COMMUNICATIONS

PERMEABILITY OF THE CORNEA AND THE BLOOD-AQUEOUS BARRIER TO OXYGEN*

BY

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INTEREST in the permeability of the cornea to oxygen arose out of recent investigations on corneal metabolism and the influence of metabolism on the hydration of the tissue. Langham (1952) reported that the concentration of lactic acid in the cornea varied inversely with the oxygen tension in the tear film and concluded that oxygen obtained directly from the atmosphere was utilized by the cornea, and that the oxygen entering from the bloodstream and the aqueous humour was unlikely to meet the requirements of the whole cornea. In studies of a rather different nature, Smelser and Ozanics (1953) and Langham and Taylor (1956) observed that the denial of atmospheric oxygen to the anterior surface of the cornea of the living animal led to an increased hydration and thickness of the cornea. Consequently, to confirm whether or not the respiratory requirements of the tissue could be met by the diffusion of oxygen from the aqueous humour, direct measures of the oxygen tension in the aqueous humour and the rate of diffusion of oxygen across the cornea have been made. At the same time, the opportunity has been taken of assessing by an independent means the conclusion of Fischer (1930) that the cornea shows a unidirectional permeability to oxygen in the inward direction.

Previous studies relating to this subject include those of de Haan (1922), Friedenwald and Pierce (1937), and Fischer (1930). The oxygen tension in the aqueous humour of rabbits was measured by de Haan (1922), using Krogh's microtonometric technique. He found the tension to be 20–30 mm. Hg, but recognized that some loss of oxygen from the aqueous humour took place during the analysis through autoxidation. Correcting for this loss, he concluded that the oxygen tension was probably nearer 40–45 mm. Hg. Friedenwald and Pierce (1937) introduced a bubble of nitrogen into the anterior chamber of dogs and, after allowing time for equilibration, analysed its oxygen content; from a series of analyses made after different time intervals they reported the mean oxygen tension in the aqueous humour to be 45 mm. Hg. Their experimental technique is, however, subject to the criticism that paracentesis is known to cause a general increase in the permeability of the blood-aqueous barrier and to disturb the intra-ocular circulation. Fischer (1930) measured the change in oxygen content within a glass chamber sealed to the anterior segment of the eye. He observed a loss of oxygen

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from the chamber, but in similar experiments in which the anterior chamber was filled with nitrogen he could not detect an outward movement of oxygen into the chamber.

In the present work, some of the principal difficulties met in previous studies have been overcome by the use of a polarographic method for the estimation of oxygen. The diffusion constant of the excised cornea in the inward and outward directions, both in the absence and presence of the epithelial and the endothelial layers, has been determined. In living animals a study has been made of the rate of accumulation of oxygen in the aqueous humour with the eye exposed to an atmosphere of oxygen.

In addition to these corneal studies, an investigation has been made of the rate of transfer of oxygen from the bloodstream into the aqueous humour. Here, the principal object was to assess the factors influencing the oxygen tension in the aqueous humour. In particular, the effect on the oxygen tension in the aqueous humour of an increased and a decreased blood flow through the ciliary processes has been measured. To do this, a reduction in blood flow was induced by the ligation of the common carotid artery and an increase in blood flow by cutting the preganglionic cervical sympathetic (Linnér, 1952).

Methods

Experimental Procedure.—Adult rabbits of both sexes weighing between 1.8 and 2.5 kg. were used. They were fed Diet 18 pellets (Associated London Flour Millers), water ad lib., and hay once a week. Samples of aqueous humour from conscious animals were withdrawn into an ice-cold 1.0-ml. tuberculin syringe, after the instillation of a 1.0 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. trichloroacetic acid, and results were rejected if more than the normal faint clouding was observed.

Arterial blood samples were withdrawn from the heat-dilated median ear artery. The oxygen tension in this blood was found to be equal to that in the common carotid artery. To avoid loss of oxygen from the blood sample, a method was used similar to that described by Berggren (1942). The blood was withdrawn into a heparinized syringe and transferred into a glass tube filled with mercury having a capacity of 1 ml. and a neck 15-mm. long of internal diameter *c.* 0.2 mm. This was carried out by inverting the tube and displacing the mercury by slowly injecting the blood sample. The tube was then surrounded by ice in a centrifuge and spun at 2,500 r.p.m. for 1 to 2 min. Finally, the plasma was withdrawn into the micro-cell through a polythene tube filled with mercury. No oxygen appeared to be lost in this procedure, for in control experiments, using a 0.05 M solution of KCl containing oxygen, the mean diffusion current before and after spinning was 1.90 and 1.88 μ amps respectively.

Measurement of Diffusion Constant of Excised Cornea Oxygen

The apparatus is shown in Fig. 1 (opposite). The small central chamber in which the oxygen accumulated had a cross-sectional area of approximately 1 sq. cm. and a volume of 1.2 to 1.4 ml.; this volume varied with the shape of the cornea. The fluid in this chamber was kept mixed by a Perspex paddle forming part of a

FIG. 1.—Apparatus for the measurement of the diffusion constant of the cornea to oxygen.

stopper which fitted very closely into the funnel. Control experiments showed that the loss of oxygen from the chamber during the course of an experiment did not exceed 5 per cent. Efficient stirring of the fluid in the outer chamber was maintained by the stream of oxygen passing through a scintered glass filter. The whole apparatus was kept at constant temperature.

The cornea was excised and transferred carefully from the eye after its thickness had been measured. An adjoining ring of scleral tissue was included in the excised tissue and this formed a firm joint to hold the cornea between the two rings of Perspex.

Studies on Living Rabbits.—The transfer of oxygen across the cornea of the living rabbit was studied using the apparatus described in a previous study (Langham, 1952). The anaesthetized animal was placed in a transparent air-tight Perspex tank, normal respiration being maintained by a tracheal tube connected to the outside atmosphere. Anaesthesia was maintained by periodic injections made intraperitoneally through a polythene tube passing into the tank from the outside. A steady stream of oxygen was passed through the tank, the pressure in the tank being kept slightly above that of the atmosphere.

Determination of Oxygen.—The sample of aqueous humour was withdrawn into a cell for analysis directly from the anterior chamber of the eye without contact with air, and without protein precipitation or any previous treatment. The apparatus employed consisted of a Tinsley polarograph (Type V722/14) and a specially constructed micro-cell capable of analysing approximately 0.15 to 0.5 ml. of solution. The diagram of this cell is shown in Fig. 2. It was found necessary to use a permanent external standard calomel half-cell as anode, connected to the micro-cell by a scintered glass disk and a salt bridge, as recommended by Beecher, Follansbee, Murphy, and Craig (1942), and by Kolthoff and Lingane (1952), the salt bridge (3 per cent. agar in saturated KCl) being renewed once a week when the apparatus was in constant use. A hypodermic needle was connected to the side arm of the apparatus by a length of polythene tubing. The dropping mercury electrode passed through a mercury seal into the cell compartment, and the height of the mercury reservoir was adjusted to give a constant drop-time (usually 4 sec.) measured at zero volts, without short circuit (*cf.* Kolthoff and Lingane, 1952), before each estimation. If this drop-time varied or became irregular, and if the error could not be rectified by cleaning the tip in 25 per cent. nitric acid and distilled water or on gentle warming, the capillary was discarded. After use the capillary was kept immersed in distilled water, and mercury from the reservoir was allowed to flow through periodically. Oxygen is reduced at a dropping mercury electrode in the potential ranges 0 to -0.3 volts and -0.5 to -1.3 volts measured against the saturated calomel electrode. The diffusion current (i_D) developed in each range, which is measured in the polarograph, is proportional to the oxygen concentration of the solution in contact with the dropping electrode, and is related to it by the Ilkovic equation (Kolthoff and Lingane, 1952).

$$i_D = 607nD^{\frac{1}{2}}C_m^{\frac{1}{2}}t^{\frac{1}{2}},$$

where i_D is the diffusion current in microamp., n is the numbers of electrons involved in the reduction of one molecule of reducible substance, D is the diffusion coefficient of the reducible substance in sq. cm. sec.⁻¹, C its concentration in millimoles l^{-1} , m the weight of mercury in mg. flowing out of the capillary per sec., and t the drop-time in sec. The observed values of i_D have to be corrected for residual current, *i.e.* the current developed between the experimental voltages in the liquid medium after the removal of oxygen by the addition of 1 per cent. sodium sulphite (Beecher and others, 1942). In the present study the diffusion current developed between -0.5 and -1.3 volts was measured, and from this the oxygen concentration was calculated. The method was found to be applicable to aqueous humour, since between 0 and -1.3 volts oxygen is the only reducible

FIG. 2.—Micro-cell for polarographic analysis.

substance present (Fig. 3, overleaf). Calibrations were made using 0.05 M KCl and aqueous humour (from excised ox eyes) both saturated with air and with oxygen, and also 0.05 M KCl saturated with mixtures of oxygen and nitrogen previously analysed in the Haldane apparatus. A linear relationship was observed between diffusion current and oxygen concentration in the two solutions, the values obtained in each case being the same after correction for residual current (Table I, overleaf). At the beginning of each day the sensitivity of the apparatus was checked using 0.05 M KCl saturated with air.

For the purposes of the present calculations it was found more convenient to express the results as partial pressures of oxygen rather than as concentrations, the following conversion factors being derived from the data of Winkler (1904):

FIG. 3.—Polarographic tracings.
(A) 0.05 M KCl saturated with air at 2° C.
(B) Sample of aqueous humour measured at 2° C.

TABLE I
CALIBRATION OF POLAROGRAPH USING 0.05 KCl AND OX AQUEOUS HUMOUR
Gas phase analysed by Haldane apparatus (last column)

System (2° C.)		i_D μamp.	mg. ³ /sec.	t^1_{90} sec.	Polar pO_2	Haldane pO_2
Oxygen	0.05 M KCl	15.0	1.706	1.22	755.6	754.7*
	Ox aqueous humour	14.8	1.706	1.22	747.7	754.7
Air	0.05 M KCl	3.1	1.706	1.22	156.1	158.4
	Ox aqueous humour	3.0	1.706	1.22	153.5	158.4
Mixture	1, KCl	6.2	1.706	1.22	312.2	333.9
	2, KCl	1.3	1.657	1.24	69.0	63.4

* Value deduced from data for water vapour pressure at 2° C. (5.29 mm. Hg) i_D has been corrected for residual current.

an aqueous solution which is 1.0 mM with respect to oxygen corresponds to a partial pressure of oxygen (pO_2) of 361 mm. Hg at 2° C. and 674.2 mm. Hg at 37° C.

To analyse a sample of aqueous humour, the cell was washed out with 0.05 M KCl, and then by means of the Agla head attached to the lower reservoir mercury was driven forward through the cell to the tip of the needle which was inserted into the anterior chamber of the eye, and approximately 0.2 ml. of aqueous humour was withdrawn. In order to obtain reproducible readings on the same aqueous humour sample, the cell was immersed in an ice-water bath at 2° C. ($\pm 0.5^\circ$ C.). The oxygen concentration of each sample was measured four times. There was sometimes a slight loss of oxygen from samples of aqueous humour but not from solutions of KCl. The decrease in the oxygen content of the aqueous

humour did not, however, exceed 5 to 10 per cent. in 20 min. and it was considered adequate to take the mean of the first three readings.

Results

Oxygen Transfer across the Cornea.—In these studies the permeability of the excised cornea to oxygen has been expressed as the volume in ml. passing through 1 sq. cm. and 1μ (0.001 mm.) thick in 1 min. for a pressure gradient of 1 atmosphere. This is in accord with the definition of the diffusion coefficient given by Krogh (1919) in studies of the permeability of muscle and connective tissue to gases.

The rates of diffusion of oxygen across the cornea in the inward and outward directions were found to be equal. At 4°C . the diffusion coefficient of freshly-excised corneae was 0.018 ± 0.0013 in eleven experiments. Six of these experiments were made with the epithelial surface and the remainder with the endothelial surface nearest the source of oxygen; the mean values of the diffusion constants were 0.017 ± 0.0013 and 0.017 ± 0.0013 respectively.

Removal of the epithelial and endothelial layer, which comprise 10 per cent. of the total thickness of the cornea, did not significantly change the diffusion coefficient. Thus at 4°C . the mean diffusion coefficient of the stroma in six experiments was 0.021 ± 0.014 . At 37.5°C . the diffusion coefficient of the stroma in six experiments was 0.031 ± 0.0015 . The oxygen uptake of an equal volume of stroma is not sufficient significantly to influence this value (Langham, 1952, 1954). The increase in the diffusion coefficient due to the increased temperature is in agreement with the observations of Krogh (1919) that it varies approximately 1 per cent. per $^{\circ}\text{C}$.

These coefficients are appreciably below those of 0.115 and 0.14 for connective tissue and muscle reported by Krogh (1919). He used connective tissue from the abdominal wall of the frog after having scraped away adherent muscular tissue. By this technique he obtained for one experiment a double layer of connective tissue 63μ thick and in a second experiment a single layer measuring only 17μ . In contrast, the cornea is approximately 400μ thick and in structure comprises densely-packed collagenous fibrils embedded

in a viscous polysaccharide matrix.

The oxygen tension in the aqueous humour of living rabbits whose eyes were exposed to an atmosphere of oxygen (713 mm. Hg) are recorded in Table II. The oxygen tension

TABLE II

OXYGEN TENSION IN AQUEOUS HUMOUR ON EXPOSURE OF ANTERIOR SURFACE OF EYE TO AN ATMOSPHERE OF OXYGEN (713 mm. Hg)

Animals anaesthetized and normal respiration maintained through a tracheal tube.

Time (hrs)	Oxygen Tension (mm. Hg)	Ratio C_1/C_2^*
0	45.5 ± 2.55 (19)	1.0 ± 0.07
1	87.2 ± 13.75 (5)	1.8 ± 0.29
2	103.0 ± 16.6 (4)	2.4 ± 0.36
3	131.0 ± 19.7 (4)	2.6 ± 0.39
4-6	131.0 ± 12.8 (6)	3.0 ± 0.23

* C_1 and C_2 = oxygen tension in experimental and control eyes respectively.

rose and approached a new steady-state value of approximately 130 mm. Hg. At this time the oxygen tension gradient across the cornea was 713—130, *i.e.* 583 mm. Hg compared with the normal gradient of approximately 150—45, *i.e.* 105 mm. Hg. The scatter in the results between animals was appreciable, and a large standard error was calculated both for the absolute values for any given period of exposure to oxygen and also in the ratio of the tensions in the experimental eyes to those in the control eyes of individual rabbits. The reason for this scatter is not known, but could be due to a variation between animals in the dimensions of the cornea and the aqueous humour.

Transfer of Oxygen into the Aqueous Humour from the Bloodstream.—In the course of these studies, results were collected on the oxygen tension in the aqueous humour of conscious and unconscious rabbits. In 35 rabbits anaesthetized with Nembutal, the mean oxygen tension was 48.2 ± 2.58 mm. Hg, and in twenty conscious animals the mean tension was 55.2 ± 2.19 mm. Hg. These results are summarized in Fig. 4, which shows that the range of values in the conscious rabbits was less than in the anaesthetized animals, and that the oxygen tension in the aqueous humour of anaesthetized rabbits tended to be below that in the conscious animal.

FIG. 4.—Mean oxygen tension of conscious rabbits and rabbits anaesthetized with Nembutal. The abscissa represents a range of tensions (mm. Hg) rising in steps of 5 mm. Hg and starting at 26–30 mm. Hg.

In the two eyes of the same animal, the oxygen tension was found to be similar. In eight anaesthetized rabbits the oxygen tension was 59.6 ± 4.4 and 60.6 ± 4.2 mm. Hg in the left and right eye respectively, the mean difference between the two eyes being 1.0 ± 1.4 mm. Hg.

The equilibration of oxygen between the aqueous humour and the blood was studied in three series of experiments. In the first, conscious animals were placed in a Perspex tank, through which a rapid stream of 95 per cent. oxygen and 5 per cent. CO_2 was passed. Animals were removed from the tank after 1, 2, and 3 hrs, and the aqueous humour was immediately withdrawn from the eyes into the micro-cell for analysis. The results of these three series of experiments are recorded in Table III. The oxygen tension in the aqueous humour rose very rapidly to approach a new steady state within 1 hour. The oxygen tension in the blood of these animals was not recorded owing to the difficulty of withdrawing samples of blood and aqueous humour before loss of oxygen had taken place. In separate experiments, however, the mean oxygen tension in the arterial blood of four rabbits treated in the same way was 630 ± 20 mm. Hg. The rapidity of equilibration of oxygen between the blood and the aqueous humour is

TABLE III
MEAN OXYGEN TENSION IN AQUEOUS HUMOUR
OF CONSCIOUS RABBITS BREATHING A MIXTURE
OF 95 per cent. OXYGEN AND 5 per cent. CARBON
DIOXIDE

Mean oxygen tension in arterial plasma 630 ± 20 mm. Hg

Time (hrs)	Mean Oxygen Tension (mm.Hg)
0	53.5 ± 3.4 (4)
1	239 ± 14.1 (6)
2	246 ± 19.0 (6)
3	258 ± 5.7 (6)

similar to that found for alcohol by Palm (1947) and Ross (1951). They observed that the alcohol concentration in the aqueous humour was within a few per cent. of equilibration with that in the blood 1 hour after an intra-venous injection of the test substance.

In the above experiments, the anterior surface of the eye was exposed to the atmosphere of oxygen and it was therefore not certain how much of the increased tension in the aqueous humour was due to oxygen passing across the blood-aqueous barrier and how much was due to diffusion across the cornea. In the corneal studies, the oxygen tension in the aqueous humour rose by only 80 per cent. in 1 hour, compared with the present value of 500 per cent., and on this basis it appeared probable that the oxygen tension in the aqueous humour was determined principally by that passing across the blood-aqueous barrier. This was supported by experiments on a series of anaesthetized rabbits in which the anterior surface of the eye was left exposed to the air and the oxygen tension in the blood was raised by giving the animal oxygen *via* a tracheal tube. In eight animals the average oxygen tension was 445 ± 36 mm. Hg in the arterial blood, and 165 ± 33 mm. Hg in aqueous humour removed 2-3 hrs after the start of the experiment.

In an attempt to determine the turnover rate of oxygen in the aqueous humour, a second series of experiments was made on conscious animals wearing a breathing mask fitted over the head. The technique was not completely satisfactory for the oxygen tension in the blood of these animals breathing 95 per cent. oxygen and 5 per cent. carbon dioxide rarely rose above 500 mm. Hg, and it showed appreciable variations between animals. Results of experiments in which the oxygen tension in the arterial plasma remained between 400 and 500 mm. Hg are recorded in Fig. 5. In seven similar experiments, in which the oxygen tension remained within these limits for 140–450 min., the mean oxygen tension in the aqueous humour was 210 ± 22 mm. Hg.

FIG. 5.—Permeability of blood-aqueous barrier to oxygen, in conscious rabbits given oxygen through a close-fitting mask. Two exponential curves, (A) $k_{in}=0.027 \text{ min.}^{-1}$ and (B) $k_{in}=0.0135 \text{ min.}^{-1}$, calculated on the basis of a maximal oxygen tension of 210 mm. Hg, and a steady-state ratio of unity.

In a final series of experiments on normal conscious rabbits, the rate of loss of oxygen in the anterior chamber was determined after it had been raised to 150–200 mm. Hg. The animals were given oxygen for 1 to 2 hrs and then the oxygen was replaced by air. In this condition the oxygen tension in the blood fell immediately from 600 to 700 mm. Hg to normal. The results recorded in Table IV (opposite) show that the oxygen tension in the aqueous humour fell at a rapid rate which decreased as the oxygen tension approached normal.

TABLE IV

DECAY OF OXYGEN IN AQUEOUS HUMOUR OF ANIMALS AFTER HAVING BREATHED A GAS MIXTURE OF 95 per cent. O₂ AND 5 per cent. CO₂.

Rate of decay calculated by assuming that the tension decreased exponentially to normal tension (53 mm. Hg)

Time (Min.)	Aq_1^*	Aq_2	$Aq_2 - Aq_1$	K_{decay}
18	207	83	124	0.092
20	137	63	74	0.109
33	122	63	59	0.060
33	101	82	19	0.032
44	205	139	66	0.039
46	165	75	90	0.070
47	136	75	61	0.042
47	172	95	77	0.022
52	131	70	61	0.030

* First column records the time at which the aqueous humour from the second eye was removed.

Effect of Change in Blood Flow through the Eye on the Oxygen Tension in the Aqueous Humour.—Two series of animals were used for this study. In the first, the blood flow through one eye relative to the other was increased by cutting the pre-ganglionic cervical sympathetic nerve on one side 24 hrs before the experiment, while in the second series the blood flow was decreased through one eye compared with the contralateral eye by ligation of the common carotid artery. In these circumstances, measurement of the blood flow through the vortex veins has shown that it is increased by sympathectomy and decreased by the ligation of the common carotid in the eye on the operated side (Linnér 1952). Similarly, evidence has been put forward that there is a corresponding increase of 20 per cent. after sympathectomy and a decrease of approximately 20 per cent. after carotid ligation in the blood flow through the ciliary processes on the operated side as compared with the unoperated side (Linnér, 1952).

The results of these operations on the oxygen tension in the two eyes are recorded in Table V. Ligation of the common carotid resulted in a decrease in the oxygen tension on the operated side as compared with the unoperated side. The oxygen tension in the aqueous humour on the operated side was

TABLE V

OXYGEN TENSION IN AQUEOUS HUMOUR OF CONSCIOUS RABBITS 24 HRS AFTER UNILATERAL LIGATION OF COMMON CAROTID ARTERY (SERIES 1) AND UNILATERAL SECTION OF PRE-GANGLIONIC SYMPATHETIC (SERIES 2)

Series	Oxygen Tension in Aqueous Humour (mm. Hg)			
	Control Eye	Experimental Eye	Cont. — Exp.	Exp. — Cont.
1	71.5 ± 3.0 (12)	58.1 ± 4.2 (12)	13.4 ± 3.29 (12)	0.81 ± 0.043 (12)
2	56.5 ± 3.4 (15)	58.1 ± 3.5 (15)	1.6 ± 3.31 (15)	1.05 ± 0.056 (15)

however essentially the same as that observed in normal conscious rabbits. After sympathectomy, no significant difference in the oxygen tension in the two eyes was observed, and the absolute values were in the same range as those in normal conscious animals.

Discussion

In these experiments oxygen has been shown to diffuse at the same rate in either direction across the cornea and not to vary after removal of the epithelium or the endothelium. Fischer (1930), however, reported that the cornea showed a unidirectional permeability to oxygen in the inward direction, and that the resistance to the passage of oxygen was due principally to the epithelium. He introduced oxygen into the anterior chamber and could not detect a passage of oxygen into a glass chamber fitted on the anterior surface of the eye. In similar experiments in which the glass chamber was filled with oxygen, he observed a loss of oxygen which increased after removal of the epithelium but not after removal of the endothelium. Unfortunately few experimental details are given, and it is difficult to understand Fischer's inability to demonstrate a passage of oxygen across the cornea from the anterior chamber. It is possible that, in the conditions of his experiments, the oxygen tension in the anterior chamber fell and that although oxygen passed into the cornea it was not sufficient to exceed the oxygen requirements and pass outward across the anterior surface.

From the present results it may be calculated that the maximal amount of oxygen that can diffuse across the cornea from the aqueous humour when the tension gradient across the tissue is 55 mm. Hg is insufficient to meet the respiratory requirements of the epithelium. Ideally, it would be desirable to assess in normal conditions the amount of oxygen that would diffuse into the epithelium from the aqueous humour when the oxygen tension in the tear film is zero, but this necessitates knowing the oxygen uptake of the component layers, the diffusion constants of each layer, and the oxygen tension gradient. Unfortunately not all this information is available. To derive the maximal rate of transfer of oxygen, it is assumed that the stroma and endothelium have no oxygen uptake and that the oxygen tension across the cornea falls from 55 mm. Hg on the endothelium side to zero at the junction of the stromal and epithelial layers. The value of 55 mm. Hg is the oxygen tension of the aqueous humour of the conscious rabbit as measured by the polarographic technique. On this basis, the amount of oxygen that will pass across the stroma plus the endothelium of average thickness 360μ is 6.2×10^{-6} ml. sq. cm. min.⁻¹. In comparison the oxygen uptake of the epithelial layer determined by the Warburg manometric technique is 1.4×10^{-4} ml. sq. cm. min.⁻¹, that is over forty times greater.

In view of this wide disparity between the amount of oxygen that can diffuse from the aqueous humour to the epithelium and the oxygen needs of the layer, it is suggested that the oxygen needs of this outer cellular layer

are met from the atmosphere and from the capillaries of the tarsal conjunctiva when the eyes are opened and closed respectively. This is consistent with previous observations (Langham, 1952) that denial to the cornea of direct access to the atmosphere caused an increase in the concentration of lactic acid in the cornea. Furthermore, it is in agreement with the conclusions of Smelser and Ozanics (1952, 1953), and of Langham and Taylor (1956), that the corneal swelling caused by wearing contact lenses is principally due to the decreased aerobic metabolism of the epithelial layer. The amount of oxygen diffusing into the cornea from the limbal vessels is likely to be very small compared with the two alternative routes, as the area of contact is relatively very small.

When the oxygen tension in the atmosphere was increased to 700 mm. Hg, the results on the cornea both of the excised eye and of the living animal showed that more than sufficient oxygen diffused across to supply the oxygen requirements of the cornea. In the living animal, an increase in the oxygen tension in the tear film from 150 mm. Hg in equilibrium with air at 37° C. to 713 mm. Hg led to an increase in the oxygen tension in the aqueous humour from an initial value of 45 mm. Hg to a final steady-state value of 131 mm. Hg. This increase is equivalent to a $2.83 \text{ ml. O}_2 \text{ l}^{-1}$ or to $8.5 \times 10^{-4} \text{ ml. O}_2$ in 0.30 ml., the average volume of aqueous humour in a normal rabbit eye.

In assessing the permeability of the blood-aqueous barrier to a substance, it is generally adequate to assume the arterial concentration to be approximately equal to that of the blood in the capillaries of the ciliary processes from which the aqueous humour is derived. However, if the compound is lost very rapidly from the circulation, a considerable concentration gradient between the arterial and venous circulations will develop, and it then becomes necessary to know the mean concentration in the capillary bed; this is true of oxygen. According to Roughton, Darling, and Root (1944), the oxygen tension in the arterial blood of human subjects breathing air is 100 mm. Hg, and it is generally accepted that the venous tension is approximately 40 mm. Hg. Now, in rabbits breathing air, the mean oxygen tension in the aqueous humour was 55 mm. Hg, and therefore the mean tension in the blood from which it was derived could not have been less than 55 mm. Hg unless secretion of oxygen took place. In similar conditions the oxygen tension in the aqueous humour rose to an equilibrium value of 210–260 mm. Hg when the arterial tension was 400–630 mm. Hg; again the mean tension in the blood of the ciliary processes could not have been less than 210 mm. Hg. Thus the maximal gradient across the blood-aqueous barrier in animals breathing air or oxygen was approximately 50 and 300 mm. Hg respectively.

The most probable explanation of this change in the oxygen tension gradient is that some loss of oxygen from the blood takes place before reaching the capillaries of the ciliary processes, and that in both cases the $p\text{O}_2$

values on either side of the barrier were approximately equal. In this event the loss of oxygen in the blood when the tension fell from 450 to 210 mm. Hg or from 630 to 260 mm. Hg should be approximately equal to that lost when the tension falls from 100 to 50 mm. Hg. In normal rabbits the oxygen-combining power of the blood is 19.1 ml./100 ml. (Langham and Lee, 1957), and if the oxygen dissociation curve of rabbit's blood is assumed to be the same as that of human blood the oxygen content at 55 mm. Hg would be 16.3 ml./100 ml. If therefore the blood were fully saturated, 2.8 ml. oxygen/100 ml. blood would be lost when the tension fell to 55 mm. Hg; if the blood were 90 per cent. saturated, the volume lost would be about 1.0 ml. oxygen/100 ml. blood. The corresponding loss of oxygen when the tension falls from 450 to 210 mm. Hg or from 630 to 260 mm. Hg is 0.61 and 1.0 ml./100 ml. blood.

The alternative explanation, that the ratio of the oxygen tension in the aqueous humour to that in the blood of the ciliary processes varies with the oxygen content of the blood, appears improbable. It would imply either that respiration varied with the oxygen tension or that large changes in the rate of entry or exit of oxygen in the aqueous humour took place.

If, in conditions of steady state, the concentration of a substance in the aqueous humour equals the mean concentration in the blood of the ciliary processes, the rates of entry and exit must be equal. This is usually expressed in the form

$$k_{in} = rk_{out},$$

where r is the ratio of concentration of the substance in aqueous humour and blood in conditions of steady state. From this equation it is evident that, provided r closely approaches unity, the error in assuming r to be unity is relatively small. On this basis it is possible to calculate k_{in} for oxygen as defined by the equation:

$$k_{in} = \frac{r}{t} \ln \left(\frac{rCpl - Caq_0}{rCpl - Caq_t} \right),$$

where Cpl is the mean plasma concentration, and Caq_0 and Caq_t the concentrations in the aqueous humour at zero time and t min. respectively. This equation is widely used in studies of intra-ocular dynamics and it will be noted that k_{in} has the dimensions of reciprocal time. From the results on the accumulation of oxygen in the aqueous humour, k_{in} for oxygen was found to equal $0.030 \pm 0.002 \text{ min.}^{-1}$. This value is of the same order of magnitude as that found for alcohol, which like oxygen has a high fat solubility (Palm, 1947; Ross, 1951). The molecular weight of oxygen is 32, and that of ethyl alcohol is 46, and their oil-water partition coefficients at 37° C. are 5.0 and 1.9 respectively. It would appear that the permeability of the blood-aqueous barrier to oxygen is consistent with the conclusion that fat solubility plays the dominant role in the penetration of the blood-aqueous barrier (see Ross, 1951; Langham, 1955a).

The aqueous humour is the sole source of the oxygen requirements of the crystalline lens, and it is of interest to consider how rapidly oxygen in the aqueous humour must be supplied to meet its requirements. There is general agreement that excised rabbit lenses utilize approximately $0.02 \mu\text{l.}$ oxygen per mg. dry tissue per hr (Kronfeld and Bothman, 1928; Field, Tainter, Martin, and Belding, 1937; Ely, 1949; Langham, 1951). For an average adult rabbit lens of 400 mg. wet weight, the oxygen requirements would be $1.3 \times 10^{-4} \text{ ml. min.}^{-1}$. Now the average volume of the aqueous humour of adult rabbits is approximately $300 \mu\text{l.}$, and at an oxygen tension of 210 mm. Hg it would contain $20.8 \times 10^{-4} \text{ ml.}$ oxygen. Consequently, in these conditions the lens would abstract 6.2 per cent. of the oxygen in the aqueous humour every minute. The turnover rate of oxygen, at the steady-state value of 210 mm. Hg, was observed to be approximately 3 per cent. per min. and this includes loss of oxygen by drainage of the aqueous humour from the eye. It is therefore possible that the oxygen uptake of the lens in the living animal is less than that observed on the excised tissue, a view which is in agreement with the conclusions of Christiansen and Leinfelder (1952) that part of the oxygen uptake of the excised lens is due to non-enzymic processes. On the other hand, it must be recognized that the oxygen requirements of the lens will be derived principally from the aqueous humour of the posterior chamber, and that here the rate of turnover of oxygen might far exceed the rate for the whole aqueous humour. This follows from the fact that for molecules which rapidly penetrate the blood-aqueous barrier the rate of accumulation in the aqueous humour is limited by the time required for mixing.

This factor might also explain the marked variation in the rate of accumulation of oxygen in the aqueous humour, for the time of mixing of compounds in the anterior chamber may be as long as 20 to 40 min. (Langham, 1955b).

Summary

(1) A method suitable for the measurement of the concentration of oxygen in the aqueous humour by a polarographic technique is described. The sample for analysis is withdrawn into a micro-cell directly from the anterior chamber of the eye without protein precipitation or contact with air.

(2) The permeability of the excised cornea to oxygen has been determined. At 4°C. the diffusion coefficient of the excised cornea was 0.018 ± 0.0013 in eleven tests. Removal of the epithelial and endothelial layers did not affect this value significantly. Oxygen was found to move freely across the cornea in either direction, and its rate of transfer increased by 1 to 2 per cent. per $^{\circ} \text{C.}$

(3) In similar experiments with living animals, the oxygen tension in the aqueous humour on exposure of the anterior surface of the eye to an atmosphere of oxygen was analysed. The oxygen tension in the aqueous humour

rose to 130 mm. Hg in conditions of steady state from an initial value of 45 mm. Hg. From these results and a knowledge of the oxygen uptake of the component layers of the cornea, it has been calculated that the oxygen diffusing into the cornea from the aqueous humour is inadequate to supply the respiratory requirements of the epithelial layer.

(4) The oxygen tension in the aqueous humour of conscious rabbits before and after exposure to an atmosphere of 95 per cent. oxygen and 5 per cent. carbon dioxide has been determined. The blood-aqueous barrier has been found to be freely permeable to oxygen, and equilibration between the blood and the aqueous humour to be attained within approximately 1 hr. In animals in which the arterial oxygen tension was over 600 mm. Hg, the tension in the aqueous humour rose to approximately 250 mm. Hg. Evidence is given that the oxygen tension in the aqueous humour is determined by blood having a composition approaching that of arterial blood.

(5) Changes in blood flow through the ciliary processes, caused by the ligation of one common carotid artery or by cutting the cervical sympathetic, resulted in no marked change in the oxygen tension of the aqueous humour. In the sympathectomized animals the oxygen tension was equal in both eyes, while in animals with a ligation of the carotid artery the oxygen tension on the operated side was significantly below that on the contralateral side but still within the same range of values as in normal eyes.

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