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Studies on the ocular hypotensive effect of Diamox

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Studies on the ocular hypotensive effect of Diamox*

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Abstract

In our studies on the hypotensive effect of Diamox by intravenous injection, we have arrived at the following conclusions. 1. Ocular tension falls and the flow of aqueous humor becomes sluggish. 2. Diamox inhibits the activity of carbonic anhydrase, and the concentrations of HCO3-, K+, Cl- and glucose are markedly altered. 3. Protein increases both in blood and aqueous humor, but no change in protein fraction can be observed in blood. 4. Diamox in no way affects the metabolism. 5. It seems that Diamox brings about the change in the specific gravity of blood, making the latter either more diluted or more concentrated. From these, we conclude that the mechanism of the loweing of ocular tension by Diamox seems to lie in the fact that it inhibits the activity of carbonic anhydrase, and that consequent alteration in the concentrations of HCO3- and other ions accompanied by the change in osmotic pressure as well as a slight decrease of water in tissue all bring about the fall in the ocular tension. However, Diamox seems to have nothing to do with aqueous humor in so far as active transport or permeability are concerned.

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STUDIES ON THE OCULAR HYPOTENSIVE EFFECT OF DIAMOX

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Ever since 1954 when Becker made his first report on the marked hypotensive effect of Diamox* against the intraocular pressure in gluacoma, many other investigators such as GRANT and TROTTER, BREININ and GÖRTZ, KLEINERT, etc., successively have written on the same subject. In Japan likewise there are many reports by IKEDA, IKUI, KINUGASA, KAMIHOSHI, KOJIMA, YUMIYAMA, KITAJIMA, etc. Indeed there appeared many reports, startig with BECKER, GRANT and TROTTER, CAMPBELL, LANGHAM, KINSEY, GREEN, KLEINERT, KOSAKI, etc., on the mechanism of lowering of intraocular pressure by Diamox, a drug that seems to have opened up a new field in the medicinal therapy for gluacoma.

In the present report are presented the results of our studies on the same subject as we believe we have in some measure clarified with following methods the mechanism of lowering the intraocular pressure by Diamox.

MATERIALS AND METHODS

For the experimental animals normal rabbits all weighing about two kg. were used. Aqueous humor was extracted ten minutes after applying a drop of 0.5% percamin on the eye, and blood sample used was arterial blood obtained by puncture of the heart. Using Diamox with its sodium chloride made into 3% physiological saline solution, with exception of cases given subconjunctival injection, all received intravenous injections by auricular vein.

Analytical:

The ocular tension was determined by the anterior chambermanometric method with the use of a manometer, an improved Wessely-Akagi type.

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For tonography, Müller's electric tonometer was used. Values of C and F were calculated by the Ballintine and the Friedenwald methods.

For the rate of accumulation of fluorescein, the Amsler-Huber method used, and as for the chemical analysis of Cl^-, K^+, and HCO_3^-, Schales and Schales method, Yamane's method (1949), and Warburg's manometry after gas-replacement of 95% N_2+5%CO_2 at 37°C, respectively. Glucose was determined by Somogyi's method. O_2-uptake by Warburg's method; TCA cycle by Tunberg methylene blue method; carbonic anhydrase by Krebs-Roughton method; cytochrome oxidase by the Nadi oxidase reaction; Protein by copper sulfate method; protein fraction by paper electrophoresis; and pH was determined by glasselectrodes manufactured by Toyo Roshi K. K.; and the specific gravity by copper sulfate method.

RESULTS

Intraocular pressures : In six cases the maximum of the average fall in intraocular pressure was 20.1 per cent while that of the blood pressure was 7. 9 per cent. Looking at the individual case, the maximum fall in intraocular pressure in two cases was 8. 5mmHg. and the rest showed the fall of 2—5 mmHg. Table (1) shows the average values in the ocular and blood pressures as measured at various intervals after intravenous injection of Diamox 30mg/kg in the six cases.

Table 1 Variations of the intraocular pressure and the blood pressure by intravenous injection of Diamox 30 mg/kg. (unit : mmHg)

	initial pressure	immediately after inj.	5 min.	10 min.	30 min.	40 min.	60 min.	90 min.	120min.
I. O. P	21.4	21.5	21.1	20	17.7	17.1	17.8	19.4	21
(%)	(100)	(100.4)	(98.1)	(93.5)	(82.7)	(79.9)	(83.2)	(90.7)	(88.1)
B. P.	92.5	93	92	91	86.8	86.7	87.3	85.8	85.2
(%)	(100)	(100.5)	(99.5)	(98.4)	(93.8)	(93.7)	(94.4)	(92.8)	(92.1)

Tonography : As for the aqueous outflow in the rabbits given intravenous Diamox injection, both C and F showed a fall 15 minutes after injection; and 120 minutes afterward the minimum outflow was shown (namely, the average of C was 52 per cent, while that of F 68 per cent), recovering to more or less normal level 240 minutes after the injection.

The rate of accumulation of fluorecein : In the rabbits concurrently injected with both Diamox and fluorescein the prolongation in the time required for the disappearance of fluorescein in the anterior chamber can be seen as shown in Table but no prolongation in the time of this appearance.

Table 2 Facility of aqueous outflow (C) and rate of aqueous flow (F) in normal and Diamox administered rabbits.

	normal		after 15min.		30 min.		60 min.		120 min.		240 min.	
	C	F	C	F	C	F	C	F	C	F	C	F
mean	0.374	5.037	0.319	2.775	0.236	2.177	0.213	1.901	0.179	1.642	0.343	3.970
pecent	100%	100%	−15%	−45%	−37%	−57%	−43%	−62%	−52%	−68%	−8%	−21%

CHEMICAL STUDIES

Chloride ions: In the determinations of variations in the concentration of the chloride ions in both aqueous humor and in blood, the concentration falls rapidly after intravenous injection of Diamox 30 mg/kg. Namely, 15 minutes after injection the concentration of chloride ions reaches its lowest level in both aqueous humor and blood, and after 30 minutes it again recovers back to the normal level. However, decreasing again slowly during period between 60 and 120 minutes, it returns back to normal 240 minutes afterward. The degree of variations in the concetration is always lesser in aqueous humor than in blood. Table (3) shows the average values in 8 cases checked at various intervals.

Table 3 Changes of concentration of chloride in aqueous humor and plasma after Diamox administration.

	normal	15 min.	30 min.	60 min.	120 min.	240 min.
aq. humor	100.0	90.5	97.0	95.7	90.4	91.8
(%)	(100)	(90)	(97)	(95)	(90)	(91)
plasma	1.047	90.8	102.4	95.2	95.2	94.0
(%)	(100)	(86)	(97)	(90)	(90)	(89)

Potassium ions: Variations in the K^+ concentration in aqueous humor and blood are similiar to chloride ions. As the result, the concentration clearly shows a fall 15 minutes after the injection, but returning almost to normal after 30 minutes, it again starts falling and 2—4 hours afterward it reaches its minimum. However, it tend sto recover again by eight hours after the injection.

The turnover method of potassium ions: With the purpose to test permeability of potassium ions through the blood-aqueous barrier, the intravenous injection of Diamox 30mg/kg is given after raising the K^+ concentration in both aqueous humor and blood by intravenous injection of 1.1% KCl solution. Then the variations in the K^+ concentration of blood

Table 4 Variation of potassium concentrations in the aqueous humor and serum after intravenous injection of Diamox 30 mg/kg. in rabbits. (unit : mg%)

	beofre	15 min.	30 min.	60 min.	120 min.	240 min.	480 min.
aq, humor	17.03	14.50	16.50	15.58	13.27	13.58	16.13
(%)	(100)	(85)	(97)	(91)	(78)	(80)	(95)
serum	18.41	17.66	17.80	17.33	17.01	16.68	17.76
(%)	(100)	(96)	(97)	(94)	(92)	(91)	(96)

and aqueous humor have been examined. As the result, very interesting phenomena have been observed; namely, when Diamox is administered, the potassium ions in blood increase whereas in contrast the same in aqueous humor decrease.

Table 5 Variation of potassium concentrations in aqueous humor and serum of rabbits injected 1.1% KCl solution 30 cc. (unit : mg%)

	normal	45 min.	60 min.	90 min.	150 min.	270 min.	510 min.
aq. humor	16.48	20.48	22.64	18.22	15.96	15.40	15.56
(%)	(100)	(124)	(137)	(111)	(97)	(93)	(94)
serum	17.78	27.64	26.38	21.42	20.06	19.50	19.36
(%)	(100)	(155)	(148)	(120)	(113)	(110)	(109)

Table 6 Variations of the potassium concentration in aqueous humor and serum of rabbits injected Diamox and 1.1% KCl. (unit. mg%)

	normal	45 min.	60 min.	90 min.	150 min.	270 min.	510 min.
aq. humor	16.04	15.20	14.46	14.34	15.62	16.46	15.92
(%)	(100)	(95)	(90)	(89)	(97)	(103)	(99)
serum	17.48	21.82	20.00	22.56	19.86	20.40	19.26
(%)	(100)	(125)	(114)	(129)	(114)	(117)	(110)

Bicarbonate ions : Fifteen minutes after the intravenous injection of Diamox a marked fall in the bicarbonate ions concentration is observed as shown in Table both in aqueous humor and blood, but after 60 minutes it recovers a little over the normal. However, two hours after the injection it reaches its minimum and during the period between 4 and 8 hours after it recovers back to the normal level. Variation in the bicarbonate ions concentration is greater in aqueous humor than in blood, as shown in Table (7) giving averages of ten cases measured at various intervals.

Glucose : The variations in the glucose concentration appear to be exactly the reverse of those of ions; namely, 30 minutes after Diamox administration the concentration increases both in blood and aqueous

Table 7 Variation of bicarbonate ions concentration in aq. humor and serum. (Diamox 30 mg/kg injekted rabbit) (unit mM/L)

	normal	15 min.	30 min.	60 min.	120 min.	240 min.	480 min.
aq. humor	31.4	26.4	26	32	24.9	25.9	30.0
(%)		(84)	(82.8)	(101.9)	(79.2)	(82.4)	(95.5)
serum	20.9	18.6	18.9	24.8	17.9	18.7	21.8
(%)		(88.9)	(89.9)	(118.6)	(85.6)	(89.4)	(104.3)

humor, and one hour later it starts to return to normal, but rising again by two hours' time, it tends to return to normal four hours afterward.

Table 8 Variation of glucose concentration in aq. humor and plasm. (Diamox 30 mg/kg injected rabbit) (unit mg%)

	normal	15 min.	30 min.	60 min.	120 min.	240 min.
aq. humor	79.0	95.7	104.6	95.1	105.3	85.5
plasm	107.5	124.3	125	111.7	117.3	109.6

Metabolism : By determining the O_2-uptake of the epithelium of ciliary body, the results as shown in Figure (1) have been obtained. Using Diamox at the concentration of $30\gamma/cc$ as an inhibitory agent throughout the entire determination process, no inhibitory action can at all be observed.

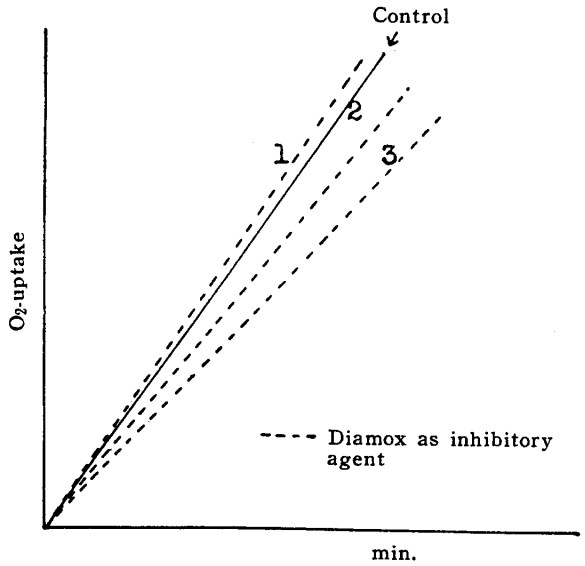

Fig. 1.

TCA cycle : It is generally belived that the energy furnished by the TCA cycle is the sole source of energy for transporting ions into the cytochrome system. As for substrates, glucose, pyruvate, α-ketoglutarate, malate, fumarate, succinate, and citrate have been used, and the decoloration time of methylene blue has been determined. As for the sample of the enzyme series in the TCA cycle, the femoral muscle of rabbit is employed, and the results are shown in Table (9). In comparison with the TCA activity in the control, hardly no significant difference can be observed in the cases given Diamox as an inhibitory agent.

Table 9 T.C.A cycle activity. (unit min.)

	glucose	pyruvate	α-keto-glutarate	malate	humarate	succinate	citrate
Diamox	36.2	21.7	20.8	31.0	30.8	16.6	27.8
control	38.2	19.8	17.0	25.3	29.2	17.8	27.8

Carbonic anhydrase : After the intravenous injection of Diamox 30 mg/kg, the carbonic anhydrase activities in the serum, anterior uvea, lens, and cornea of the removed eyes have been determined by the Krebs-Raughton method. As the result it has been clearly demonstrated that the activity is markedly inhibited as compared with that of the control group, and Table (10) shows the average of six samples.

Table 10 Carbonic anhydrase activity.

	serum	ant. uvea	lens	cornea
Diamox	0.26	0.30	1.05	0
contral	0.56	0.96	1.05	0.22

Cytochrome oxidase : With the Nadi oxidase reaction the existence of cytochrome oxidase in the eye tissue has been explored, and in the same reaction performed on the rabbits given Diamox, no difference can be recognized between the two groups.

Proteins : In the determination of the total protein of plasma, the hematcrit, and the hemoglobin contents by the copper sulfate method, the contents tend to decrease fifteen minutes after the intravenous injection of Diamox as shown in Table (11), and it seems that either blood is highly concentrated or some change has occurrred in the protein metabolism.

Paper electrophoresis : No difference can be observed between the fraction of the normal rabbit-serum and that of the rabbbit-serum given

Table 11 Diamox effect on total protein of plasma, hematcrit and hemoglobin in rabbits.

	normal	15 min.	30 min.	60 min.	120 min.	240 min.
total protein of plasma	6.216 (gr/dl)	5.735	6.327	6.475	6.512	6.586
hematcrit	42.42 (%)	41.40	41.35	41.72	41.64	41.89
hemoglobin	14.38 (gr/dl)	14.03	14.01	14.14	14.11	14.20

Diamox.

The hydrogen-ion concentration : In the periodical examinations of the pH in the rabbits treated with Diamox, both the pH of aqueous humor and blood tend to decrease, and thus giving rise to an acidotic state. This phenomenon endorses the results of bicarbonate ions previously mentioned.

Table 12 Effect of Diamox on pH of aqueous humor and blood in rabbits.

	normal	15 min.	30 min.	60 min.	120 min.	240 min.
aq. humor	7.866	7.866	7.800	7.683	7.733	7.716
blood	7.775	7.766	7.691	7.666	7.708	7.675

Specific gravity : The specific gravity of both blood and aqueous humor increases during the period between 15 minutes and 60 minutes after the Diamox injection as shown in Table, but thereafter it returns to normal. Table (13, 14) shows the average value of six rabbits.

Table 13 Effect of Diamox on the specific gravity of blood and serum in rabbits.

	normal	15 min.	30 min.	60 min.	120 min.	240 min.
aq. humor	1.0068	1.0076	1.0075	1.0070	1.0068	1.0068

Table 14 Effect of Diamox on the specific gravity of aqueous humor in rabbits.

	normal	15 min.	30 min.	60 min.	120 min.	240 min.
aq. humor	1.0068	1.0076	1.0075	1.0070	1.0068	1.0068

DISCUSSION

Although there are still many conflicting opinions concerning the mechanism of the lowering action on the intraocular pressure by Diamox, BECKER and his co-workers explain the mechanism of this action based on

the theory of KINSEY-FRIEDENWALD. In 1942 FRIEDENWALD made it clear experimentally that a great deal of cytochromes existed on the ciliary epithelium and he assumed that O_2 activated by cytochromes of the ciliary epithelium are turned into OH^-ions after reaction with H_2O, and that these OH^-ions in turn formed HCO_3^- by combining with CO_2. In other words, in the formation of HCO_3^- in the H_2O-CO_2 system it was thought that in the case of erythrocytes, carbonic anhydrase participates, and in aqueous humor, moreover, it was assumed that cytochromes were also associated with the formation of bicarbonate ions. By 1950 after his verification of the concentration of bicarbonate ions in the aqueous humor to be higher than that of plasma of rabbits, KINSEY advocated the secretary-diffusion theory for the formation of aqueous humor.

BECKER (1954) hypothecated that carbonic anhydrase was involved in the combination of CO_2 and OH^-ions produced by the cytochrome system. Namely, he believes that the cytochrome system and carbonic anhydrase form bicarbonate ions; and he assumes that the mechanism of the lowering action on the intraocular pressure by Diamox lies in the fact that the production of HCO_3^- is inhibited by the inhibition of carbonic anhydrase with resultant fall in the osmotic pressure of aqueous humor so that the amount of water penetrating through the blood-aqueous barrier is lessened, thereby bringing about the down-fall in the intraocular pressure. BECKER et al. presented many experimental results in support of this view, but the significance of the mechanism of aqueous humor production being still ambiguous, their results still lack in the experimental basis suffciently enough to uphold this view.

In their studies on the concentrations of Na^+ and K^+ in blood and urine as well as on the intraocular pressure and the amount of urine in the normal persons and in gluacoma patients, both groups given oral administration of Diamox 250 mg, CAMPBELL (1955) and his colleagues found the fluctuation curve of the amounts of urine, sodium ions and potassium ions were correlated to the fluctuation curve of intraocular pressure so that they believed micturation brought about the lowering of intraocular pressure. However, in this instance they have not conducted comparative studies with other micturation agents.

From their determinations of Na^+ concentration, total CO_2 content and pH in aqueous humor, WEINSTEIN and FORGAS (1955) concluded that due to the fall in osmotic pressure of aqueous humor by Diamox action the intraocular pressure declined because aqueous humor was conversely absorbed by the venous plexus of ciliary body.

KLEINERT (1954) stated that the disappearance of edema in the outflow

route was the cause of the fall in ocular tension of glaucoma, but this in a broader sense can be included in the theory of CAMPBELL et al.

Besides these there are reports by GREEN (1954) concerning carbonic anhydrase, intraocular pressure, bicarbonate ions and the concentration of Diamox in aqueous humor, and LANGHAM's detailed report (1957) on the relationship between ammonia chloride and Diamox. As for the fall in the intraocular pressure by intravenous injection of Diamox, there are papers by BECKER, KINSEY, TAKAOKA, etc., and also the results of our studies clearly indicate the fall in eye pressure. If we should list the points thought to be given a serious consideration for the clarification of the mechanism of the fall in the ocular tension, they will be : a) variations in blood pressure, b) the active transport of aqueous humor, c) changes in osmotic pressure, d) permeability through the blood-aqueous barrier, and e) changes in the water content.

From the manometrical results we can see a slight tendency of fall in blood pressure, but considering the relationship $IOP = K\{(OP_{AQ} - OP_{pI}) + Cap_P\}$, such a fall can be practically dismissed. Moreover, because the manner of the fall in blood pressure does not parallel that of ocular tension, we, therefore, believe the two are hardly related with each other.

As for the results of tonography, one to two hours after Diamox administration about 60 per cent decrease can be observed in the content of both C and F. This indicates a marked diminution in the aqueous outflow. As for the rate of fluorescein accumulation, no prolongation of appearance time can be observed, but this seems to be due to the concurrent intravenous injection of Diamox and fluorescein into rabbits.

BECKER et al., on the other hand, recognized a marked prolongation of the appearance time, but we have observed significant differences in the prolongation of disappearance time, indicating the slowing down of aqueous flow.

Briefing these phenomena, it may be said that the hydraulic movement of aqueous flow has slackened due to a fall in the ocular tension. In other words, it appears that the production of aqueous humor has been curtailed.

In dynamic chemical studies of Cl^-, K^+, HCO_3^- and glucose, Cl^-, K^+, and HCO_3^-, behaving about the same, all show the most pronounced change at the period between 15 and 60 minutes after intravenous injection of Diamox. This seems to be a part of the general ion changes.

According to KINSEY or BECKER, HCO_3^- plays the rôle of a pilot in the production of aqueous humor, but we can not quite agree to such an interpretation from the following standpoints: 1) HCO_3^- or total CO^2 concentration contained in aqueous humor of man, cattle (Salit), and horse

(Duke-Elder) is lesser than either one of these in the serum or plasm; 2) as will be mentioned later, the distribution of cytochrome oxidase in the eye tissues is not necessarily specific to the ciliary body epithelium; 3) the reaction, $OH^- + CO_2 \rightarrow HCO_3^-$, is almost negligible unless pH is over 11; and 4) because the eye tissue contains a large quantity of carbonic anhydrase, the reaction $CO_2 + H_2O \rightarrow H_2CO_3$ readily takes place without the presence of activated oxygen.

However, it is may be said that the inhibitory action of Diamox on carbonic anhydrase induces changes in the HCO_3^--concentration, but it is not right to attribute specificity only to the eye tissues, hence it will be more proper to consider that whether in the eye tissues or in blood the same mechanism inhibits the HCO_3^--production. When these fluctuations in the ions concentration are viewed from the standpoint of time, all show W-type of fluctuations. This seems to be due to the fact that at the time when Diamox chases various ions out of the tissue, migration of ionizing water is started, thus bringing about a transient hemohydraulic state; and that in the next stage the tissue fluid as well as blood will become concentrated. In any case the phase-contrast differences between the change of water content and those ions seem to be the cause for such complex variations. Variations in glucose content seem to be not so much associated with the fall in ocular tension.

At present the concept of active transport in the production of aqueous humor is widely accepted despite various interpretations of the concept. Nevertheless, a common point in the concept is an assumption that a part of aqueous humor is produced by consuming the energy emanating from the so-called metabolism. From this point of view, relationships between Diamox on one hand and O^2-uptake, TCA cycle, and cytochrome oxidase on the other, have been studied, but no effect of Diamox at all can be observed.

In short, it is true that with the fall in the ocular tension the aqueous flow slows down and that as for the background for these, changes in concentrations of HCO_3^-, Cl^- and K^+ take place, but the mechanism for these does not coincide with what BECKER contends. Moreover, Diamox seems to have nothing whatsoever to do with the mechanism in the production of a portion of aqueous humor by the energy consumption.

Total proteins in blood are decreased by Diamox for the first 15 minutes, but later they tend to increase with the lapse of time; and judging these phenomena along with the results of paper electrophoresis, it would be more reasonable to assume that these fluctuations are dependent upon the changes in water metabolism rather than upon the changes of

protein metabolism. The specific gravity of aqueous humor, though slightly, is increased, indicating an increase in the aqueous humor protein. As the result these phenomena seem to appear not due to the activation of permeability through the blood-aqueous barrier but rather due to the retention of aqueous humor. What is more, these phenomena are conclusive proof that the curtailment in the aqueous production is not caused by the weakening of the barrier permeability. The variations of pH concentration, endorsing the fluctuation of bicarbonate ions, bring about a transient acidosis. Moreover, the specific gravity, being determined as an aid in knowing changes in water content, clearly demonstrated its variations.

From these foregoing findings, the fall in ocular tension is definite, and the rate of hydroaulic movement is diminished. However, the source of the energy consumed by active transport of aqueous humor is not affected by Diamox nor the permeability in blood vessels.

In the turnover method of potassium ions, apparently the blood-aqueous barrier is affected, but this phenomenon seems to be not an essential interferance of the barrier. Carbonic anhydrase is inhibited by Diamox so that the production of HCO_3^- is curtailed; and again ion are dispatched from the kidneys. It is then easily conceivable that ions concentration is altered in order to maintain ion equilibrium so that anions emigrate from tissue. Of course, we believe, H_2O, which is bipolar molecule, adjusted itself secondarily in consonant with these alterations in the ion concentration, is deployed out of the tissue into blood and then it is again expelled out of the kidneys. Such a movement has a certain gradient having the kidneys as its center, and the eye is also included in the current of that extensive gradient. Furthermore, the osmotic pressure gradient would be higher than that of tissue fluid and aqueous humor from the standpoint of the osmotic pressure. When potassium ions are determined by the turnover method, they are not at all increased in aqueous humor. This seems to be not due to the inhibitory action of the so-called barrier but rather due to the inability on the part of potassium ions to overcome the ion current to penetrate into aqueous humor.

We have drawn the above assumptions from the determinations of the specific gravity of blood and aqueous humor because it has been proven to be extremely difficult to determine the variation of water content.

CONCLUSIONS

In our studies on the hypotensive effect of Diamox by intravenous

injection, we have arrived at the following conclusions.

1. Ocular tension falls and the flow of aqueous humor becomes sluggish.

2. Diamox inhibits the activity of carbonic anhydrase, and the concentrations of HCO_3^-, K^+, Cl^- and glucose are markedly altered.

3. Protein increases both in blood and aqueous humor, but no change in protein fraction can be observed in blood.

4. Diamox in no way affects the metabolism.

5. It seems that Diamox brings about the change in the specific gravity of blood, making the latter either more diluted or more concentrated.

From these, we conclude that the mechanism of the loweing of ocular tension by Diamox seems to lie in the fact that it inhibits the activity of carbonic anhydrase, and that consequent alteration in the concentrations of HCO_3^- and other ions accompanied by the change in osmotic pressure as well as a slight decrease of water in tissue all bring about the fall in the ocular tension. However, Diamox seems to have nothing to do with aqueous humor in so far as active transport or permeability are concerned.

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