same time. The average normal vitreous of cats was found to be 64 mg. per cent. with a blood sugar of 135 mg. per cent. The concentration of sugar in the vitreous of the two eyes of the same animal is identical. A number of tests showed that the method of analyzing the vitreous was com-

parable to that used in analyzing the blood or the aqueous, and introduced no factor which could account for the low figures found.

Experiments showed that the diffusion of sugar from the anterior into the posterior chamber took place very slowly, if at all, at normal intra-ocular pressures. The iris-lens contact probably forms a water-tight barrier to the passage of fluid from the anterior into the posterior chamber. This is compatible with the difference in the sugar concentration normally found between aqueous and vitreous.

The concentration of sugar is not uniform throughout the entire vitreous. The posterior layers in contact with the retina contain less sugar than the anterior layers.

The low concentration of sugar in the vitreous is probably due to the marked glycolytic power of the retina. All the fluid passing from the blood-vessels into the vitreous must pass the retina first except that portion which is supposed to seep backward from the anterior chamber along the canal of Cloquet, representing a hypothetic lymph stream. In its passage into the vitreous, therefore, the major portion of the fluid has sugar extracted from it by the retina. Further, the retina keeps drawing on the vitreous in contact with it for sugar, and hence these outer layers are relatively depleted of sugar. The utilization of sugar by the tissues bounding the anterior chamber is relatively small, hence the aqueous sugar level is more nearly that of the blood.

4. Experiments were performed to determine the relative rates at which the aqueous and vitreous sugar levels came into equilibrium with changes in blood sugar. It was found that aqueous promptly followed the rise and fall of blood sugar, when this was altered by an intravenous injection of glucose. On the other hand, the vitreous showed a definite lag in equilibrating its sugar level with both rises and falls of blood sugar. This may play a part in the development of refractive changes in diabetes. It does not explain all the

phenomena observed clinically, for example, the changes in astigmatism, but it affords a ready explanation for the observed fact that the refractive changes occur only following a change in the blood sugar level, and that these changes are transitory.

5. The tissues of the eye, like most other structures in the body, have the power of splitting glucose into lactic acid. The relative power of glycolysis of these various tissues has been measured. It was found that the retina can split up more glucose than any of the other tissues—in fact, its glycolytic power is double that of any other tissue in the eye. The physiologic significance of this is unknown, but it may have something to do with the thermodynamics of the transformation of light energy into the nerve impulse. On the other hand, it may represent the need of a constant source of ready energy on the part of a tissue poorly designed for the storage of food-stuffs in its own cells; compared, for example, with the storage of glycogen in the muscle cell.

6. An attempt has been made to determine the influence of light on the splitting of glucose by the retina. The results of these experiments are too inconsistent to draw any conclusions. Further experiments carried out under more carefully controlled conditions are under way to test this hypothesis.

REFERENCES

1. Osborne: J. Physiol., 1919, lii, p. 347.
2. Cohen, Killian, and Metzgar: Proc. Soc. Exper. Biol. & Med., 1925, xxii, p. 445; Contributions to Ophthalmic Science, Jackson Volume, 1926, p. 216.
3. Yudkin, Krause, Goldstein, and Berman: Proc. Soc. Exper. Biol. & Med., 1927, xxiv, p. 451.
4. Duke-Elder: Biochem. J., 1927, xxi, p. 66; J. Physiol., 1927, lxii, p. 315.
5. de Haan and Creveld: Biochem. Ztschr., 1921, cxxiii; cxxiv, p. 172.
6. Abel, Rountree, and Turner: J. Pharmacol. & Exper. Therap., v, p. 275; 1914, p. 611.
7. Hess and McGuigan: J. Pharmacol. & Exper. Therap., vi, p. 45.
8. Rona and Michaelis: Biochem. Ztschr., 1908, xiv, p. 476.
9. Van Creveld: Cited in de Haan and Creveld.
10. Rusznyak: Biochem. Ztschr., 1921, cxiii, p. 52.
11. Ask: Acta Ophth., 1927, v, p. 23.
12. Ask: Biochem. Ztschr., 1914, lix, p. 1; p. 35.

13. Duke-Elder: J. Physiol., 1929, lxviii, p. 155.
14. Folin-Wu: J. Biol. Chem., 1929, lxxxii, p. 83.
15. Wright: Bull. No. 9, Ayer Clinical Laboratory of the Penna. Hospital, October, 1925.
16. Hagedorn-Jensen: Biochem. Ztschr., 1923, cxxxv, p. 46.
17. Cole: Practical Physiological Chemistry, Ed. 8, 1928, W. Heffer and Sons, Cambridge.
18. Fales: Inorganic Quantitative Analysis, Century Co, 1925, p. 300.
19. Tychowski and Crowell: Arch. Int. Med., 1926, xxxvii, p. 567.
20. Heesch: Arch. f. Augenh., 1929, c–ci, p. 680.
21. Adler and Landis: Arch. Ophth., 1925, liv, p. 265.
22. Richards and Wearn: Am. J. Physiol., xcvi, p. 209; J. Biol. Chem., xcvi, p. 247.
23. Takahashi: Graefe's Arch. f. Ophth., 1926, cxvii, p. 479.
24. Cohen, Kammer, and Killian: Tr. Am. Ophth. Soc., 1927, xxv, p. 284.
25. Duke-Elder: Brit. J. Ophth., 1925, ix, p. 167.
26. Hagen: Graefe's Arch. f. Ophth., 1921, cv, p. 243.
27. Elschnig: Med. Klin., 1923, No. 19, p. 17.
28. Meyerhoff: Pflüger's Arch. f. d. ges. Physiol., 1919, clxxv, p. 82; 1921, clxxxviii, p. 114; 1919, clxxv, p. 20; J. General Physiol., 1926, viii, p. 531.
29. Hill: Proc. Physiol. Soc. J. Phys., 1914, xliii, p. 10; Proc. Roy. Soc. Med., 1926, c, p. 87.
30. (a) Warburg: J. Cancer Research, 1925, ix, p. 161.
30. (b) Warburg, Posener, and Negelein: Biochem. Ztschr., 1924, clii, p. 309; 1925, clx, p. 307.
31. Cohen: Arch. Ophth., 1929, ii, p. 529.
32. Kronfeld: Deutsch. ophth. Gesellsch., 1927, xlvi, p. 230.
33. Friedenwald, Jonas S.: Personal Communication.
34. Kahn: Graefe's Arch. f. Ophth., 1918, xcv.
35. Seidel: Graefe's Arch. f. Ophth., 1918, xcv, p. 210.
36. Adler: Arch. Ophth., 1924, liii.
37. Friedenwald, H.: Tr. Am. Ophth. Soc., 1902; Tr. Ophth. Soc. U. Kingdom, 1924, xliv, p. 310.
38. Seidel: Graefe's Arch. f. Ophth., 1923, cxi, p. 162.