The pH-temperature coefficient of rabbit anterior chamber aqueous humor

Bernard Schwartz

The pH-temperature coefficient of rabbit anterior chamber aqueous humor was determined for the range of 20 to 40° C. For two series of measurements at 3° C. intervals, the average coefficient was found to be -0.00603 pH units per degree centigrade.

The recent determination of the ocular temperature gradient indicated the necessity to define those ocular physiologic and metabolic functions, especially of the anterior segment of the eye, which are most temperature dependent. One such important function is that of pH or hydrogen ion activity of the anterior chamber aqueous humor. With the recent introduction of extremely stable pH meters as well as anaerobic, capillary glass electrodes requiring small volumes, the reproducibility of electrometric pH measurements has become more precise. The recent publication of revised standard values for phosphate buffers at different temperatures and the formulation of an additional phosphate buffer to cover the physiologic pH range have provided valuable references for obtaining pH measurements that are meaningful to a thousandth of a pH unit. Since the only data available on the pH-temperature coefficient for rabbit aqueous humor are those reported by von Saliman and Di Grandi for only two different temperatures, and the coefficient obtained by Langham and Lee for three different temperatures in the range 15 to 37.5° C., it was considered worthwhile to re-evaluate the pH-temperature coefficient.

Methods and materials

The Metrohm capillary glass electrode* was used for the electrometric pH determinations. The electrode is surrounded by a water jacket which was maintained at the desired constant temperature by a Haake water bath pump.† The bath temperature was calibrated against a National Bureau of Standards thermometer. The constancy of the bath temperature determined with a thermistor was found to vary not more than 0.25° C. over a 3.5 hour period at 35° C. The reference electrode was of the saturated calomel type with a diffusion pore opening of small diameter. The liquid junction between the reference electrode and the capillary electrode was maintained by means of a saturated potassium chloride solution. The volume of the capillary electrode was approximately 100 μl. The pH meter was of the chopper amplifier null-balance type‡ and its readability was a thousandth of a pH unit.

From the Division of Ophthalmology, Department of Surgery, State University of New York, Downstate Medical Center, Brooklyn, N. Y.

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*Model EA 128, distributed by Brinkmann Instruments, Inc., Great Neck, N. Y.
†Ultra Thermostat Type F, distributed by Brinkmann Instruments, Inc., Great Neck, N. Y.
‡Instrumentation Laboratory Inc., Boonton 15, Mass.
Standard phosphate buffers were made up from certified samples obtained from the National Bureau of Standards. The phosphate salts were dried and dissolved in boiled carbon dioxide-free, double distilled water of average specific conductivity of 0.9 micromhos per centimeter at 25° C. Both the 6.865 and the 7.413 at 25° C. phosphate buffers, which are of equivalent ionic strength, were used as primary reference standards. In addition, a third phosphate buffer of pH 7.806 at 35° C. of similar ionic strength was used as a secondary standard7 to check the linearity of the pH electrode. Before each pH measurement the glass electrode was standardized at least against the two primary standards and following each pH measurement was rechecked against the 6.865 standard for drift during the pH determination. Values of these buffers at different temperatures were taken from the recently published tables of Bates,7 and interpolated values were derived for those temperatures not specifically stated.

New Zealand albino male rabbits of 2.0 to 2.5 kilograms in weight were used as the experimental animals. They were maintained on a pellet diet supplemented by cabbage leaf once per week.8 Anterior chamber aqueous humor was aspirated in a Silicone greased syringe after two drops of 0.5 per cent Ophtheticf (proparacaine hydrochloride) were applied to the cornea and the excess blotted dry. Anterior chamber aqueous samples were pooled in the same syringe, care being taken not to expose the sample to the atmosphere and to remove all residual air bubbles from the syringe. Within each syringe was a small glass rod within which was embedded an iron wire. This was used to mix the contents of the syringe by passing the syringe back and forth through the poles of a horseshoe magnet.8 The number of passes required to obtain a sufficiently mixed sample had been previously determined by testing similarly mixed samples of 6.865 phosphate buffer and 0.1N sulfuric acid so that consecutive samples taken from the syringe gave identical pH readings.

After the aqueous humor samples had been obtained and mixed adequately within the syringe, the capillary electrode was calibrated with the reference buffer standards at the lowest selected temperature. At least two independent determinations were then taken of the pH of the aqueous humor. The temperature of the water bath was then elevated by 3° C. steps until the maximum temperature selected was reached. At each temper-ature the standardization of the glass electrode was repeated for the proper temperature values for the phosphate buffers both before and after the pH measurement of the aqueous humor. After the pH was measured at the highest temperature, the bath was then cooled to the initial temperature level and the pH of the aqueous humor reetermined after the glass electrode had been restandardized with the phosphate buffers. This procedure not only determined glass electrode stability in regard to thermal stress but also constancy of the aqueous humor sample over the experimental period. The total time taken for each of the two series of measurements was approximately 3 hours. All determinations were conducted in an air-conditioned room of average temperature of 24° C. pH values were read to the thousandth of a pH unit and the pH corrected for slight drifts of the electrode during pH determination. All aqueous humor samples were obtained in the late afternoon during the midsummer.

Results

The reproducibility of the method was determined by pooling and mixing the anterior chamber aqueous humor samples of 3 rabbits and obtaining 7 independent pH readings at 35° C. The mean value for the 7 determinations was 7.584 with a standard deviation of ±0.004.

Two series of pH readings of anterior chamber aqueous humor were then determined with 14 rabbits, 7 rabbits in each series. Nineteen determinations were done for the first series and 17 determinations for the second series (A and B, respectively) as shown in Fig. 1. The initial pH readings for Series A at 20° C. were 7.624, 7.618, and 7.619. The final pH readings at the end of the determinations at 20° C. were 7.619 and 7.629. The initial readings at 22° C. for Series B were 7.665 and 7.663. The final readings for Series B at 22° C. were 7.668 and 7.664. Essentially there is little difference between the initial and final readings of the two series.

The least square equations for both series were:

Series A, \( Y = 7.74368 - 0.0059062 \(X) \)

Series B, \( Y = 7.80122 - 0.0060549 \(X) \)

where \( Y = \) pH and \( X = \) temperature in degrees centigrade. The standard deviation
of the slopes are 0.0006807 for A and 0.0002928 for B, with an average of 0.0004868. The slopes of these equations differ by less than 1 per cent and therefore over the range of 20 to 40° C. the average pH-temperature coefficient is -0.00603 pH units per degree centigrade.

Comment

The pH-temperature coefficient obtained above differs by only 6 per cent from that reported by Langham and Lee⁵ of -0.0064 pH units per degree centigrade. However, both these coefficients are quite different from those calculated from von Sallman's and Di Grandi's data of -0.0074 pH units per degree centigrade.

It is apparent from the initial and final values obtained in both series that the temperature effect is reversible over this temperature range, which is similar to the observation made by Langham and Lee.⁵ Also, the relation between pH and temperature appears to be linear with the slope being constant for the temperature range studied.

In view of the previous reported data regarding the ocular temperature gradient in the rabbit, 33° C. is a more appropriate temperature for the anterior chamber at a room temperature of 22 to 24° C. Therefore, from Fig. 1, the pH of anterior chamber aqueous humor at 33° C. was 7.546 for Series A and 7.602 for Series B. These values are similar to those obtained previously by the author as well as those reported on a review of the literature.⁶ Slight differences in technique or possible air exposure of the samples may account for the difference in pH values between the two series.

From the data obtained for the ocular temperature gradient,¹ it appears that at least a difference of 1° C. exists across the anterior chamber. Therefore, it is to be expected that the aqueous humor at the posterior surface of the cornea will be at least 0.006 of a pH unit less than the aqueous humor bordering the anterior surface of the lens, assuming complete mixing of the anterior chamber contents. This represents a difference of $0.04 \times 10^{-3}$ gram moles per liter of hydrogen ion or a 1.4 per cent change. The ocular temperature gradient thus creates a hydrogen ion concentration gradient.

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REFERENCES