Temperature gradients in the rabbit eye

Bernard Schwartz and Martin R. Feller*

Measurements of the temperature of the various parts of the rabbit eye under both local and general Nembutal anesthesia were made with the thermistor as the temperature-measuring device at a room temperature of 22 to 24° C, and a relative humidity of 33 to 53 per cent. The equipment and its calibration are described in detail. The existence of a temperature difference of 6° C. (corrected for the depressant effect of Nembutal anesthesia on rectal temperature) was shown to exist between the orbit and the central corneal surface along the pupillary axis. No significant difference was noted between right and left eyes except for the central corneal surface temperature under local Ophthaine anesthesia, when the temperature of the left eye was found to be higher than that of the right by 0.5° C. An estimation of the temperature gradients indicates that at the mid-lens position the gradient increases sharply toward the corneal surface. Some of the possible physiologic effects of the ocular temperature gradient are discussed, such as the choice of temperature for study of the metabolism of ocular tissues in vitro, the role of the temperature gradient in the maintenance of the intraocular pressure on the basis of the irreversible thermodynamic approach, and its role in the transfer of fluid across the cornea.

Studies of ocular temperature have generally fallen into four areas: superficial temperature determinations of conjunctiva and cornea, thermal circulation of anterior chamber aqueous, the effect of radiation on eye temperature, and the determination of various intracocular temperatures. Dohnberg1 initiated the ocular temperature studies which were concerned with determinations of the superficial temperatures of the conjunctiva and cornea. The early studies were reviewed and continued by Holmberg.2 Recently Amano,3,4 Piper and Beyer,5 Huber,6 and Matthias7 have investigated superficial conjunctival and corneal temperatures further. With the introduction of the slit lamp, the thermal circulation of anterior chamber aqueous was confirmed and attributed to a difference in temperature between the posterior surface of the cornea and the anterior surface of the iris. Studies on the thermal circulation were reviewed by Amsler and co-workers.8 Numerous experiments that describe the action of ultrasonic waves and infra-red, shortwave, and microwave radiation on the tissues of the eye through their temperature effect were reviewed by Duke-Elder9 and Nordmann.10 Recent studies by Carpenter and associates11 on microwaves, by Irvine and Knoll12 on shortwave diathermy, and by Langley and co-workers13 on infra-red have also included temperature measurements of the eye. Although earlier studies14,15 have demonstrated that a temperature gradient

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exists in the eye, there have been few systematic determinations of temperature of its various parts, and, in particular, of gradients within the eye. Andrew,16 Kotsuka,17 Walther and associates18 and, more recently, the Japanese authors, Amano19 and Nakao,19 have presented such data.

The experiments described in this report were undertaken to confirm and measure the temperature gradient in the individual rabbit eye with the thermistor. Data were to be obtained particularly regarding the temperature of the lens. These values would then be used to establish a physiologic norm for the temperature required for the study of lens metabolism in a perfusion culture system.29 It is also the purpose of this paper to point out the physiologic significance of the ocular temperature gradient.

Materials and methods

The thermistor rather than the thermocouple was used as the temperature-measuring device because it was available in many sizes and shapes; a bead as small as 0.015 inch in diameter could be purchased. It was also relatively inexpensive and required simple auxiliary electronic equipment, and did not need a "cold junction" kept at constant temperature as did a thermocouple. Its major disadvantage was that a large electrical tolerance exists in small-sized thermistors so that each one had to be calibrated individually which made temperature probes uninterchangeable. Another disadvantage was the need for recalibration when a thermistor was subjected to repeated large temperature cycles because of aging. A Wheatstone bridge similar to the design employed by Gray and Axelrod21 was used for measurement of resistance of the thermistor. The output of the bridge was then fed into a two-tube differential d. c. amplifier, and then into a Sanborn pen recorder. With this equipment the maximum sensitivity obtained was approximately 1° C. full-scale deflection. Any one-degree segment could be selected for display on the recorder by means of the bridge-balancing control.

Several different types of temperature probes were constructed with Veco* thermistors. These were: a small bead thermistor mounted at the tip of a right-angled Lucite probe with a handle approximately 5 inches long for measuring the temperature of the center of the cornea and the conjunctival sac; a bead thermistor mounted in a long, flexible plastic covering for ambient air temperature measurements; a bead thermistor mounted in a 4 inch length, stainless steel tubing approximately ¥ inch in diameter for rectal temperature measurements; and a very small bead thermistor mounted in the tip of a 22 gauge stainless steel hypodermic needle 3 inches long* for measurement of temperature in various parts of the eye. The probes were calibrated in a water bath with a mercury thermometer graduated to 0.05° C., which had been calibrated against a United States National Bureau of Standards thermometer as a reference. The Wheatstone bridge was balanced by means of its 10 turn precision potentiometer for each probe for a given temperature. A plot of temperature versus meter reading was obtained for each probe. The linearity obtained was excellent, and the resetability of the bridge was on the order of 0.02° C. The bridge itself gave a sensitivity of approximately 5° C. full-scale meter deflection with all probes, except the needle probe, the sensitivity with the latter was 10° C. full scale.

The experimental animals were New Zealand white albino male rabbits which weighed between 4.5 and 5.5 pounds. For the experiments conducted with local anesthesia, each eye was anesthetized with 2 drops of 0.5 per cent Ophthaine (2-dimethylaminoethyl-3-amino-4-propoxybenzoate hydrochloride). Air temperature, relative humidity with a wet bulb thermometer, rectal temperature, and pupil size by millimeter rule were all measured and recorded. The animals were kept in a prone position with the head erect throughout the entire procedure to maintain eye exposure to room temperature. Temperature measurements were taken at the center of the corneal surface and in the inferior conjunctival sac of each eye; sometimes the right eye was measured first, and other times the left, in an attempt to determine if the order of measurement had an effect upon the temperature. Before each measurement the eyelid was closed for one minute, then opened for one minute, then closed for one minute, and then reopened for one minute. This procedure was found to be necessary in order to give reproducible measurements by standardizing the effect of lid closure upon corneal temperature. Measurements were repeated several times in each eye and the average recorded. The standard deviation of the conjunctival and corneal temperatures was estimated by measuring the differences between duplicates according to Youden22 and was found to be 0.20° C. for the conjunctiva and 0.26° C.

*Victory Engineering Corporation, Union, N. J.
for the cornea in 6 and 14 pairs of duplicate determinations, respectively.

The animals were then put under general anesthesia with an intraperitoneal dose of Nembutal,\(^*\) approximately 25 mg per kilogram body weight, alter which air temperature, relative humidity, pupil size, and rectal temperature were again measured and recorded. The temperature of the center of the corneal surface and that of the inferior conjunctival sac were again measured with the same lid-closure cycle. The needle probe was used to penetrate the eye from the center of the cornea along the pupillary axis; temperatures were recorded at each structure within the eye. Indication of intraocular position beyond the midlens point was obtained by estimating the depth to which the needle was penetrating as well as the resistance encountered within each structure. The probe was left in each structure until a constant temperature was recorded, then advanced to the next structure. During the passage of the needle probe, the Sanborn recorder was used to give a continuous recording. Calibration of the equipment was repeated after the experiments; no measurable change was found. All experiments were performed during the late afternoon and early evening. For statistical analysis, the Student t test of significance was used in comparing the difference of means.

Results

The environmental conditions under which the data on temperature gradients in the rabbit eye were obtained were fairly narrow in range. The laboratory air temperature for the 7 major experiments varied from 22.0 to 24.0° C. In addition, air temperatures 6 inches lateral to each eye and one inch in front of each eye were also measured. The lateral temperatures ranged from 23.2 to 24.2° C. for the right eye, and from 22.9 to 24.1° C. for the left eye. The air temperatures one inch in front of the right eye ranged from 23.2 to 24.3° C., whereas those for the left were 23.0 to 24.3° C. The relative humidity, as measured by the wet bulb method, ranged from 33 to 53 per cent.

At the 5 per cent probability level Nembutal anesthesia had no effect on the temperature of the central corneal surface; the local Ophthaine temperatures minus those for Nembutal were, for the same eye, 

\[ -0.19 \pm 0.63° \text{C.} \] (10) (mean ± standard deviation, number of determinations).

However, there was a significant difference at the 5 per cent level for temperatures of the inferior conjunctival sac, which dropped slightly under Nembutal anesthesia. Temperatures for the local anesthesia (Ophthaine) minus those for general anesthesia (Nembutal) for the same eye were

\[ +0.26 \pm 0.31° \text{C.} \] (12). It was also noted that the mean rectal temperature for 7 rabbits prior to Nembutal anesthesia was 39.73° C. whereas during anesthesia it was 39.13° C. This is a significant difference, for every animal showed a drop in temperature with anesthesia. Similarly, there was a small change in pupil size; the average pupil diameter prior to Nembutal anesthesia was approximately 5 mm., whereas with anesthesia there was an increase in diameter of 1 to 2 mm.

An analysis of the data for determining if temperature differences existed between the right and left eyes in the same animal with local and general anesthesia indicates that, at the 5 per cent level of significance, only the temperature of the central corneal surface showed a significant difference under local anesthesia, and that for the left eye it was greater by 0.53 ± 0.51° C. (19). No significant differences were noted for the inferior conjunctival sac, mid-

\[ \text{Fig. 1. Recording of intraocular temperature as thermistor needle probe is advanced through the eye from midanterior chamber (A.C.) to anterior lens surface (A.L.) to mid-lens (M.L.) to midvitreous humor (M.V.H.) to retina-choroid (R.C.) and to ORBIT. (Ordinate-temperature [° C.] and abscissa-time.)} \]
anterior chamber, anterior lens surface, mid-lens, mid-vitreous humor, retina-choroid, and orbit.

Since there appeared to be no significant difference in temperatures between eyes except for the central corneal surface, those

Table I. Ocular temperatures (°C.)

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean ± standard deviation</th>
<th>No. of determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local, Ophthaine anesthesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central corneal surface</td>
<td>32.16 ± 0.76</td>
<td>12</td>
</tr>
<tr>
<td>Right eye</td>
<td>31.88 ± 0.73</td>
<td>6</td>
</tr>
<tr>
<td>Left eye</td>
<td>32.42 ± 0.74</td>
<td>6</td>
</tr>
<tr>
<td>Inferior conjunctival sac</td>
<td>35.77 ± 0.79</td>
<td>14</td>
</tr>
<tr>
<td>General, Nembutal anesthesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central corneal surface</td>
<td>32.30 ± 0.49</td>
<td>9</td>
</tr>
<tr>
<td>Inferior conjunctival sac</td>
<td>38.74 ± 0.54</td>
<td>12</td>
</tr>
<tr>
<td>Mid-anterior chamber</td>
<td>32.98 ± 0.74</td>
<td>13</td>
</tr>
<tr>
<td>Anterior lens surface</td>
<td>33.60 ± 1.01</td>
<td>10</td>
</tr>
<tr>
<td>Mid-lens</td>
<td>35.42 ± 1.00</td>
<td>13</td>
</tr>
<tr>
<td>Mid-vitreous humor</td>
<td>35.56 ± 0.90</td>
<td>13</td>
</tr>
<tr>
<td>Retina-choroid</td>
<td>37.02 ± 0.86</td>
<td>13</td>
</tr>
<tr>
<td>Orbit</td>
<td>37.68 ± 0.71</td>
<td>13</td>
</tr>
</tbody>
</table>

Table II. Regional ocular temperature differences in the individual eye (°C.)

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean difference ± standard deviation</th>
<th>No. of determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local, Ophthaine anesthesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior conjunctival sac minus central corneal surface</td>
<td>+ 6.84 ± 0.49</td>
<td>12</td>
</tr>
<tr>
<td>General, Nembutal anesthesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior conjunctival sac minus central corneal surface</td>
<td>+ 6.35 ± 0.49</td>
<td>10</td>
</tr>
<tr>
<td>Mid-anterior chamber minus central corneal surface</td>
<td>+ 0.80 ± 0.74</td>
<td>9</td>
</tr>
<tr>
<td>Anterior lens surface minus mid-anterior chamber</td>
<td>+ 0.77 ± 0.48</td>
<td>10</td>
</tr>
<tr>
<td>Mid-lens minus anterior lens surface</td>
<td>+ 1.57 ± 0.89</td>
<td>10</td>
</tr>
<tr>
<td>Mid-vitreous humor minus mid-lens</td>
<td>+ 1.14 ± 0.57</td>
<td>13</td>
</tr>
<tr>
<td>Retina-choroid minus mid-vitreous humor</td>
<td>+ 0.47 ± 0.52</td>
<td>13</td>
</tr>
<tr>
<td>Orbit minus retina-choroid</td>
<td>+ 0.65 ± 0.42</td>
<td>13</td>
</tr>
<tr>
<td>Orbit minus central corneal surface</td>
<td>+ 5.49 ± 0.91</td>
<td>10</td>
</tr>
</tbody>
</table>

Table III. Temperature gradients of the individual rabbit's eye

<table>
<thead>
<tr>
<th>Site</th>
<th>°C./mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-anterior chamber to central corneal surface</td>
<td>0.53</td>
</tr>
<tr>
<td>Anterior lens surface to mid-anterior chamber</td>
<td>0.77</td>
</tr>
<tr>
<td>Mid-lens to anterior lens surface</td>
<td>0.39</td>
</tr>
<tr>
<td>Mid-vitreous humor to mid-lens</td>
<td>0.14</td>
</tr>
<tr>
<td>Retina-choroid to mid-vitreous humor</td>
<td>0.13</td>
</tr>
<tr>
<td>Orbit to retina-choroid</td>
<td>1.30</td>
</tr>
</tbody>
</table>

for each structure were grouped together to give mean values (Table I). It must be emphasized again that the measurements of intraocular temperature (Table I) were made along the pupillary axis of the eye. The data indicate a gradual increase in temperature extending from the central corneal surface to the orbit. The temperature levels are presented graphically in Fig. 1, an actual recording of the temperature in a rabbit's eye as the needle probe was advanced from the anterior chamber to the orbit along the pupillary axis.

Table II shows the regional ocular temperature differences calculated for each eye. The greatest difference within the eye appeared to exist from the mid-lens position to the anterior surface of the lens. Under Nembutal anesthesia the difference of +5.49° C. from the orbit to the central corneal surface would be slightly higher if a correction were added for the depressant effect of Nembutal anesthesia upon body temperature. Since the rectal temperatures were, on the average, decreased by 0.6° C., this amount, when added to the orbit to central corneal surface difference, would give a value of about 6° C.

Table III shows the ocular temperature gradients calculated from the data in Table II in terms of the distance in millimeters along the pupillary axis with the central corneal surface area as the zero reference point. The dimensions for the rabbit's eye were taken from Davson. These data are presented graphically in Fig. 2 in which the mean temperatures for Nembutal anesthesia (Table I) were plotted against distance in millimeters along the pupillary
The temperature-gradient curve of the rabbit eye appears to have three different slopes.

Discussion

The temperature measured in each part of the eye is determined by the balance of the various modes of gain and loss of heat. It is intuitively obvious that the corneal surface of the eye is cooler than the posterior portion which is highly vascular. The corneal temperature is, thus, undoubtedly strongly dependent upon air temperature and humidity. In these experiments the range of both humidity and air temperature was small so that this dependency could not be measured. In preliminary experiments the effect of lid closure was found to have a distinct influence on the surface temperature of the central cornea. In order to standardize this factor, the lid-closure cycle described under Methods and Materials was adopted.

The effect of Nembutal anesthesia on conjunctival temperature indicates the large role of the blood stream in providing heat to the eye. It appeared that the decrease in temperature of the conjunctiva was primarily due to the decrease in temperature of the body core, and, thus, the temperature of the blood stream, as evidenced by the depression of rectal temperature. The decrease in body temperature under Nembutal anesthesia is well known, it was also noted for the eye by von Sallmann and di Grandi. They observed a 0.2° C. depression of temperature in the anterior chamber with Nembutal anesthesia in the chinchilla rabbit, whereas the rectal temperature depression we obtained was 0.6° C.

No adequate explanation appears to be available for the significant difference in temperature between the right and left central corneal surface. In surveying the literature on determinations of surface temperature of the eye, only Amano has indicated a difference between the two eyes. He found that the temperatures of both the palpebral conjunctiva and the skin lid of the left eye were consistently greater than those of the right in human beings.

It is difficult to compare the absolute temperature measurements reported in Table I with those in the literature since the factors known to influence ocular temperature have to be considered, factors such as relative humidity, pupil size, lid closure, protrusion of the globe, rectal temperature, and position of the animal. It is also important to know whether calibrated standard thermometers have been used as a reference. Since the temperatures in this study were measured only in the pupillary axis, comparison with peripheral temperatures reported in the literature is impossible. Although absolute temperatures cannot be compared, the differences between various parts of the eye can be. Nakao's careful measurements with thermocouples correspond well to the data reported upon here. He found a difference of 1.78° C. on successive measurements from the corneal surface to the anterior part of lens, whereas the corresponding data in Table II show a temperature difference of 1.57° C. Similarly, he reported that the total temperature difference from the corneal surface to the posterior pole of the vitreous is 4.65° C., whereas the corresponding data from Table II including the retina-choroid measurement indicate a difference of 4.75° C. Although the differ-
ences in technique and environmental conditions were considerable, a review of the literature shows that all authors have reported the existence of a temperature gradient within the eye, with the anterior portion lower than the posterior.

Table III and Fig. 2 indicate that the sclera acts as a good insulator since there is a steep slope to the gradient from orbit to retina-choroid. In the vitreous cavity there appears to be little change in temperature gradient, probably because the vitreous is surrounded by a shell of vascular tissue that radiates heat and maintains a homogenous temperature. However, beginning with the mid-lens position and extending to the anterior surface of the eye, there was a sharp drop in the temperature gradient. Considerable heat loss with exposure of the eye to the atmosphere occurs here.

The original purpose of this paper was to determine the temperature at which cultures could be made of the rabbit lens in a perfusion system. In earlier studies of the mitotic activity of lens epithelium in a closed-culture system, Constant\textsuperscript{25} noted an effect of small differences of temperature. It appears that, almost across the lens, there is a mean temperature difference in the pupillary axis of 1.57° C. (Table II). One must also consider that the peripheral portion of the lens may have a slightly higher temperature.\textsuperscript{20} Assuming that the measured temperatures are close to the physiologic norm, the problem of maintaining the temperature gradient across the lens is difficult in any culture system. It therefore appears that an average temperature close to the temperature of the lens in the rabbit eye should be chosen. The midlens temperature of approximately 35° C. appears to be a reasonable temperature at which future lens cultures should be maintained: this is in contrast to the previously chosen temperature of 37° C.\textsuperscript{20}

The physiologic significance of the ocular temperature gradient

The evolutionary adaptation of the vertebrate eye to aerial vision is aptly discussed by Walls.\textsuperscript{33} However, one unconsidered problem faced by the homeothermic vertebrate was the development of an ocular temperature gradient. The closure of the lids with their warm palpebral conjunctiva, the presence of a nictitating membrane, and the spread of warm tears across the corneal surface all help to decrease this gradient. However, tears in themselves lead, perhaps more to an increase in the gradient because of the loss of heat by vaporization. Such a gradient in the eye of the homeothermic vertebrate may play a significant part in many of its functions, especially those of the anterior portion where the gradient is steepest.

One of the most obvious effects of temperature is the change in rate of the metabolic reaction. For most biological systems, an increase in temperature of 10° C. approximately doubles the reaction rate.\textsuperscript{30,37} It is apparent, therefore, in view of the existing temperature gradient, especially in the anterior portion of the globe, that in any attempt to study ocular tissues in vitro the corresponding temperature in vivo should be used. A drop in temperature for culture of the lens of 2° C. may possibly decrease the metabolic rate of the lens in the order of 20 per cent.

Similarly, an evaluation of the metabolism of the cornea in vitro should ideally be made not only at a temperature of about 32° C. (Table I), but should also include some allowance for a temperature difference across its structure, the anterior surface of which is approximately 0.8° C. cooler than the posterior surface (Table II).

Another physiologic effect of the temperature gradient may be on the transport of substances within as well as out of the eye. The difference in temperature existing, especially across the anterior surface of the iris, may be an effective force in the movement of water and ions across the surface.
From the equations of irreversible thermodynamics taken from Lifson and co-workers, water transport or flux per unit area \((J_w)\) across a given plane in a membrane may be considered to be of two parts, the first due to a force acting directly upon water molecules, and the second due to coupling of water transport to other driving forces, particularly those for solutes and heat.

\[
J_w = -k_w c_w \frac{dp}{dx} + J_{w,c}
\]

where \(-\frac{dp}{dx}\) = total chemical potential gradient of water, \(c\) = concentration of water molecules, \(k_w\) = permittivity of the membrane to water, and \(J_{w,c}\) = water flux due to all coupling effects. In the first part of the above equation, the total chemical potential \((\mu)\) is a function primarily of pressure, concentration, electrical potential, and temperature. For uncharged particles, such as water, the electrical potential term becomes zero and thus the direct total driving force on water consists mainly of three force gradients: the hydrostatic pressure, \(\frac{dp}{dx}\); the osmotic pressure in dilute solutions, \(\frac{ds}{dx}\); and the temperature difference, \(cS \frac{dT}{dx}\), where \(S\) is the molar entropy.

The force gradients of hydrostatic and osmotic pressure have already been considered in the equations for the movement of ions and water into and out of the aqueous humor. Since these equations are formulated on the basis that the eye exists as an isothermal system, one force which has not been estimated as important is that of the temperature gradient. The importance of this force on the movement of water has been indicated by Spanner. On a theoretical basis, it appears to be of considerable magnitude being able to produce a pressure of approximately 132 atmospheres per degree centigrade on the low temperature side of the membrane. However, as Spanner has pointed out, there are other factors which appear to decrease the estimate of this particular high value. In view of the lack of osmotic effect on intraocular pressure shown recently, the temperature-gradient force may have a significant role in maintaining intraocular pressure. Some discussion on this subject by Fremont-Smith has appeared recently. The decreased ocular tension obtained by Pollack, Becker, and Constant and Davson and Spaziani in their experiments on hypothermia could be partially explained by the decrease in the ocular temperature gradient.

Not only may ocular temperature gradients play a part in the transport of substances across the blood-aqueous barrier, but they also may play a part in the transport of substances across the cornea. A mean temperature difference of approximately 0.8°C exists across the cornea (Table II). Recent experiments by Mishima and Maurice on measuring corneal thickness in the rabbit indicated that upon opening the lids, there is a small but significant decrease in corneal thickness. This seems to be related to the degree of evaporation of the tear film. The temperature gradient thus created may be the mechanism for partial maintenance of the physiologic state of corneal dehydration.

REFERENCES
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