Passive radiotelemetry of intraocular pressure in vivo: calibration and validation of continual scleral guard-ring applanation transensors in the dog and rabbit

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Continual monitoring of intraocular pressure (IOP) by passive radiotelemetry, with a miniature scleral applanating device, mounted in a haptic contact lens (applanating transensor) is reported in the rabbit and dog. Several prototype transensors were calibrated in vivo in the rabbit and dog; a consistent linear output was obtained for each eye, although reproducibility of the slope between eyes over a period of time was less than ideal. Accuracy of determination of IOP was found to be better for the rabbit and will have to be improved before the instrument can be applied to the human. Multiple regression analysis of calibration studies in six rabbits established that the transensor output was not significantly affected by the small changes in body temperature and atmospheric pressure encountered during the study. Continual scleral tonometry with a standard Mackay-Marg tonometer showed that ocular rigidity has a significant effect on calibration curves, in contradistinction to standard Mackay-Marg corneal tonometry. A time-dependent decay in tonometer readings was more evident in canine eyes with low ocular rigidity. Since the transensor is also a guard-ring applanating device, calibration may be necessary for individual eyes and species.

Key words: biotelemetry, passive radiotelemetry, continual intraocular pressure monitoring, intraocular pressure, transensor calibration, guard-ring applanation, Mackay-Marg tonometry of sclera, Mackay-Marg tonometry of cornea

Continual noninvasive monitoring of intraocular pressure (IOP) would be beneficial for management of the glaucomas. Previous attempts to develop a noninvasive method with contact lens—mounted strain-gauge transducers have required wires to transmit pressure information to a receiving system. One of these systems has been tested for tolerance in the human. A short-term method of monitoring with a tube-connected pressure transducer has recently been described with a hard corneal contact lens. Passive radiotelemetry, on the other hand, requires no direct wiring or tubing to connect sensor and monitoring device and no internal power source and is ideal for miniaturization. It has been used in the past for invasive monitoring of IOP in the rabbit.

Where monitoring of diurnal IOP variations is required, a corneal applanating device should be avoided. We have developed a
Fig. 1. Passive radiotelemetry of IOP: transensor incorporated in a polymethylmethacrylate haptic contact lens.

guard-ring applanating transensor (AT) that continually applanates the sclera, while held in a haptic contact lens in the conjunctival fornix, as first suggested by Mackay. We have found this system capable of continual monitoring of IOP by passive radiotelemetry in vitro. One of us (D. G. B.) has developed an automatic continual frequency monitor (ACFM) for tracking and recording of the resonant frequency (Rf) of the AT. This Rf has been found to be linearly related to IOP in vitro.

The present study was undertaken to evaluate the behavior of several prototype ATs in vivo, with reference to the linearity of calibration curves over a period of time and the effect of variations of temperature and atmospheric pressure on the transensor output.

Since this device acts on sclera as a guard-ring applanator, it was necessary to investigate the validity of continual guard-ring applanation of the sclera. Many data are available for instantaneous guard-ring applanation, but these are not applicable to continual transscleral monitoring of IOP.

Materials and methods

Adult rabbits and dogs were used for all experiments. Transensors, previously described, were mounted in polymethylmethacrylate (PMMA) cases (Western Australian Contact Lens Manufacturing) with either a soft-molded (Xantropren) silicone rubber or a PMMA haptic element (Fig. 1). The AT was placed in the upper conjunctival fornix, and the aerial coil assembly of the ACFM positioned and glued to the outer surface of the eyelid with Dow-Corning adhesive B, in such a position that pulsations of IOP due to heart beat were observed on the oscilloscope used for monitoring output of the ACFM (Fig. 2). The Rf of the AT was monitored by a digital frequency counter set to count over 1 sec.

The anterior chamber was cannulated with a 19-gauge needle connected to a variable-height reservoir containing heparinized normal saline, and a water manometer calibrated in millimeters of mercury. For each calibration experiment, IOP was varied in stepwise fashion by upward and downward movements of the reservoir. After at least 30 sec at each pressure point, mean Rf of at least four readings was obtained under open-stopcock conditions. Cul-de-dac and rectal temperatures were recorded from the rabbits used for the study of body temperature effects. Atmospheric pressure was recorded in millimeters of mercury from the local meteorological office.

The effect of temperature on transensor output was determined by immersion of the AT in a variable-temperature saline bath. Rf was recorded for each change in temperature.

Validity of continual scleral guard-ring applanation was studied by clamping a Mackay-Marg transducer (Berkeley Bioengineering, Inc., San Leandro, Calif.) in contact with the sclera or cornea of the anesthetized rabbit or dog. IOP was varied under stopcock conditions, and the output of the instrument recorded in millimeters of chart reading. At least two up-down sequences were made between 0 and 30 mm Hg. Prolonged scleral Mackay-Marg readings at constant IOP were also recorded from one dog and one rabbit eye.

Statistical methods. Mean values of Rf were

Fig. 2. Passive radiotelemetry of IOP in vivo: experimental calibration apparatus.

Fig. 3. Passive radiotelemetry of IOP in vivo: calibration curve and linear regression constants for both eyes of the same rabbit, 1 week apart. The same transensor was used for each experiment. Each point represents the mean of 10 readings ± 2 × S.E.M.

plotted against IOP. Linear regression of Rf against IOP was calculated by the method of least squares. The coefficient of determination ($r^2$) was calculated for each regression line. The coefficient of determination is defined as the proportional reduction of total variation of Rf of the transensor, associated with the use of the independent variable IOP, with which Rf is compared. If there is an absolute correlation between Rf and IOP, $r^2 = 1.0$, and conversely, where there is no correlation, $r^2 = 0$. The correlation coefficient (R) is defined as the square root of the coefficient of determination but does not have such a clear cut significance as $r^2$, when applied to regression analysis.10

The accuracy of the monitor was assessed by examination of the scattergrams in order to assess the range of IOP values that would be expected from any one value for Rf. This is an approximate method of determining the inverse confidence interval, the calculation of which is complex and has serious theoretical limitations. For comparison, the 99% confidence interval for the determination of Rf from the regression line of Rf on IOP (or IOP on Rf) has fewer theoretical limitations and was calculated for one case.10

Regression constants calculated for each calibration experiment relate Rf to IOP as a linear model of Rf on IOP:

$$Rf = A + B \cdot P$$

where $Rf$ = resonant frequency in megahertz (MHz); $A$ = intercept of the extrapolated regression line with the ordinate (this constant is assumed to be the baseline Rf of the AT (in MHz) at cul-de-sac temperature and zero IOP); $B$ = slope
Fig. 4. Passive radiotelemetry of IOP in vivo: calibration curves and regression constants for (A) rabbit and (B) dog. The same transensor, but with different molded haptics, was used for each experiment. Each point is the mean of five to 10 readings ±2 x S.E.M. •, Upper lid; a, lower lid. (Lower lid points shown for comparison only, not used in regression.)

Fig. 5. Temperature characteristics of transensor. The best-fit curve by linear regression of Rf on ambient temperature, in degrees Centigrade is drawn, together with the regression constants.

Results

Linearity of response of the AT is illustrated in Fig. 3, where data are recorded from two separate experiments, 1 week apart, in the same rabbit. The same AT was used for each experiment.

The regression constants obtained for each experiment were as follows:

Week 1: Rf = 27.834 + 0.013 P, r² = 0.93
Week 2: Rf = 27.820 + 0.013 P, r² = 0.86
All data: Rf = 27.823 + 0.013 P, r² = 0.89

The estimated standard deviation of Rf around this regression line was 0.049 MHz.

The 99% confidence interval was calculated for the regression line shown in Fig. 3, for four levels of IOP: 10, 20, 30 and 40 mm Hg. The calculated confidence interval ranged from 0.0123 to 0.0201 MHz. This implied that, for the worst case, 99% of the Rf readings lay between the limits ±0.0201 MHz. Since the slope of the regression line...
Fig. 6. Passive radiotlemetry of IOP: computer-generated scattergram of mean Rf readings for 328 points of IOP obtained from six different rabbits, over 2 months, with the same AT (polymethylmethacrylate haptic). Figures denote coincident points. These data formed the basis for a multiple regression analysis of Rf on IOP, body temperature, and atmospheric pressure. See text.

was 0.013 MHz/mm Hg, this represented a range of values for determination of IOP of ±(0.0201/0.013) mm Hg = 1.55 mm Hg for 99% of Rf readings.

IOP was also plotted against Rf, with single determinations of Rf for each value of IOP. The regression line had the following regression constants:

\[
\text{IOP} = 64.36 \text{Rf} - 1787.32
\]

\[
\text{r}^2 = 0.85
\]

The estimated standard deviation of IOP around this regression line was 3.41 mm Hg. The 99% confidence interval was calculated for four levels of Rf from 27.051 to 28.348 MHz. The confidence interval varied from 0.89 to 1.85 mm Hg. This meant that for any value of Rf, 99% of the values of IOP fell between the limits ±1.85 mm Hg for the worst case. However, by inspection of the scatter of results, it could be seen that for any one level of Rf, the range of IOP was approximately 15 mm Hg. This gave an inverse confidence interval in the order of ±7.5 mm Hg for the determination of IOP for any value of Rf.

The effect of species difference on the calibration slope and baseline is shown in Fig. 4, in which data for two calibration experiments are plotted for the rabbit (A) and the dog (B). The same AT was used for each experiment, but with different haptics,
Table IA. Multiple regression of Rf on IOP, body temperature, and atmospheric pressure

<table>
<thead>
<tr>
<th>Variable related to Rf</th>
<th>Regression constants</th>
<th>Standard error of constant</th>
<th>(F)*</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear regression:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IOP</td>
<td>0.018 MHz/mm Hg</td>
<td>0.014 MHz/mm Hg</td>
<td>172.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Intercept</td>
<td>27.915 MHz</td>
<td>0.026 MHz</td>
<td>1150710</td>
<td>0.0001</td>
</tr>
<tr>
<td>Multiple regression:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IOP</td>
<td>0.018 MHz/mm Hg</td>
<td>0.0014 MHz/mm Hg</td>
<td>168</td>
<td>0.0001</td>
</tr>
<tr>
<td>Body temperature</td>
<td>-0.024 MHz/°K</td>
<td>0.015 MHz/°K</td>
<td>2.48</td>
<td>0.116†</td>
</tr>
<tr>
<td>Atmospheric pressure</td>
<td>0.006 MHz/mm Hg</td>
<td>0.0034 MHz/mm Hg</td>
<td>2.95</td>
<td>0.087†</td>
</tr>
<tr>
<td>Intercept</td>
<td>31.003 MHz</td>
<td>4.76 MHz</td>
<td>42.37</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*F, see text.
†Not significant.

Table IB. Correlation coefficients (R)* for each variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean Rf</th>
<th>IOP</th>
<th>Body temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP</td>
<td>0.588</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Body temperature</td>
<td>0.021</td>
<td>0.122</td>
<td>—</td>
</tr>
<tr>
<td>Atmospheric pressure</td>
<td>0.135</td>
<td>0.130</td>
<td>0.299</td>
</tr>
</tbody>
</table>

*See text.
moulded to fit each animal. Best fit curves were as follows:

Rabbit: \( R_f = 30.014 + 0.014 P \), \( r^2 = 0.92 \)

Dog: \( R_f = 29.351 + 0.017 P \), \( r^2 = 0.78 \)

The data suggested that there was a better linear fit for the rabbit than the dog. Accuracy of determination of IOP for any value of Rf for the dog was considerably worse than for the rabbit, the confidence interval for which was in the order of ±7.5 mm Hg as determined by inspection of the scattergram Fig. 4, (A). For the dog, the confidence interval could not be assessed easily because of the lack of data points.

The difference in baseline Rf was largely a result of the difference in haptic size. Haptic mass had a profound effect on the resonant circuit, by virtue of its dielectric properties.

The temperature characteristic of the AT is shown in Fig. 5, as a plot of Rf in response to temperature changes, with atmospheric pressure constant. The best fit curve was calculated as follows:

\( R_f = 28.200 - 0.030 C \), \( r^2 = 0.96 \)

Table II. Comparison of calibration slopes with a Mackay-Marg tonometer under continual and instantaneous conditions for dog and rabbit eyes in vivo

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Regression constants</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont. scleral</td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>dog eyes</td>
<td>1.48</td>
<td>0.80</td>
<td>0.89</td>
</tr>
<tr>
<td>rabbit eyes</td>
<td>-1.39</td>
<td>0.37</td>
<td>0.92</td>
</tr>
<tr>
<td>Cont. corneal</td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>dog eyes</td>
<td></td>
<td>-0.55</td>
<td>0.43</td>
</tr>
<tr>
<td>rabbit eyes</td>
<td></td>
<td>-1.24</td>
<td>0.41</td>
</tr>
<tr>
<td>Instant. corneal</td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>dog eyes</td>
<td></td>
<td>-0.67</td>
<td>0.48</td>
</tr>
<tr>
<td>rabbit eyes</td>
<td></td>
<td>-0.77</td>
<td>0.52</td>
</tr>
<tr>
<td>Human calibration</td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>supplied for cornea</td>
<td></td>
<td>0</td>
<td>0.50</td>
</tr>
</tbody>
</table>

For explanation of regression constants see text; n = the number of readings taken for each IOP; \( r^2 \) is coefficient of determination (see text).

where \( C \) = temperature of the saline bath (in degrees Centigrade).

The order of change in Rf for each degree change in temperature was thus 0.030 MHz. The AT used in this experiment was found to have a calibration slope of 0.018 MHz/mm Hg, when used over 2 months in six rabbits. This means that an error of 1.63 mm Hg would be expected for each degree Centigrade change in cul-de-sac temperature.

For the multiple regression analysis of the effect of body temperature, atmospheric pressure, and IOP on Rf of the AT, the data...
consisted of 328 separate determinations of mean \( R_f \) for each level of IOP, in 24 experiments using six rabbits. The data are plotted in the scattergram in Fig. 6. The constants calculated for each variable together with their correlation coefficient \( R \) are shown in Tables IA and IB. Although IOP gave a highly significant relationship with \( R_f \), of 0.018 MHz/mm Hg, rectal temperature gave a negative slope of 0.024 MHz per degree Kelvin, which was not significant \( (p = 0.116) \). (Mean rectal temperature was 38.74\(^\circ\) C \( \pm0.57, \) S.D., \( n = 40 \); mean cul-de-sac temperature was 38.20\(^\circ\) \( \pm0.58, \) S.D., \( n = 10 \)) Atmospheric pressure was related to \( R_f \) by 0.006 MHz/mm Hg, which was also not significant \( (p = 0.087) \). (Mean atmospheric pressure was 762.59 \( \pm 2.96, \) S.D.) mm Hg, \( n = 19 \).)

These data suggest that the scatter of the results shown in Fig. 6 was not significantly related to temperature or atmospheric pressure, but to other sources of error such as mechanical properties of the AT or the coupling between the AT and sclera or to physical properties of the sclera.

Regression constants were also calculated separately for each experiment, and these demonstrated that the slopes were similar for each day but varied between animals. Some of the variation could be related to fever, and therefore temperature variations. For each experiment, there was a strong linear relationship between \( R_f \) and IOP, with \( r^2 \) varying from 0.79 to 0.99. The inverse confidence interval for the determination of IOP by inspection of the individual scattergrams varied between \( \pm 7.5 \) and \( \pm 10 \) mm Hg.

**Continual Mackay-Marg tonometry.**

Comparison of calibration curves obtained from continual Mackay-Marg tonometry of canine and rabbit eyes is shown in Table II. Mackay-Marg chart paper readings, expressed in millimeters, were related to manometric pressure in millimeters of mercury by linear regression, so that

\[ \text{MM} = a + bP \]

where \( \text{MM} = \) reading (in mm of Mackay-Marg chart paper), \( a = \) intercept of regression line with ordinate, \( b = \) slope of regression line (in mm of chart reading per mm Hg), and \( P = \) manometric pressure (in mm Hg).

Calibration slopes for continual scleral Mackay-Marg tonometry in dog eyes were more than twice those obtained for the rabbit eyes: 0.80 mm/mm Hg for the dog and 0.37 mm/mm Hg for the rabbit. There was no significant difference between calibration slopes for continual corneal tonometry between the same animals: 0.43 mm/mm Hg for the dog and 0.41 mm/mm Hg for the rabbit. There was only a small difference between instantaneous corneal readings for the two species and the standard calibration supplied with the instrument for human eyes. These results indicated a fundamental difference between eyes of the two species. It is likely that this difference is related to ocular rigidity; the dog eye has a much lower value for ocular rigidity than the rabbit.\(^{19}\) The high value of the calibration slope for the dog implied that during the reading of each level of IOP, the tonometer recorded forces in the sclera that were not directly related to IOP, at least over the period of recording for each pressure level.

A 6 min recording of continual scleral tonometry by the Mackay-Marg transducer proved that a decay in readings occurred toward true IOP, which was held constant at 22 mm Hg. This decay was exponential, with a half-time decay constant in the order of 350 sec. A 3 min recording of the rabbit eye under similar conditions showed no visible decay in reading, however.

**Discussion**

In vivo calibration of several AT prototypes confirmed our previous finding of linearity of their response to variations of IOP while applanating sclera.\(^{9}\) Validation of this linearity was achieved for this method of continual scleral applanation with a commercially available guard-ring applanating tonometer.

In order to assess accuracy of the AT, conditions were idealized as far as possible. The suspension system was stabilized to the limits
available in our laboratory, and the haptic element was constructed to fit individual rabbits.

Factors affecting accuracy of AT output include temperature, atmospheric pressure, coupling of the AT to the eye, physical properties of the sclera, mechanical instability within the AT, physical properties of the AT such as permeability to saline, and the geometric relationship between the AT and aerial system. Under the conditions of in vivo monitoring, body temperature and atmospheric pressure cannot easily be controlled and were thus included in the multiple regression analysis with IOP as independent variables. This analysis demonstrated the linearity of AT output in response to IOP variations, without significant effects from the small temperature and atmospheric variations encountered during the study. The significant linear response obtained in all experiments implies that coupling between the AT and sclera was stable for most of the calibrations and therefore that the scatter of baseline and slopes seen from the results in Fig. 6 was due to the other factors enumerated above.

Diurnal body temperature variation in the human is of the order of 1.5°C, which would introduce an expected diurnal temperature-dependent error of IOP determination of approximately 2.45 mm Hg, as calculated from the characteristics of the AT used in the rabbit experiments.

Some of the lower calibration points in Fig. 6 were obtained from rabbits with fever (body temperature of more than 39°C). The trend in determination of Rf was still linear in these animals, but there was a significant fall in baseline Rf, as would be expected from the temperature characteristic shown in Fig. 5.

Large atmospheric pressure variation would affect AT output. This can be overcome by atmospheric pressure monitoring and signal processing. A modification in the structure of the AT is possible for negation of this source of error.

The principle of instantaneous or fast guard-ring applanation tonometry and its validity are well established for the human and animal cornea. In the continual applanating mode, it has also been validated and shown to give a lower indication for IOP than the instantaneous method, due probably to the elastic properties of the cornea. It is thought that during the 0.4 sec required to record IOP by the fast method, residual corneal forces are still acting on the transducer and that these forces decay over about 4 sec toward a true indication of IOP with continual corneal applanation. We found that this time-dependent phenomenon occurred, during continual scleral applanation, to a much greater extent in the dog than in the rabbit.

Recent accurate determination of an exponential time-dependent expansion strain in response to increases in IOP for the monkey eye was found to be at least twice that obtained from the rabbit eye. One explanation of the decay in Mackay-Marg readings when applied continually to canine sclera could be ascribed to such a time-dependent relaxation of the sclera in response to applanation and IOP changes. The difference between dog and rabbit results may be due to the low ocular rigidity of the former eye, in comparison to the rabbit. This is an important observation which reduces the chance of successful IOP monitoring in the dog through conjunctiva. The effect is likely to be insignificant in the rabbit. Since human scleral rigidity lies between those of the rabbit and the dog, a careful assessment of the effects of continual scleral applanation will have to be made on human eyes. That it does not have a great influence on this form of applanation in the human has been suggested by one report.

For the management of glaucoma patients, a continual monitoring device should be capable of measuring IOP to within ±2.5 mm Hg and should have a baseline drift of less than 2.5 mm Hg over 24 hr. The mechanical and physical properties of our transensor do not yet meet these constraints; by inspection of the scattergrams, any value of Rf would predict IOP to within ±7.5 mm Hg for the rabbit. This confidence interval was about
three times the calculated 99% confidence interval and therefore represents a worst-case situation. The effects of the geometric relationship between the AT and monitoring system have been largely overcome by novel design techniques. Coupling of the AT to the eye should be easier for the human, with well-tried methods of haptic contact lens fitting. The effect of scleral rigidity on output may mean that the AT will have to be calibrated for each eye. Work is in progress to develop a more stable AT and methods for such individual calibration.

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REFERENCES