COMMUNICATIONS

ACTION OF DIAMOX AND AMMONIUM CHLORIDE ON FORMATION OF AQUEOUS HUMOUR*

BY

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THE investigation was undertaken to establish the cause of the decrease in intra-ocular pressure induced in normal rabbits by 2-acetyl-amino-1, 3, 4 thiadiazole 5-sulphonamide†, an inhibitor of the enzyme carbonic anhydrase. At the same time, it was hoped that a comparison of these results with the known ability of Diamox to reduce the intra-ocular pressure in glaucomatous patients for extensive periods would help in assessing whether the intra-ocular dynamics of normal and pathological eyes are affected in a similar manner and to a comparable extent.

The lowering of the intra-ocular pressure by Diamox has led to the suggestion that the enzyme carbonic anhydrase is essential to the formation of the aqueous humour, and this idea has found expression in the hypothesis that the reduction of the intra-ocular pressure by Diamox is due to an inhibition of secretion across the blood-aqueous barrier (Becker, 1955a; Friedenwald, 1955a, b). This view had its origin in the studies of Friedenwald and Stiehler (1938) on the transfer of acidic and basic dyes across the epithelial layers of the ciliary processes. Friedenwald (1949) suggested that hydroxyl ions were secreted into the aqueous humour, and that an excess of bicarbonate was produced from the reaction of the hydroxyl ions with carbonic acid. This view was supported by Kinsey (1950), who observed in rabbits that the concentrations of hydroxyl and bicarbonate ions in the aqueous humour exceeded those in the plasma. The participation of carbonic anhydrase in this secretory activity was supported by the reports of the presence of the enzyme in extracts of the iris and the ciliary processes (Wistrand, 1951; Kauth and Sommer, 1953; Green, Capper, Bocher, and Leopold, 1954; Gloster and Perkins, 1955a).

Experiments designed to test either this hypothesis or alternative explanations of the action of Diamox in reducing the intra-ocular pressure have been reported by several investigators (Becker, 1955a, b, 1956; Becker and Constant, 1955; Green, Bocher, and Leopold, 1955; Langham and Lee, 1955a, b, c). There is general agreement that the reduction in the intra-ocular pressure is accompanied by a decreased rate of formation of aqueous humour, but the means by which this is brought about is still unresolved.

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† Diamox, Lederle Laboratories.

Becker (1955a, b, 1956) and Becker and Constant (1955) reported that their experimental results were consistent with the view that Diamox lowers the intra-ocular pressure by directly inhibiting the secretion of aqueous humour and in particular the transfer of excess bicarbonate into the aqueous humour. However, evidence that the ratio of the concentrations of bicarbonate between the aqueous humour and the blood was unchanged in animals given Diamox, in spite of the absolute decrease in the concentrations, led Langham and Lee (1955a, b) to question whether the production of excess bicarbonate in the eye in fact depends on a secretory mechanism involving carbonic anhydrase.

In the present study three aspects of this problem have been considered. First, the pH and the distribution of bicarbonate ions between the blood and the aqueous humour before and after the administration of Diamox have been determined. In particular, attention has been focused on possible differences between the composition of the fluids in transient and steady-state conditions. This was undertaken in view of the rapid decrease in the intra-ocular pressure that takes place immediately after giving Diamox (Becker, 1955a; Gloster and Perkins, 1955). Similar studies were made on animals with the renal arteries tied off and on animals given ammonium chloride in view of the interesting observations of Becker (1955a) that neither the administration of ammonium chloride nor nephrectomy made animals unresponsive to Diamox. Technically, this problem involved measurements of pH by the glass electrode technique and the analysis of total carbon dioxide in small volumes of fluid. Previously published papers relevant to this question include those of Kinsey (1950), Green, Bocher, and Leopold (1955), and Becker (1955). Kinsey (1950) measured the pH of the aqueous humour and the plasma by a dye technique, and the total carbon dioxide by the manometric technique of van Slyke (1926), and reported that the pH and the total concentration of carbon dioxide in the aqueous humour exceeded that in the plasma. Green and others (1955) reported that Diamox did not affect the concentration of total carbon dioxide in either the aqueous humour or the blood, and Becker (1955b) reported that Diamox reduced the concentration of total carbon dioxide in the aqueous humour but not in the blood. It is not at all evident from these last two papers why there should be a discrepancy between the results, but it is hoped that the more detailed approach used in the present study has avoided the errors inherent in the techniques used in previous studies. In particular, care has been taken to analyse whole arterial blood rather than plasma derived from a mixed arterial-venous sample obtained by cardiac punctures.

The second aspect of the problem concerns the effect of Diamox and ammonium chloride on the rate of formation of aqueous humour. For this purpose, fluorescein has been used as the test substance, and the concentration of fluorescein in the anterior chamber has been continuously recorded by a technique described in an earlier paper (Langham and Wybar, 1954).

The use of fluorescein to measure changes in the rate of formation of the aqueous humour by observing the time taken for the dye to pass through the posterior chamber was first suggested by Linnér and Friedenwald (1954). Since then the method has been employed in several studies (Becker, 1955c; Kornblueth and Linnér, 1955; Langham and Lee, 1955a, b). That fluorescein may be used in this way has found confirmation in a separate study (Langham and Wood, 1956).

The third aspect deals with the changes in the intra-ocular pressure in animals given Diamox or ammonium chloride during the period leading to a new condition of steady-state. This has assumed an increasing importance in view of observations that the effect of Diamox on the rate of formation of aqueous humour and distribution of bicarbonate ions across the blood-aqueous barrier was minimal in conditions of steady-state.

Methods

Adult rabbits of both sexes were used. They were fed Diet 18 pellets (Associated London Flour Millers), water *ad lib.*, and hay once a week. Samples of aqueous humour were taken after instilling a 1 per cent. solution of pantocaine into the conjunctival sac. All samples of aqueous humour were tested for the presence of protein by the addition of an equal volume of a solution of 8 per cent. (w/v) trichloroacetic acid, and the results were rejected if more than the normal faint clouding was observed. Blood samples were removed from the heat-dilated median artery of the ear. The Diamox for injections was prepared as a 10 per cent. solution of the sodium salt. Intravenous injections of fluorescein were made, using a 10 per cent. solution of the sodium salt.

The rate of accumulation of fluorescein in the anterior chamber was studied in rabbits anaesthetized with urethane (1.75 g./kg. body weight). Urethane in a 25 per cent. solution was slowly injected intravenously and 15 min. later 1.0 ml. of a 10 per cent. solution of sodium fluorescein was injected intravenously into the marginal ear vein within a period of 10 sec.

The ascorbic acid was dissolved in water, partially neutralized with a solution of sodium hydroxide to a pH of 6.5 to 7.2 and made up to an isotonic concentration. Raised concentrations of ascorbic acid in the plasma were maintained by an initial intravenous injection followed by hourly intramuscular injections for 6 hrs. A period of 6 hrs is sufficient to allow a steady-state to be reached in the aqueous humour to within a few per cent. (Bárány and Langham, 1955).

Analytical.—There are both theoretical and practical reasons for using whole blood rather than plasma to determine the pH and the concentration of total carbon dioxide in the plasma. Thus whole blood may be analysed immediately, and provided the haemoglobin and water content are known, the plasma values may be derived directly from the nomograms of van Slyke and Sendroy (1928). In contrast, to obtain plasma, the blood has first to be centrifuged, which involves difficulties in maintaining the composition and pH constant. Consequently, all determinations were made on freshly withdrawn samples of arterial blood. To enable the pH and the total carbon dioxide measurements to be made on the aqueous humour of each rabbit, samples from the two eyes were withdrawn into the same syringe and the pooled sample analysed immediately. In this way it was possible to take duplicate determinations of the pH and the total carbon dioxide on each sample. The alternative method of using the aqueous humour of one eye for both analyses was rejected in view of the larger errors involved in making analyses on volumes of less than 0.15 to 0.20 ml.

The pH of the blood and the aqueous humour was estimated using a Cambridge model pH meter with a micro glass electrode and cell. Dead space in the cell was reduced to approximately 0.75 ml., and the sample was introduced from a syringe directly into the chamber through a capillary tube connexion. The cell was immersed in a saturated solution of KCl which acted as a water bath as well as a junction to the calomel half-cell.

To obtain stable pH readings on the small samples of aqueous humour, it was found imperative to dry the micro-cell and prevent any mixing of the aqueous humour in the cell with the KCl in the connecting capillary. The glass electrode cannot, however, be dried, and it is important to wash the electrode with CO₂-free glass-distilled water of pH 7.0 in view of the limited buffering power of the aqueous humour. The temperature correction factor was estimated separately (see Results). In these conditions the pH of the blood and the aqueous humour was found to remain unchanged for at least 3 min. pH readings were taken within 1 to 2 min. of removing the sample from the animal. Standard buffers of known pH were run at the beginning and end of each run of readings.

The total CO₂ of the blood and the aqueous humour was determined by the manometric technique of van Slyke and Neill (1924). To derive the concentration of total CO₂ in the plasma, use was made of the nomogram compiled by van Slyke and Sendroy (1928); this relates pH to the oxygen capacity of the blood and gives the factor by which the CO₂ content of whole blood may be converted to plasma CO₂. For these calculations the oxygen capacity of rabbit blood was determined separately on a group of six animals, and a mean value of 19.1 ± 0.3 ml./100 ml. was recorded. The conversion factor changes less than 1 per cent. for a change in the oxygen capacity of from 19 to 20 vols per cent.

The concentrations of bicarbonate and CO₂ were calculated from the total CO₂ concentration and the blood pH by using the Henderson-Hasselbach equation. In this equation the pK for the bicarbonate system in blood was taken as 6.10 and in aqueous humour as 6.13. The latter figure is that given by Warburg (1922) for a protein-free bicarbonate solution containing 150 m. moles NaCl per kg. H₂O, which is an equivalent ionic concentration to the aqueous humour.

The CO₂ tension in the blood and the aqueous humour was calculated from the CO₂ concentration; $\text{mM}(\text{H}_2\text{CO}_3) = \frac{1,000\alpha\text{CO}_2}{22.4} \times \frac{p\text{CO}_2}{760}$ where αCO_2 represents the ml. CO₂

dissolved per g. H₂O at 760 mm., and $p\text{CO}_2$ represents the tension in mm. Hg. The value of CO₂ substituted in the above equation was 0.533 (van Slyke, Sendroy, Hastings, and Neill, 1928); the equation simplifies to $\text{mMH}_2\text{CO}_3/\text{kg. H}_2\text{O} = 0.0315p\text{CO}_2$.

The mean water content of the aqueous humour was taken as 99 per cent., and in a series of ten determinations the water content of whole blood was found to be 82.3 ± 0.30 per cent. Direct determination of the concentrations of total carbon dioxide in the plasma agreed with the values derived from the analysis of whole blood.

Ascorbic acid in samples of aqueous humour were analysed immediately after withdrawal by the procedure previously described (Bárány and Langham, 1955).

The concentration of sodium in the aqueous humour and the plasma was determined by the standard flame photometric technique by the procedure previously described (Langham and Taylor, 1956).

Measurement of the Intra-Ocular and Blood Pressures.—Rabbits were anaesthetized by the intraperitoneal injection of a 25 per cent. solution of urethane (1.75 g./kg.). The femoral artery on one side was cannulated for the blood pressure record. The intra-ocular pressure was measured by the technique of Greaves and Perkins (1952).

Measurement of the intra-ocular pressure in conscious animals was made by a tonometric technique using a standard Schiötz X tonometer. The animal was placed in a canvas bag tied around the neck to restrain undue movement and one drop of pantocaine (1 per cent.) was placed in each eye. This was washed off almost immediately with saline to prevent corneal drying. Two readings were recorded on each eye.

Results

Chemical Studies.—The *pH* of the blood and the aqueous humour were measured at room temperature and the values corrected to body temperature. Rosenthal (1948) determined the *pH*-temperature coefficient for whole rabbit blood and reported a value of 0.0147 ± 0.00020 *pH* units per °C.; von Sallman and di Grandi (1946) reported the *pH* of the aqueous humour of 24 rabbits to be 7.53 at 36.5°C. and the *pH* of the aqueous humour of ten rabbits to be 7.63 at 23°C. In the present study the *pH*-temperature coefficients of freshly-drawn arterial blood and of the aqueous humour of twelve conscious rabbits were determined. The mean value for blood was 0.0147 ± 0.00020 *pH* units per °C. and for the aqueous humour it was 0.0064 ± 0.00025 *pH* units per °C. Throughout the temperature range studied, namely 15 to 37.5°C., the temperature coefficients for blood and for aqueous humour were found to be constant and the *pH* effect fully reversible. In the following results the *pH* of the blood and the aqueous humour have been corrected by the use of these factors; the mean temperature of the aqueous humour has been taken as 36.5°C. (von Sallman and di Grandi, 1946).

The mean *pH*, CO₂ tension, and concentrations of total CO₂ and bicarbonate in the arterial plasma and aqueous humour of conscious rabbits are recorded in Table I. The concentrations of bicarbonate and hydroxyl ions in the aqueous humour exceeded those in the arterial plasma and are in general agreement with the observations of Kinsey (1950). In addition, the calculated value for the CO₂ tension in the aqueous humour agreed with the mean value of 35.1 mm. Hg reported by Pierce, Friedenwald, and Freeman (1933). These workers introduced a bubble of nitrogen into the anterior chamber of dogs, and analysed the CO₂ content in samples of the bubble after increasing time intervals. The observation that the CO₂ tension in the blood and the aqueous humour are approximately equal was to be expected in view of the relatively small molecular size, the lipid solubility of carbon dioxide, and the ease with which lipid-soluble molecules penetrate the blood aqueous barrier (Ross, 1951; Langham, 1955a).

The effect of Diamox on the composition of arterial blood and aqueous

TABLE I
COMPOSITION OF ARTERIAL PLASMA AND AQUEOUS HUMOUR OF NORMAL CONSCIOUS RABBITS

Plasma values calculated from analysis on arterial blood
Concentrations expressed in mM/kg.H₂O and CO₂ tension in mm.Hg

	Plasma	Aqueous Humour	Aq-Pl	Aq/Pl
<i>pH</i>	7.43 ± 0.012 (12)	7.60 ± 0.020 (12)	0.17 ± 0.02	—
H ⁺	3.71×10^{-8}	2.51×10^{-8}	-1.20×10^{-8}	0.68
Total CO ₂	24.2 ± 1.05 (12)	31.6 ± 1.19 (12)	7.4 ± 0.96	1.31
HCO ₃ ⁻	23.1 ± 1.07 (12)	30.5 ± 1.20 (12)	7.4 ± 0.90	1.32
<i>p</i> CO ₂	35 ± 1.2 (12)	35 ± 1.2 (12)	0.0 ± 0.8	1.00

humour was studied approximately 15 and 150 minutes after an intravenous injection. The time interval of 15 minutes was chosen in view of the known ability of Diamox given intravenously to cause a significant reduction in the intra-ocular pressure within this time (Gloster and Perkins, 1955b). The period of 150 minutes was based on preliminary experiments which showed that the fall in the pH and in the concentration of bicarbonate in the aqueous humour and the blood after the administration of Diamox approached to within 10–20 per cent. of the steady-state values in 1 to 2 hrs. Fig. 1 shows the concentration of total carbon dioxide in the arterial blood of individual rabbits given repeated doses of Diamox. It was found that the concentration of total carbon dioxide in the plasma decreased by approximately 6 mM/kg. H_2O in 30 to 60 minutes.

FIG. 1.—Concentration of bicarbonate in arterial plasma of conscious rabbits given Diamox intravenously (50 mg./kg. initially followed by 25 mg./kg. hrly) and ammonium chloride orally (270 mg./kg.).

The composition of the plasma and aqueous humour of conscious rabbits given an intravenous injection of Diamox 15 minutes previously are recorded in Tables II and III (opposite). The pH of the blood was significantly below that in the control series but the concentration of bicarbonate remained unchanged. The calculated value of pCO_2 in the blood indicated a significant increase. A similar elevation in the CO_2 tension and a fall in the pH of arterial blood in human subjects 30 to 60 minutes after the oral administration of Diamox in doses of 25 mg./kg. has been reported by Cranston, Sanderson, and Stapleton (1955). Further indications that Diamox can cause a temporary elevation of arterial CO_2 tension is to be seen in the experimental results in dogs of Maren, Wadsworth, Yale, and Alonso (1954).

The pH in the aqueous humour was also lower than in the control series

TABLE II
EFFECT OF DIAMOX (50 mg./kg. BODY WT.) ON COMPOSITION OF
ARTERIAL PLASMA AND AQUEOUS HUMOUR
Samples were removed 15 min. after intravenous injection of Diamox

	Plasma	Aqueous Humour	Aq-Pl	Aq/Pl
pH	7.34±0.013 (12)	7.45±0.020 (12)	0.11±0.02 (7)	—
H ⁺	4.57×10 ⁻⁸	3.55×10 ⁻⁸	-1.02×10 ⁻⁸	0.77
Total CO ₂	25.3±0.58 (12)	26.0±0.50 (11)	0.70±0.55 (7)	1.03
HCO ₃ ⁻	23.9±0.63 (12)	24.8±0.51 (11)	0.80±0.47 (7)	1.03
pCO ₂	44±1.8 (12)	38±1.9 (11)	-6±2.35 (7)	0.86

TABLE III
EFFECT OF 20 mg. DIAMOX/kg. BODY WT. ON COMPOSITION OF
ARTERIAL PLASMA AND AQUEOUS HUMOUR OF CONSCIOUS RABBITS
Samples withdrawn 15 minutes after intravenous injection of Diamox

	Plasma	Aqueous Humour	Aq-Pl	Aq/Pl
pH	7.33±0.014 (9)	7.51±0.025 (9)	0.18±0.023	—
H ⁺	4.68×10 ⁻⁸	3.10×10 ⁻⁸	-1.58×10 ⁻⁸	0.66
Total CO ₂	26.2±1.01 (9)	28.5±1.60 (9)	2.3±1.30	1.09
HCO ₃ ⁻	24.7±1.02 (9)	27.4±1.57 (9)	2.3±1.77	1.11
pCO ₂	46±2.5	35±1.3	-11±3.6	0.76

but it remained significantly above that in the blood. The $p\text{CO}_2$ was not significantly above that in the control series, but the differences in the values of the aqueous humour and blood of individual rabbits showed a greater scatter than in the control series. In general the $p\text{CO}_2$ in the blood was higher than in the aqueous humour. The concentration of bicarbonate, unlike that in the blood, was significantly lower than in the control series and closely approached that in the plasma. Thus, the excess concentration of bicarbonate in the aqueous humour of the two series of rabbits given Diamox dropped nearly to zero within 15 minutes.

The composition of the arterial blood and aqueous humour of conscious rabbits 2.5 hrs after an initial intravenous injection of Diamox are recorded in Tables IV and V (overleaf). Two series of experiments were made using different dosages of Diamox to see whether the effect varied appreciably with the amount of drug given. In the first series an initial injection of 20 mg./kg. followed by 10 mg./kg. at hourly intervals was administered, and in the second series the dosage was increased five times. In spite of this difference the response in the two series appeared to be very similar. The dosage of 100 mg./kg. is at least twenty times that which will induce an appreciable fall in the intra-ocular pressure of rabbits (Gloster and Perkins, 1955b). The pH of the blood was similar to that measured 15 minutes after giving Diamox, but the concentration of bicarbonate in the plasma showed a significant decrease. The $p\text{CO}_2$ was similar to that in normal rabbits and in this respect differed from the elevated values shown in Tables II and III. Thus the increase of the $p\text{CO}_2$ was only a transient effect seen in the period immediately after Diamox was given.

TABLE IV
EFFECT OF DIAMOX GIVEN INTRAVENOUSLY 2.5 HRS PREVIOUSLY TO
CONSCIOUS RABBITS

Initial intravenous dose of 20 mg./kg. body wt. followed by 10 mg./kg. body wt. 1 and 2 hrs later

	Plasma	Aqueous Humour	Aq-Pl	Aq/Pl
pH	7.34 ± 0.022 (8)	7.44 ± 0.018 (8)	0.10 ± 0.03	—
H ⁺	4.57×10^{-8}	3.63×10^{-8}	-2.31×10^{-8}	0.79
Total CO ₂	19.2 ± 0.88 (8)	25.3 ± 0.95 (8)	6.1 ± 0.30	1.32
HCO ₃ ⁻	18.2 ± 0.87 (8)	24.2 ± 0.92 (8)	6.0 ± 0.26	1.33
pCO ₂	34 ± 1.3	35 ± 1.7	1 ± 2.1	1.03

TABLE V
EFFECT OF DIAMOX ON COMPOSITION OF ARTERIAL PLASMA AND
AQUEOUS HUMOUR

Diamox 100 mg./kg. given intravenously followed by 50 mg./kg. after 60 and 120 minutes.
Samples removed at 2.5 hrs

	Arterial Plasma	Aqueous Humour	Aq-Pl	Aq/Pl
pH	7.36 ± 0.01 (12)	7.50 ± 0.02 (11)	0.13 ± 0.03	—
H ⁺	4.36×10^{-8}	3.16×10^{-8}	-1.20×10^{-8}	0.72
Total CO ₂	17.6 ± 0.50 (12)	23.1 ± 0.30 (10)	5.4 ± 0.46	1.31
HCO ₃ ⁻	16.7 ± 0.50 (12)	22.2 ± 0.30 (10)	5.5 ± 0.38	1.33
pCO ₂	29 ± 1.1 (12)	29 ± 1.2 (10)	0 ± 1.9	1.0

The pH of the aqueous humour was similar to that observed 15 minutes after the administration of Diamox, and again exceeded that in the plasma. The concentration of bicarbonate in the aqueous humour also showed no change compared with the value after 15 minutes. However, owing to the decrease in the concentration of bicarbonate in the plasma, there was an excess concentration of bicarbonate in the aqueous humour similar to that in untreated animals.

The concentrations of total carbon dioxide in the aqueous humour and plasma after the administration of Diamox have been reported by Green, Bocher, and Leopold (1955) and by Becker (1955b). Unfortunately these authors' results do not agree with the present results. Green and others (1955) reported that Diamox caused no change in the concentrations of total CO₂ in the aqueous humour or plasma of rabbits. No measurements of pH were taken, but it is evident from their results that many of their animals were in varying stages of acidosis before the start of the experiments, for over half of the animals injected with Diamox had an initial concentration of total CO₂ in the plasma of less than 20 mM/l. This contrasts with the values of 24.2 ± 1.05 (12) mM/kg. H₂O in the plasma of normal rabbits having a pH of 7.43 ± 0.012 (12). Their method of analysis is also open to criticism (Grant, 1956). In an agreement with the present observation, Becker (1955b) found that Diamox decreased the concentration of bicarbonate in the aqueous humour. However, Becker also reported that Diamox

had no significant effect on the concentration of bicarbonate in the plasma. This is difficult to understand, in that it is widely recognized that Diamox, even in a very low dosage, will cause an excretion of bicarbonate into the urine and a consequent decrease in the concentration of bicarbonate in the blood. The explanation may, however, lie in the method of analysis. The samples of plasma analysed by Becker were taken from the heart and would therefore comprise a variable mixture of arterial and venous blood. In comparison, the present analyses were made immediately on samples of arterial whole blood freshly drawn from the heat-dilated median artery of the ear.

Effect of Diamox on the Composition of the Blood and the Aqueous Humour of Rabbits in which Kidney Function has been Eliminated.—In the first series of rabbits, the blood and aqueous humour of untreated animals were analysed 3 hours after the operation; in the second series 50 mg. Diamox/kg. was injected intravenously 30 minutes after the operation and samples of aqueous humour and arterial blood were taken 2.5 hours later. The results in the untreated animals are recorded in Table VI. The pH and the concentration of bicarbonate in the blood and aqueous humour were significantly below those in normal animals. The concentration of bicarbonate in the aqueous humour of individual rabbits was, however, significantly above that in the blood and similar in value to that in normal rabbits.

TABLE VI

COMPOSITION OF ARTERIAL PLASMA AND AQUEOUS HUMOUR OF ANIMALS
3 HRS AFTER LIGATION OF RENAL ARTERIES

	Plasma	Aqueous Humour	Aq-Pl	Aq/Pl
pH	7.29±0.02 (5)	7.39±0.02 (5)	0.10±0.02	—
H ⁺	5.13×10 ⁻⁸	4.26×10 ⁻⁸	-1.13×10 ⁻⁸	0.83
Total CO ₂	20.0±1.15 (5)	26.6±0.35 (5)	6.6±1.25	1.33
HCO ₃	18.8±1.06 (5)	25.2±0.35 (5)	6.4±1.18	1.34
pCO ₂	38±3.5	44±0.7	6±3.4	1.16

The effect of Diamox on the operated rabbits is recorded in Table VII. The pH of the blood and aqueous humour dropped below the mean values recorded in the untreated series, and calculation of the CO₂ tension showed this to increase to high values compared with the tensions in untreated

TABLE VII

EFFECT OF DIAMOX (50 mg./kg.) ON COMPOSITION OF ARTERIAL PLASMA AND
AQUEOUS HUMOUR OF RABBITS 3 HRS AFTER LIGATION OF RENAL ARTERIES

Diamox given intravenously 30 min. after ligation of renal arteries

	Plasma	Aqueous Humour	Aq-Pl	Aq/Pl
pH	7.10±0.05 (5)	7.25±0.03 (4)	0.17±0.06	—
H ⁺	7.94±10 ⁻⁸	5.62×10 ⁻⁸	-2.32×10 ⁻⁸	0.71
Total CO ₂	25.1±1.9 (5)	29.4±2.0 (4)	5.2±1.0	1.22
HCO ₃	22.8±1.8 (5)	27.4±2.0 (4)	6.0±0.83	1.28
pCO ₂	73±4.1	64±1.5	-7±5.9	0.88

rabbits. The concentrations of bicarbonate in the blood and aqueous humour were higher than in the untreated animals. In individual animals, the concentration of bicarbonate in the aqueous humour again exceeded that in the arterial plasma by an amount similar to that seen in the control and the normal series of rabbits.

The condition of acidosis observed in these animals is at variance with the observations of Becker (1955b). He reported that the concentrations of total carbon dioxide in the plasma and the aqueous humour of rabbits nephrectomized under pentobarbital anaesthesia 18 to 24 hours previously did not differ from those in unoperated animals. In the present study the decision to use animals 3 rather than 24 hours after the operation was prompted by the desire to keep pathological changes to a minimum. Thus at 24 hours the blood pressure and the intra-ocular pressure are below normal and the blood-aqueous barrier abnormally permeable to fluorescein. In view of Becker's observations, however, samples of the aqueous humour and blood of animals nephrectomized 24 hours previously were also made. The results did not differ significantly from those recorded in Tables VI and VII. In three control rabbits the mean concentrations of total carbon dioxide in the plasma and in the aqueous humour were 17.5 and 24.5 mM/kg. H₂O respectively, and in the animals given Diamox (50 mg./kg. 2.5 hrs previously) the corresponding values were 16.5 and 21.0 mM/kg. H₂O.

Chemical analysis of the blood and aqueous humour of rabbits after the administration of ammonium chloride was made in order to help interpret the observations of Becker (1955a) that Diamox lowers the intra-ocular pressure of animals pre-treated with high doses of ammonium chloride by mouth. A similar amount of ammonium chloride (270 mg./kg.) to that used by Becker and recommended by Maren and Wadsworth (1954) to block the renal effect of Diamox, was given to conscious rabbits. Repeated analysis of the concentration of total CO₂ in the blood showed a fall in concentration to approximately 16 mM/kg. H₂O within 60 minutes and little further change in the following 2 hours.

The composition of the plasma and aqueous humour of conscious rabbits 2.5 hours after the oral administration of 270 mg. NH₄Cl/kg. are recorded in Table VIII. The concentration of bicarbonate in the aqueous humour of individual animals exceeded that in the arterial plasma, but the excess

TABLE VIII
ANIMALS GIVEN 270 mg. AMMONIUM CHLORIDE/kg. BY STOMACH TUBE
2.5 HRS EARLIER

	Plasma	Aqueous Humour	Aq-Pl	Aq/Pl
pH	7.14±0.02 (12)	7.29±0.02 (12)	0.15±0.29	—
H ⁺	7.25×10 ⁻⁸	5.12×10 ⁻⁸	-2.13×10 ⁻⁸	0.70
Total CO ₂	18.7±0.84 (12)	21.5±0.82 (12)	2.8±0.90	1.15
HCO ₃	17.1±0.76 (12)	20.1±1.83 (12)	3.0±0.86	1.18
pCO ₂	51±1.9	44±2.8	-7±2.9	0.86

was considerably less than in untreated animals. The *pH* and the concentrations of bicarbonate in the plasma and the aqueous humour were well below those in untreated animals, and the calculated value for the CO_2 tension exceeded that in normal animals. The increased acidity was probably caused mainly by the acid nature of ammonium chloride and differs in this respect from the more prolonged action of ammonium chloride in producing a hyperchloraemia and a fully compensated type of metabolic acidosis (Haldane, 1921; Haldane, Linder, Hilton, and Fraser, 1928).

The composition of the plasma and aqueous humour of animals given ammonium chloride 270 mg./kg. by mouth followed by Diamox intravenously is recorded in Table IX. The concentration of bicarbonate in the aqueous humour exceeded that in the arterial plasma by an amount similar to that seen in the previous series of experiments; the *pH* of the blood and aqueous humour appeared to be slightly higher than in the previous series, and the bicarbonate concentrations were essentially unchanged. The calculated value for the CO_2 tension in the blood and aqueous humour showed them to be similar to those in normal rabbits.

TABLE IX

ANIMALS GIVEN 270 mg. AMMONIUM CHLORIDE/kg., 50 mg. DIAMOX/kg. BEING INJECTED INTRAVENOUSLY AFTER 1.5 HRS AND A FURTHER 25 mg./kg. AFTER 1 HR

Samples removed 3.5 hrs after administration of ammonium chloride

	Plasma	Aqueous Humour	Aq-Pl	Aq/Pl
<i>pH</i>	7.22 ± 0.015 (12)	7.40 ± 0.072 (12)	0.18 ± 0.02	—
H^+	6.04×10^{-8}	3.98×10^{-8}	-2.06×10^{-8}	0.66
Total CO_2	17.5 ± 0.85 (12)	20.3 ± 0.84 (12)	2.8 ± 0.93	1.16
HCO_3	16.3 ± 0.81 (12)	19.3 ± 0.82 (12)	3.0 ± 0.92	1.18
pCO_2	38 ± 1.6	32 ± 0.9	-6 ± 3.4	0.84

Effect of Diamox on the Concentration of Sodium in the Blood and Aqueous Humour.—In view of the observations that Diamox caused a decrease in the concentration of bicarbonate in the aqueous humour of approximately 6 mEq/kg. H_2O , studies were made to see if a corresponding decrease occurred in the concentration of the main cation, sodium. In ten control rabbits the mean concentration of sodium in the first eye was 135.6 ± 0.99 mEq/kg. H_2O , and in the second eye, removed 15 min. later, it was 135.9 ± 1.02 mEq/kg. H_2O . In the same series the mean difference in the concentration of sodium between the second and first eyes of individual rabbits was 1.0 ± 0.21 mEq/kg. H_2O .

The effect of Diamox on the concentration of sodium in the aqueous humour and arterial plasma of four conscious rabbits given Diamox is recorded in Table X (overleaf). In two animals the concentration of sodium in the plasma and aqueous humour showed an increase, while in the other two the changes were not significant.

TABLE X
CONCENTRATION OF SODIUM IN PLASMA AND AQUEOUS HUMOUR OF CONSCIOUS RABBITS BEFORE (Pl_1 AND Aq_1) AND 20 MINUTES AFTER (Pl_2 AND Aq_2) INTRAVENOUS INJECTION OF DIAMOX (50 mg./kg.)
Results expressed as mEq/kg. H_2O

Pl_1	Pl_2	Aq_1	Aq_2	Aq_1/Pl_1	Aq_2/Pl_2
151	155	136	139	0.89	0.90
144	145	137	136	0.95	0.94
149	151	136	134	0.91	0.89
151	156	139	144	0.92	0.92

In separate experiments the concentrations of sodium in the blood and aqueous humour of untreated rabbits were compared with analyses made on conscious rabbits given Diamox (50 mg./kg.) intravenously 2.5 hours previously. The results, summarized in Table XI, showed that there was no significant change in the concentration of sodium in the plasma or aqueous humour.

TABLE XI
EFFECT OF DIAMOX ON CONCENTRATION OF SODIUM IN ARTERIAL PLASMA AND AQUEOUS HUMOUR OF CONSCIOUS RABBITS
Series A represents a control group of rabbits.
Animals in Series B were given 50 mg. Diamox/kg. intravenously and a further 25 mg./kg. 1 and 2 hrs later.

Samples withdrawn 2.5 hrs after initial injection

Series	Plasma	Right Aqueous	Left Aqueous	Aq/Pl
A	143.6 ± 1.64 (10)	135.6 ± 0.99 (10)	135.9 ± 1.02 (10)	0.945 ± 0.01 (10)
B	144.9 ± 1.30 (10)	136.1 ± 1.18 (10)	136.8 ± 1.33 (10)	0.945 ± 0.01 (10)

Effect of Diamox and Ammonium Chloride on the Transfer of Ascorbic Acid across the Blood-Aqueous Barrier.—In view of the observation that in certain respects ammonium chloride affects the formation and composition of the aqueous humour in a similar way to Diamox, it was considered of value to compare its action with that of Diamox on the accumulation in the aqueous humour of a compound known to be actively transported across the blood-aqueous barrier, namely, ascorbic acid. These results have been reported in a preliminary communication to the Physiological Society (Langham and Lee, 1955c).

In rabbits, guinea-pigs, and humans, it has been shown that a rise in the concentration of ascorbic acid in the plasma leads to an increase in the concentration in the aqueous humour, even though the raised plasma level may be considerably below that of the aqueous humour (Kinsey, 1947; Langham, 1950; Linnér, 1952; Bárány and Langham, 1955). In rabbits the secretion of ascorbic acid into the aqueous humour reaches a maximum at a plasma concentration of 2–3 mg./100 ml. and gives a mean concentra-

tion of 67.5 ± 2.1 mg./100 ml. in the aqueous humour (Bárány and Langham, 1955).

The effect of Diamox and ammonium chloride on the secretion of ascorbic acid into the aqueous humour of conscious rabbits is recorded in Table XII. The concentration of ascorbic acid in the plasma of these animals was kept at a mean value of 5–10 mg./100 ml., which well exceeds the level of 2–3 mg./100 ml. required to ensure a maximal rate of transfer in normal rabbits. This was done to exclude a possible vasomotor action of Diamox in influencing the rate of secretion of ascorbic acid; thus the rate of transfer of ascorbic acid may be varied by a change in uveal blood flow (Linnér, 1952; Langham, 1955b). The aqueous humour from one eye was removed 3 hours after the initial injection of ascorbic acid. At this time the concentration of ascorbic acid in the aqueous humour had approached the maximum steady-state value of approximately 70 mg./100 ml. (Table XII). Then Diamox or ammonium chloride was given to the two experimental series of animals, and the aqueous humour in the second eye was removed 3 hours later. The results showed that the secretion of ascorbic acid had been maintained in these conditions. The analyses of Becker (1956) of the concentration of ascorbic acid in the aqueous humour of nephrectomized rabbits after giving Diamox are similar to those recorded here.

TABLE XII

EFFECT OF DIAMOX (50 mg./kg. FOLLOWED BY 25 mg./kg. AT HOURLY INTERVALS) AND AMMONIUM CHLORIDE (270 mg./kg.) ON CONCENTRATION OF ASCORBIC ACID (mg./100 ml.) IN AQUEOUS HUMOUR OF CONSCIOUS RABBITS

Ammonium chloride (orally) and Diamox (intravenously) were given after removal of A_{q1} . All animals were given an initial intravenous injection of a solution of sodium ascorbate (pH 7.0), followed by intramuscular injections hourly to maintain the concentration of ascorbic acid in the plasma at 5–10 mg./100 ml.

Series	A_{q1} (3 hrs)	A_{q2} (6 hrs)	$A_{q2}-A_{q1}$
Controls	62.5 ± 2.6 (8)	69.9 ± 3.2 (8)	7.4 ± 0.9
NH_4Cl	61.3 ± 2.4 (8)	76.0 ± 2.6 (8)	13.7 ± 1.5
Diamox	65.3 ± 1.6 (8)	83.3 ± 2.1 (8)	18.0 ± 2.1

Effect of Diamox and Ammonium Chloride on Transfer of Fluorescein from the Bloodstream into the Anterior Chamber.—The concentrations of fluorescein in three areas of the anterior chamber after an intravenous injection of a solution of sodium fluorescein are shown in Fig. 2 (overleaf). It took about 4 minutes for fluorescein to pass in a significant amount into the anterior chamber but then the concentration in front of the lens started to increase rapidly. The concentrations of fluorescein in the central and corneal areas of the anterior chamber lagged behind that in the lens area for approximately 30 minutes. In the period of approximately 4 minutes before the dye was recorded in a significant concentration in the anterior chamber, the aqueous humour, illuminated by the focal vertical beam of light from the slit lamp, changed from black to a faint greyish and then just perceptibly

green colour. This was, however, too little to record on the fluorophotometer and was probably due to the occasional streamline of fluorescein which passes through the pupil (see Friedenwald and Becker, 1955).

FIG. 2.—Concentration of fluorescein in three areas of aqueous humour after intravenous injection of 1.0 ml. of a 10 per cent. solution of sodium fluorescein to an anaesthetized rabbit.

In a series of ten anaesthetized rabbits the mean time taken for fluorescein to appear in the area of the anterior chamber immediately in front of the lens was 248 ± 26 seconds. This value, which is based on the curve of accumulation, exceeds those reported by Linnér and Friedenwald (1954) and Kornblueth and Linnér (1955), who recorded the times at which a green colour was first perceptible at the pupillary margin.

The ability of Diamox to modify the appearance time of fluorescein was studied in rabbits 15, 60, 180, and 360 minutes after an intravenous injection. The results are recorded in Table XIII. At 15 and 60 minutes after the administration of Diamox, the appearance times were significantly above the mean value of the control series, but at 180 and 360 minutes the values did not exceed the controls, even though Diamox had been administered at hourly intervals.

TABLE XIII

TIME TAKEN FOR FLUORESCIN TO PASS FROM THE BLOODSTREAM INTO THE AREA OF THE ANTERIOR CHAMBER IMMEDIATELY IN FRONT OF THE LENS

Diamox given intravenously in the stated dosage and repeated hourly at half this dosage

Series		Time (min.) after Diamox	Appearance Times (sec.)
Controls		—	248 ± 26 (10)
Diamox (mg./kg.)	50	15	328 ± 24.5 (6)
	20	15	334 ± 32 (5)
	50	60	348 ± 21 (9)
	50	180	208 ± 15 (9)
	50	360	187 ± 30 (6)

A typical result of an animal given Diamox 1 hour previously is seen in Fig. 3. This shows the increased appearance time and also that the time taken for fluorescein to become evenly distributed in the anterior chamber exceeded that in a control experiment (Fig. 2). This latter observation was emphasized by the unequal distribution of fluorescein after 30 minutes in all animals given Diamox compared with the equal distribution of fluorescein observed in the majority of untreated animals (Langham and Lee, 1955a).

FIG. 3.—Concentration of fluorescein in aqueous humour in a rabbit given 50 mg. Diamox/kg. body wt 1 hr previously. 1.0 ml. of a 10 per cent. solution of sodium fluorescein was injected intravenously.

The dosage of Diamox given to the animals well exceeded that needed to reduce the intra-ocular pressure and it is considered that the observed effects on the appearance times reflect its maximum response. It was, however, considered of interest to test whether a low dosage would influence the appearance time since Gloster and Perkins (1955b) observed a lowering of the intra-ocular pressure in animals receiving 5 mg. Diamox/kg. body weight; this is also a similar dosage to that given to patients with glaucoma. In five rabbits given 5 mg. Diamox/kg. body weight 15 minutes previously, the mean appearance time was 300 ± 25 seconds, which significantly exceeds the value in untreated animals and is similar to that given greater amounts of the drug.

The effect of Diamox on rabbits in which the renal arteries had been tied was found more difficult to assess owing to an increased permeability of the iris vessels to fluorescein and protein in some animals. Control animals were given fluorescein 2 hours after tying the renal arteries and the mean appearance time was 218 ± 40 (5) seconds, which is similar to that found in normal animals. In similar experiments, Diamox (50 mg./kg.) was given 1 hour after the operation and fluorescein 1 hour later. The appearance time in these conditions was 280 ± 31 (7) seconds. Although this value

exceeds that in the control series, it cannot be considered statistically different in view of the large standard error.

In a final series of experiments, the fluorescein appearance time of rabbits given ammonium chloride by mouth 1 hour previously was determined. The mean value was 335 ± 30 seconds and is significantly higher than the appearance time in untreated animals.

The observation that the rate of formation of the aqueous humour is decreased 1 hour after administration of Diamox agrees with the observations of Becker and Constant (1955). Using the electric tonographic method of Grant (1950) on conscious rabbits, these authors reported that the rate of formation of the aqueous humour was decreased by approximately 63 per cent. 45–60 minutes after intravenous injections of Diamox (100 mg./kg.).

Intra-Ocular Pressure Studies.—In a preliminary series of experiments, the observations of Gloster (1955) that Diamox given intravenously to rabbits reduces the intra-ocular pressure within a few minutes were fully confirmed. A typical result in which the intra-ocular and blood pressures were measured are shown in Fig. 4.

FIG. 4.—Effect of Diamox (25 mg./kg. body wt) given intravenously on intra-ocular pressure of a rabbit.

In similar experiments, Diamox was administered intravenously 2.5 hours after the administration of ammonium chloride, and again Diamox was found to reduce the intra-ocular pressure in all six animals studied. Fig. 5 records a typical result and shows that the fall in the intra-ocular pressure followed a pattern similar to that seen on animals not pre-treated with ammonium chloride. These observations confirm the findings of Becker (1955a), who measured the intra-ocular pressure of rabbits by a tonometric technique after the administration of ammonium chloride.

FIG. 5.—Effect of Diamox (25 mg./kg. body wt) given intravenously on intra-ocular pressure of a rabbit 2.5 hrs after oral administration of ammonium chloride (270 mg./kg. body wt).

The conventional Schiötz X tonometer was used to observe the influence of Diamox and ammonium chloride on the intra-ocular pressure over a period of several hours. The manometric technique could not be employed for, although it is more accurate than tonometry, it is unsuitable for recording slow changes in pressure in the eye. In a control series of experiments on anaesthetized rabbits, the intra-ocular pressure was found to fall slowly during a period of 5 hrs, and it was found increasingly difficult to obtain reproducible tonometric readings. Attempts were therefore made to record

tonometric readings on conscious rabbits. In these conditions it was found that the tonometric readings remained essentially unchanged in the two eyes during a period of 5 hours provided the application of local anaesthetic to the eye was kept to a minimum. To achieve an adequate anaesthesia of the corneal epithelium surface one drop of a 1 per cent. solution of panto-caine was instilled into the conjunctival sac and the excess was washed away with an isotonic saline solution 1 minute later. If, however, the local anaesthetic remained on the eye and readings were taken at intervals of 30 minutes there was a tendency for the intra-ocular pressure to fall and remain low. Consequently, the number of readings in these experiments was kept to a minimum.

Fig. 6 shows the relative changes in the intra-ocular pressure of conscious rabbits given Diamox. The pressure fell rapidly and averaged 2 to 3 divisions on the Schiötz scale within 30 to 60 minutes of the intravenous injection. Later, however, there was a significant recovery of the intra-ocular pressure towards its initial value in spite of further hourly injections of Diamox.

FIG. 6.—Effect of Diamox on intra-ocular pressure of conscious rabbits. Standard Schiötz X tonometer used to measure intra-ocular pressure.

Ammonium chloride given orally (270 mg./kg. body weight) to conscious rabbits also caused a decrease in the intra-ocular pressure corresponding to a change of 2 to 3 divisions on the Schiötz scale. In contrast to the effect of Diamox, the intra-ocular pressure remained low over the experimental period of 5 hrs. Attempts to confirm this hypotensive action of ammonium chloride by the manometric technique were not successful owing to its action on the blood pressure when injected intravenously.

Discussion

In assessing how Diamox influences the composition of the aqueous humour and intra-ocular dynamics several observations merit special mention. First, Diamox caused a reduction in pH and also in the concen-

trations of bicarbonate in the aqueous humour and the blood, but only a temporary reduction in the *ratio of the concentrations* of bicarbonate between the aqueous humour and the blood. Secondly, there was a rapid but only temporary decrease in the rate of flow of the aqueous humour. Thirdly, Diamox neither inhibited the secretion of ascorbic acid across the blood-aqueous barrier nor significantly affected the distribution of sodium between the two fluids. Lastly, there was a rapid and significant fall in the intra-ocular pressure followed by a partial or complete recovery. It would seem to follow that, in the continued presence of Diamox, there is little or no effect on the intra-ocular dynamics of the normal rabbit eye. At the same time, in confirmation of previous investigations, our experimental results show that Diamox can cause transient changes in the intra-ocular pressure and in the formation of the aqueous humour. For this reason, the animals employed in this study could not be considered resistant to the action of Diamox. These views are not, however, in agreement with the conclusions of Becker (1956) and Becker and Constant (1955); in this discussion the discrepancies may to some extent be resolved.

It now appears well established that in rabbits the concentrations of hydroxyl and bicarbonate ions in the aqueous humour exceed those in the plasma and that the two fluids are not in thermodynamic equilibrium in so far as the distribution of these two ions is concerned. In an ultrafiltrate of plasma, Greene and Power (1931) reported that the concentrations of hydroxyl and bicarbonate ions were approximately 4 per cent. higher than in the plasma water and in agreement with the theoretical van Slyke ratio of 1.04. In studies of the dialysis of horse serum against a solution of salt at 38° C., Hastings, Salvesen, Sendroy, and van Slyke (1927) reported ratios of 1.13 for the hydroxyl ion and 1.03 for the bicarbonate ion, and concluded that the bicarbonate ion approached more closely to the theoretical van Slyke ratio than chloride, sodium, and hydrogen ions. On this basis the excess of hydroxyl and bicarbonate ions in the aqueous humour above that consistent with passive ultrafiltration or dialysis of the plasma is 20–30 per cent. In absolute amounts the excess of bicarbonate in the aqueous humour was equal to 7mEq/kg.H₂O. This exceeds that observed for other ions and is well above that required to account for the hypertonicity of the aqueous humour which is generally agreed not to exceed 2 to 3 mMNCl/kg.H₂O. Consequently, the excess bicarbonate must be partially or completely compensated by a reciprocal deficit of another anion.

The origin of the high concentrations of hydroxyl and bicarbonate ions in the aqueous humour remains unsolved. One explanation that either or both of these ions are secreted across the stromal epithelial barrier of the ciliary process was suggested by Friedenwald, Buschke, and Michel (1943) on the the evidence of the transfer of dyes across the ciliary processes. Friedenwald (1949) postulated that the primary product of the secretory mechanism was the accumulation of hydroxyl ions in the epithelium and

hydrogen ions in the stroma, and that an excess of bicarbonate ions would accumulate on the epithelial side of the barrier through the reaction of hydroxyl ions with carbonic acid. There is, however, an alternative explanation—that the excess of these ions is dependent on the composition and metabolic activity of the corneal, lens, and retinal tissues. The ions and water constituting the aqueous humour are freely diffusible with those in the cornea across the endothelium. Therefore, in considering the nature of the aqueous humour and its thermodynamic equilibration with the plasma, the membrane-effect acting across the corneal-aqueous barrier cannot be ignored. The extracellular volume of the corneal stroma has a high concentration of indiffusible anions and the concentration of sodium exceeds that in the aqueous humour by some 20 to 30 per cent. (Langham and Taylor, 1956). This membrane-effect would tend to increase the *pH* and the concentration of all anions in the aqueous humour of the anterior chamber, above that found in an ultrafiltrate of plasma. Thus, in the presence of this factor, the anatomical complexities of the posterior and anterior chambers the presence of a constant circulation of the aqueous humour, and the low maximal concentration gradient of 1.2×10^{-8} mEq of hydroxyl ion/kg. H_2O between the plasma and the aqueous humour, it might be considered surprising if the hydroxyl ion were in complete thermodynamic equilibrium with the plasma. It is, therefore, of particular interest that Davson and Luck (1956) have shown that the distribution of bicarbonate between the aqueous humour and the plasma varies in different species and that in some animals, including man, the concentration in the aqueous humour is less than that in the plasma.

The effect of Diamox on the *pH* and on the concentration of bicarbonate in the plasma is consistent with its recognized action in increasing the *pH* and excretion of bicarbonate in the urine. The fall in the concentration of bicarbonate in the plasma of 6 to 8 mEq/kg. H_2O from an initial mean value of 24 mEq/kg. H_2O agrees well with similar observations on dogs (Maren and others, 1954) and man (Galdston, 1955). This took place with doses of Diamox of 20 mg./kg body weight and was not increased at concentrations of 100 mg./kg body weight. The main decrease in the concentration of bicarbonate in the plasma took place within the first 2 hours, although it is interesting to note that at 15 minutes the concentration had not altered significantly (compare Tables I, II, and III). This would seem to indicate that the loss of bicarbonate through the kidney had not reduced the body bicarbonate sufficiently to affect the plasma concentration during this period. It is therefore of special interest that there was in the same 15-minute period an apparent fall in the *pH* of the blood and an increase in the arterial CO_2 tension. From general physiological principles it is unlikely that Diamox could cause a decrease in the *pH* and an increase in the CO_2 tension of the blood within 15 min. Therefore these analyses could reflect changes in the *pH* of the blood at the time of withdrawal. This explanation is supported

by the calculation that the *pH* of the blood equals that in untreated animals if the CO_2 tension is assumed to equal that in the aqueous humour.

In the same initial period of 15 minutes the *pH* decreased and the CO_2 tension of the aqueous humour increased and, in addition, there was a marked decrease in the concentration of bicarbonate. This could well be due to the increased acidity, for the very low buffering power of the aqueous humour is determined almost completely by its bicarbonate content. On the other hand, it could be argued that the decrease is consistent with a hypothetical inhibitory effect of Diamox on a process of secretion of bicarbonate into the aqueous humour. This, however, appears unlikely, since the excess concentration of bicarbonate in the aqueous humour became re-established within 2 to 3 hours in spite of further injections of Diamox.

Thus, although Diamox caused a temporary disturbance of the normal distribution ratio for bicarbonate, the excess of both hydroxyl and bicarbonate ions in conditions of steady-state were proportionately the same as in normal untreated rabbits. It is therefore evident that Diamox, even though it may completely inhibit the carbonic anhydrase activity in the ciliary processes (Green, Capper, Bocher, and Leopold, 1954), does not alter the normal distribution ratios of these ions. Consequently, it is unlikely that carbonic anhydrase activity in the eye is essential to the accumulation of excess bicarbonate in the aqueous humour.

The report of Becker (1956) that the normal distribution of bicarbonate between the aqueous humour and the blood is altered by Diamox hinges on the fact that he did not detect a decrease in the concentration of bicarbonate in the plasma of rabbits given Diamox during a period of 6 hours. His results on the aqueous humour, which were determined in a similar manner to those described in this paper, are in accord with the present observations; and this strengthens our opinion that a similar agreement would have been obtained in the plasma analyses had the more detailed procedure used in this study been followed. It is to be remembered that a decrease in the concentration of bicarbonate in the plasma of animals given Diamox for several hours agrees with the observations of many workers in this field (*e.g.* Maren and others, 1954; Galdston, 1955), and indeed constitutes the accepted mode of action of Diamox in increasing the renal excretion of sodium bicarbonate (Berliner, 1951).

The analyses of the sodium content of the aqueous humour show that the marked loss of bicarbonate immediately after giving Diamox is not accompanied by an equivalent decrease in the concentration of the main cation, sodium. It would seem that there was no marked reduction in the rate of transfer of sodium into the eye either during this period or in conditions approaching steady-state. This conclusion agrees with the findings of Kinsey, Camacho, Cavanaugh, and Constant (1955), who reported that Diamox did not affect the turnover rate of sodium in the aqueous humour.

The constancy of the steady-state ratios for the sodium, hydroxyl, and bicarbonate ions lends support to the experimental evidence that the intra-ocular dynamics remain essentially unchanged after the administration of Diamox.

The transfer of ascorbic acid across the blood-aqueous barrier was studied in the belief that, if Diamox affected the secretory mechanism in the manner postulated by Friedenwald (1955a, b), the transfer of ascorbic acid would presumably also be modified. Friedenwald (1955a) suggested that carbonic anhydrase acts by facilitating the neutralization of hydroxyl ions on the epithelial side and hydrogen ions on the stromal side of the ciliary process. In the former area the excess of hydroxyl ions would react with carbonic acid to form bicarbonate, and in the latter area hydrogen ions would react with bicarbonate to form carbonic acid. Diamox, it was postulated, would slow down these reactions of neutralization and lead to a change in pH which could inhibit the enzymes necessary for the secretory process. Now ascorbic acid is the only compound which is universally agreed to be secreted across the ciliary processes, and to it has been ascribed a cardinal role in the secretory mechanism (Friedenwald, 1949). It is therefore of some significance that the transfer of this compound across the blood-aqueous barrier underwent no significant change in the presence of Diamox. It is true that the small increase in the concentration of ascorbic acid in the aqueous humour of animals given Diamox 3 hours previously is consistent with an increase in the rate of secretion; but, on the other hand, it may be more simply ascribed to the transient decrease in the rate of flow of the aqueous humour during the first 1 or 2 hours.

The results with ascorbic acid are in general agreement with those reported by Becker (1956) in nephrectomized rabbits. In this paper he analysed the concentration of ascorbic acid in the posterior and anterior chambers and in the plasma, and used these values to derive information about the rate of flow of the aqueous humour. This approach was based on the equations of Kinsey and Palm (1955) in which the rate of accumulation of a substance in the anterior chamber was expressed in the form

$$\frac{dC_{aq}}{dt} = K_f(C_\pi - C_a) + K_d(C_p - C_a),$$

Where C_π , C_a , and C_p represent, respectively, the momentary concentrations of the test substance in the fluid passing through the pupil (π), in the anterior chamber (a), and in the plasma (p). K_f represents the rate of flow per minute as a fraction of the volume of the anterior chamber; K_d represents the corresponding rate of diffusional exchange between the anterior chamber and the plasma. In conditions of steady-state,

$$\frac{dC_a}{dt} = \text{zero, and } \frac{K_f}{K_d} = \frac{C_a - C_p}{C_\pi - C_a}.$$

Using both equations, Becker found that, in animals given Diamox, the rate flow of the aqueous humour was depressed 60 per cent. in conditions of steady-state.

This conclusion is in apparent disagreement with the observations recorded in the present study that the rate of flow is unchanged in conditions of steady-state. It should, however, be pointed out that the experiments of Becker were carried out on nephrectomized rabbits; the conclusion that Diamox inhibits the rate of flow of the aqueous humour by 60 per cent. in normal animals rests on the assumption that rabbits in which the renal arteries have been ligated 18 to 24 hours previously are normal. This, however, is not so, for the present experiments show that changes occurred in the composition of the aqueous humour, in the permeability of the blood-aqueous barrier, and in the intra-ocular pressure of rabbits after ligation of the renal arteries. Furthermore, Diamox caused an irreversible lowering of the intra-ocular pressure in these animals.

There is general agreement that fluorescein can be used as a test substance to measure relative changes in the rate of flow or in the formation of the aqueous humour (Linnér and Friedenwald, 1954; Becker, 1955b; Langham and Wood, 1956). The experimental results in this paper showed that the time taken for fluorescein to pass from the bloodstream and into the anterior chamber *via* the posterior chamber increased by approximately 50 per cent. 14 minutes after the administration of Diamox, and that this increase in the time of flow remained high for at least 1 to 2 hours. Subsequently, however, there was a return of the flow-time to normal in spite of the administration of further amounts of Diamox. An increase in the flow-time of 100 per cent. would be equivalent to a decrease in the rate of flow of at least 50 per cent., and the observed increase of 50 per cent. in appearance time would be equivalent to a decrease in the rate of flow of approximately 20–25 per cent. The changes in the rate of flow of the aqueous humour after the administration of Diamox is of interest, in that the initial decrease in the rate of flow occurred at the same time as the loss of bicarbonate in the aqueous humour. Furthermore, the recovery of flow to normal was concurrent with the return of the bicarbonate distribution ratio to normal. Presumably the action of Diamox in decreasing the rate of flow of the aqueous humour is associated with a disturbance of the normal osmotic balance between the aqueous humour and the blood, and these results are consistent with the view that the loss of bicarbonate in the aqueous humour was responsible for the temporarily decreased rate of formation.

The variation observed in this study in the level of the intra-ocular pressure in normal rabbits given Diamox is in agreement with the results of previous investigations. The change was rapid and reached a maximum within 30 to 60 minutes. Becker (1955a) and Becker and Constant (1955) reported that the normal intra-ocular pressure in rabbits fell rapidly and reached a minimal value within 20 to 40 minutes and that in some rabbits the intra-

ocular pressure returned to normal within 2 hours. By the tonographic technique of Grant (1950), they found evidence that during this time there was an increase in the resistance to outflow which tended to minimize the full action of the Diamox. This increase in the resistance to outflow cannot however explain the return of the intra-ocular dynamics to normal in conditions of steady-state, for if the rate of formation is unchanged, an increase in the resistance to outflow would cause the intra-ocular pressure to rise above its normal value. It is therefore likely that any change in the resistance to outflow takes place only when the intra-ocular pressure and the rate of formation of the aqueous are depressed during the period immediately following the administration of Diamox.

The conclusion emerging from these studies—that the intra-ocular dynamics of the normal eye are almost, if not completely, undisturbed by Diamox in conditions of steady-state—is not inconsistent with the well-proven fact that, in similar conditions, the intra-ocular pressure is decreased and controlled in the glaucomatous eye. It is reasonable to assume that the immediate action of Diamox on the body is basically the same, and that the essential difference in the final equilibrium is determined by the different properties of the hypertensive eye. Theoretically, there are several factors which could make the eye with a high intra-ocular tension react more dramatically than the normal eye. First, there is the obvious reason that a given change in flow will lead to a greater absolute fall in the intra-ocular pressure if the initial pressure is high. Secondly, there is the possibility that homeostatic mechanisms are operating in the eye with a normal intra-ocular pressure and not in the eye with a pressure above a certain critical level. In this case, Diamox may reduce the pressure sufficiently to the level at which the homeostatic mechanism acts. Thirdly, it is possible that the relative contributions of filtration and osmotic pressures in the maintenance of the flow of the aqueous humour are not the same in the two cases. In this event Diamox could affect the rate of formation of the aqueous humour in the normal and abnormal eye disproportionately.

There are few reports of the action of Diamox on normal human eyes. Grant and Trotter (1954) observed that Diamox in doses used clinically did not decrease the intra-ocular pressure of normal eyes more than 1 or 2 mm. Hg, and they also made the interesting observation that in glaucomatous eyes which were at comparable initial pressures due to the action of miotic drugs, the change in intra-ocular pressure after Diamox was only 3 mm. in four out of five eyes. These results referred to conditions of steady-state. Similar observations on normal and glaucomatous human eyes have been reported by Campbell, Tonks, and Jones (1956). The lack of change in the intra-ocular pressure of these subjects contrasts with the observations of Becker (1955c) on fifteen normal eyes of ten subjects given a high dosage of Diamox by mouth. Approximately 3 hours after giving 500 mg. Diamox by mouth the intra-ocular pressure was found to be decreased from a mean value of

17.6 to 14.6 mm. Hg, and the rate of flow to be decreased by a mean value of 55 per cent. as measured by the tonographic technique.

The assessment of how and to what extent Diamox acts on the eye is handicapped by our lack of knowledge of the contribution that secretion and the hypertonicity of the aqueous humour play in the movement of water through the anterior chamber. It is widely accepted that the osmotic pressure of the aqueous humour is 1 to 2 per cent. higher than that of the blood (Benham, Duke-Elder, and Hodgson, 1938; Roepke and Hetherington, 1940; Davson, Duke-Elder, and Maurice, 1949; Bárány, 1947; Kinsey, 1951). This osmotic excess is equivalent to a hydrostatic pressure of 100–200 mm. Hg. and would mean that the hydrostatic pressures acting across the blood-aqueous barrier contributes in a minor way to the movement of fluid through the eye. On the other hand, the estimate of the osmotic pressure of the aqueous humour may be high, since these determinations were made at temperatures below 37.5° C. Now the pH-temperature coefficient is not the same for blood and for the aqueous humour and, as a consequence, a given decrease in temperature will disturb the two fluids disproportionately. In this connexion, the studies of Ingraham, Lombard, and Visscher (1933) have shown that the distribution ratio for sodium and potassium ions between plasma and its ultrafiltrate decreases with an increased pH; the value for sodium fell from 0.928 to 0.913 and for potassium from 0.901 to 0.816 when the pH increased from 7.0 to 7.70. Consequently, in determinations of the relative osmotic activity of these two fluids at temperatures below that of the body, an error is introduced which causes an increase in the osmotic pressure of the aqueous humour in relation to that of the blood.

In bringing together these findings on normal and glaucomatous eyes, the evidence is consistent with the view that Diamox modifies the osmotic balance between the blood and the aqueous humour independently of its renal action. The mechanism remains unsolved, but would appear to be related to an increased acidity in the eye. In animals, however, this increased acidity relative to that of the blood was only temporary. This could have been due to a disproportionate inhibition of carbonic anhydrase activity in different regions of the eye through a slow penetration of Diamox, or it could have reflected the ability of the eye to reassert the normal concentration ratios of hydroxyl and bicarbonate ions between the aqueous humour and the blood. Apart from the changes in the concentration of bicarbonate in the aqueous humour immediately after giving Diamox, no evidence has been found to support the view that Diamox inhibits the secretion of bicarbonate into the eye. The possibility that Diamox directly or indirectly inhibits the active transport of some other ion such as sodium or chloride cannot be answered from this study.

Summary

(1) The pH and the concentration of total carbon dioxide in the aqueous humour and in the arterial blood of normal rabbits have been determined. The pH temperature coefficient was determined separately and found to be 0.0147 ± 0.0002 and 0.0064 ± 0.00025 pH units per $^{\circ}C$. for the blood and the aqueous humour respectively. The pH and the concentration of bicarbonate in the aqueous humour exceeded those in the blood and the calculated values of the pCO_2 tensions in the two fluids were found to be equal.

(2) The composition of the aqueous humour and the blood has been analysed at varying times after an injection of Diamox. A rapid fall in the pH and a concurrent increase in the CO_2 tension in the two fluids was observed. At the same time there was a decrease in the concentration of bicarbonate in the aqueous humour and a temporary decrease in the normal excess of bicarbonate. In conditions of steady-state, however, the distribution ratio (C_{aq}/C_{pl}) was equal to that in control animals.

(3) Ammonium chloride, given in amounts that produced a marked acidosis, resulted in a prolonged decrease in the excess of bicarbonate in the aqueous humour. Ligation of the renal arteries induced a state of progressive acidosis, but left the normal distribution ratio for bicarbonate unchanged. The administration of Diamox to these animals resulted in a further increase in the CO_2 tension and a decrease in the pH of the aqueous humour.

(4) Analysis of the concentrations of sodium in the aqueous humour and the blood after administration of Diamox showed that the initial fall of approximately 6 mEq HCO_3/kg . H_2O in the aqueous humour was not accompanied by any significant change in the normal distribution ratio of the sodium ion.

(5) The effect of Diamox and ammonium chloride on the secretion of ascorbic acid across the blood-aqueous barrier has been studied. Both compounds led to an increase in the concentration of ascorbic acid in the aqueous humour compatible with a decrease in the rate of flow of the aqueous humour. It may be concluded that neither compound inhibited secretion of ascorbic acid.

(6) The circulation of the aqueous humour and its rate of flow in rabbits given Diamox or ammonium chloride have been studied using fluorescein as a test substance. The time taken for fluorescein to pass from the blood-stream through the posterior chamber to the pupillary area immediately in front of the lens was 238 ± 23 (11) seconds; 1 hour after the administration of Diamox this increased to 348 ± 21 (9) seconds. In animals maintained on Diamox for 6 hours, the appearance-times were normal. Ammonium chloride also led to an increase in the fluorescein appearance-time which

was maintained over several hours. It is concluded that Diamox temporarily reduced the rate of flow of the aqueous humour.

(7) Intra-ocular pressure observations, using both the manometric and the tonometric methods of measurements, are reported. In confirmation of other investigations, the intra-ocular pressure was found to fall rapidly after giving Diamox and then to increase toward normal. Ammonium chloride in doses that gave frank acidosis caused a more prolonged lowering of the intra-ocular pressure.

(8) It is concluded from these studies that the continued presence of Diamox gives rise to little or no effect on the intra-ocular dynamics of the normal rabbit eye. It is also suggested that the temporary reduction in the intra-ocular pressure, the rate of formation of the aqueous humour, and the excess of bicarbonate in the aqueous humour immediately following the administration of Diamox are associated with the action of this substance in inducing a rapid fall in the pH of the aqueous humour.

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