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Ostrin, Lisa A
Wildsoet, Christine F

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A Comparison of Applanation and Rebound Tonometers in Young Chicks

Lisa A. Ostrin^a and Christine F. Wildsoet^b

^aCollege of Optometry, University of Houston, Houston, Texas, USA; ^bSchool of Optometry and Vision Science Program, University of California Berkeley, Berkeley, California, USA

ABSTRACT

Purpose: To assess the validity of and compare applanation and rebound tonometry readings of intraocular pressure in alert normal chicks from ages 3 to 45 days.

Methods: Intraocular pressures (IOPs) were measured weekly in awake White Leghorn chicks, from ages 3–45 days ($n = 22$ –30 per age group), with both applanation Tono-Pen and rebound TonoLab tonometers. Three repeated measurements on individual eyes were used to derive variance data for both instruments at each age. Calibration curves were also derived for each instrument and each age, weekly from ages 10–45 days ($n = 3$ –4 per age group), from *in situ* manometry data collected over IOP settings of 0 to 100 mmHg in 5 mmHg steps in cannulated eyes.

Results: The TonoLab showed less within measurement variability, but more variability with age, than the Tono-Pen. The coefficient of variation ranged from 3.8–8.3% for the TonoLab, compared to 11.0–19.7% for the Tono-Pen across all ages. For the youngest, 3 day-old chicks, mean IOPs recorded with the Tono-Pen and TonoLab were not significantly different (17.0 ± 5.6 and 15.2 ± 3.7 mmHg, respectively, $P = .27$). However, with increasing age, IOP readings significantly increased for the TonoLab ($P < .001$), whereas Tono-Pen readings did not. Compared to manometry settings, the Tono-Pen tended to underestimate IOPs while the TonoLab overestimated IOPs over the range 20–60 mmHg, saturating thereafter; there were also age-dependent differences for the TonoLab.

Conclusions: Both the Tono-Pen and TonoLab gave IOP readings that differed from manometry settings in normal young chicks over some or all of the ages tested. These results reinforce the importance of calibrating clinical tonometers in animal studies involving IOP as a key variable.

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Young chicken have been widely used in studies of eye growth regulation and myopia,^{1–4} and, to a lesser extent, as a model of glaucoma.⁵ Intraocular pressure (IOP) has been evaluated in a number of these studies,^{6–8} with early studies of IOP making use of either applanation tonometry^{7,9–11} or direct manometry of cannulated eyes.¹² More recently, rebound tonometry has been become available as an additional option for measuring IOP,^{13,14} with commercially available models specifically designed for use with small animals (TonoLab for mice and rats), large animals (TonoVet for rabbits, cats, dogs and horses), and humans (ICare and ICare Home). These instruments have the additional advantage over more traditional tonometry devices of not requiring corneal anesthesia.¹⁵

Early studies in chicks suggest that IOP plays a critical role in normal eye enlargement in developing eye.^{16,17} As in humans, chicks exhibit a diurnal IOP rhythm, with an amplitude of approximately 8 mmHg.⁸ In the eyes of normal (untreated) chicks, IOP has also been reported to vary in phase with diurnal changes in axial length, with IOP peaking during the day and dropping during the night when eyes are reported to be at their shortest.^{18,19} Interestingly, studies that include IOP data for young chicks report a wide range in normal IOPs, from 13 to 27 mmHg.^{7–9,11} While time of day of the measurement, age, and strain are likely contributing

factors to such differences, the instruments used to record IOP are another potential source of variability between studies.

The only method allowing direct, accurate measurement of IOP is manometry. However, manometry comes with its own limitations because it is invasive, requiring cannulation of eyes, typically performed under general anesthesia, which is known to affect IOP.^{20,21} Alternative clinical tonometers for measuring IOP have thus been developed, yet all represent indirect methods and also come with their limitations and assumptions. For measuring IOP in human clinical settings, Goldmann tonometry, an applanation method, represents the traditional, still widely used approach and is considered the gold standard. Readings assume Imbert-Fick law,²² yet are known to be influenced by corneal biomechanics, thickness, and curvature.^{23–26} However, more versatile, hand-held and thus portable instruments have seen increasing use in the clinical setting, with the Tono-Pen, which is a applanation tonometer based on the Mackay-Marg principle,²⁷ being one of the earliest such devices developed for human use. The Tono-Pen has been reported to be more robust against corneal thickness variations, potentially due to a smaller applanation area compared to Goldmann tonometry.^{28,29} The Tono-Pen has also been used with a variety of animals, although without calibration, readings cannot be assumed to accurately represent their IOP due to

species differences in corneal dimensions and biomechanical properties.

Rebound tonometry represents the most recent addition to this field, with results from healthy human adults reported to be in good agreement with those from Goldmann applanation tonometry,^{30,31} and those from children being only slightly higher.^{32,33} Rebound tonometers specifically designed for animal use, for example, in veterinary practice and research, are also now available. To acquire IOP measurements, the probe is electromagnetically expelled to the cornea, and the deceleration is detected by the instrument. A key advantage of rebound tonometry is that topical anesthesia is not required. In measuring animals, general anesthesia can generally also be avoided and so the potential effects on IOP of ketamine^{21,34,35} and isoflurane.²⁰ Rebound tonometers have now been validated in humans, as well as many of the animal models commonly used in vision research.^{36–40}

Rebound tonometry has been utilized in two studies involving chicks.^{6,35} In one of these studies involving 3-week old chicks, investigators found that IOP was significantly associated with corneal thickness and body weight.³⁵ However, rebound tonometry has not yet been validated or calibrated in chicks for the range of ages often utilized in eye growth studies. The goals of the current study were to compare rebound and applanation tonometry as methods for measuring IOP in normal chicks up to 6 weeks of age and to examine the validity of such *in vivo* readings through their direct comparison with readings obtained via *in situ* manometry using cannulated eyes.

Methods

In vivo tonometry

IOP was measured using both a TonoLab rebound tonometer (iCare, Helsinki, Finland) and a Tono-Pen XL applanation tonometer (MentorNorwell, MA, USA) in normal (untreated) White Leghorn chicks over the age range of 3 to 45 days. Measurements on individual chicks were made at weekly intervals, with some chicks measured first at 3 days of age, and others at ages 10 or 17 days. Not all cohorts were followed out to 45 days of age. Overall, data for each age group represented 22–30 chicks.

Chicks were reared in a temperature-controlled facility with 12-hour light/dark cycle. Food and water were available *ad libitum*. Experiments were carried out in accordance with the ARVO statement for the use of animals in ophthalmic and vision research and with institutional approval from the University of California Berkeley.

For IOP measurements, chicks were alert and gently restrained. IOP measurements were performed between 1:00 and 5:00 pm to minimize effects of diurnal variation, with right eyes being measured first. All measurements were taken by the same observer to avoid technique-dependent influences on results. In all cases, IOP was measured first with the TonoLab, which does not require corneal anesthesia. The TonoLab calibration was set to the “rat mode.” The instrument used in this study has a probe corneal contact area of approximately 1 mm and provides a weighted average of 6 readings. Three such

measurements were recorded from each eye and averaged. Following these measurements, a further set of IOP readings were collected with the Tono-Pen after the instillation of a drop of topical 2.5% proparacaine. To prevent blinking during measurements during the latter measurements, a custom-made eye lid speculum was inserted to hold the eyelids open without applying pressure to the globe. The Tono-Pen has a corneal contact area of approximately 3 mm. Several readings were taken, from 3 to 10 depending on the variability in the readings, and the average, as displayed on the digital readout, recorded. Again, three such measurements were recorded per eye and averaged.

In situ manometry

For manometric studies, 3–4 eyes each were measured for ages 10, 17, 24, 31, 38, and 45 days. Chicks were sacrificed with carbon monoxide and then immediately placed prone on a stage with their head in a beak holder. One eye was cannulated with a 27 gauge needle inserted into the vitreous chamber near the equator, thereby avoiding contact with the crystalline lens. The needle was connected via a tube to a three-way tap, which was also connected via tubing to a saline-filled syringe and a sphygmomanometer. Starting at 0 mmHg, IOP was increased in 5 mmHg increments to 100 mmHg by injecting saline into the cannulated eye. At each step, immediately after fluid was injected into the system and the manometer pressure gauge read, IOP was measured first with the TonoLab, and then with the Tono-Pen. Here also, three measurements were recorded and averaged for each instrument. The manometer pressure gauge was monitored during each measurement period to ensure that the pressure was stable.

Data analysis

Data were analyzed with MedCalc Statistical Software version 18.2.1 (MedCalc Software, Ostend, Belgium). All data are reported as mean $\pm$ standard error. Normality was confirmed with the Shapiro Wilk test. *In vivo* IOP measurements for right and left eyes were analyzed with a paired t-test and the intra-class correlation correlation (ICC) for between-eye comparisons⁴¹ was determined for each instrument. Additionally, *in vivo* IOP measurements were analyzed with a two-factor repeated measures ANOVA, with age and instrument as variables. Post-hoc pairwise comparisons were assessed using paired t-tests, with Bonferroni correction for multiple comparisons (corrected significance at $P \leq 0.007$). Bland-Altman analysis was used to examine the limits of agreement between the instruments.⁴² Linear regression analyses were also performed on the manometric data across the 8 to 40 mmHg range, to generate calibration functions for each instrument and each age over the physiological range of IOPs.

Results

In vivo tonometry

For the Tono-Pen, over all ages, the mean IOPs for right and left eyes were 16.28 ± 5.05 and 15.36 ± 4.78 mmHg,

Table 1. Summary of intraocular pressures (mean $\pm$ standard error), measured with Tono-Pen and TonoLab; coefficient of variation (CoV) for three repeated measurements for each instrument; instruments were compared in terms of biases and limits of agreement (LOA).

Age (days)	N	Tono-Pen (mmHg)	TonoLab (mmHg)	Bias [#] (mmHg)	LOA [#] (mmHg)	P value
3	23	17.0 $\pm$ 5.6 (CoV 19.7%)	15.2 $\pm$ 3.7 (CoV 8.3%)	+1.9	−13.2 to 16.9	.27
10	27	17.6 $\pm$ 5.7 (CoV 18.0%)	24.8 $\pm$ 2.2 (CoV 5.5%)	−7.2	−17.8 to 3.4	<.001*
17	30	16.8 $\pm$ 4.2 (CoV 14.0%)	26.9 $\pm$ 4.4 (CoV 4.8%)	−10.2	−20.4 to 0.1	<.001*
24	30	13.3 $\pm$ 4.0 (CoV 12.8%)	30.3 $\pm$ 4.4 (CoV 4.2%)	−17.1	−24.6 to −9.53	<.001*
31	29	15.7 $\pm$ 4.3 (CoV 12.4%)	30.9 $\pm$ 4.4 (CoV 4.9%)	−15.1	−23.6 to −6.6	<.001*
38	22	17.5 $\pm$ 3.5 (CoV 11.0%)	32.4 $\pm$ 4.3 (CoV 5.1%)	−15.0	−23.1 to −6.9	<.001*
45	30	17.8 $\pm$ 6.2 (CoV 11.5%)	33.4 $\pm$ 2.9 (CoV 3.8%)	−15.6	−29.1 to −2.1	<.001*

[#]from Bland-Altman analysis

($IOP_{\text{Tono-Pen}} - IOP_{\text{TonoLab}}$); *significant at Bonferroni corrected level of 0.007; P values shown for paired t-test comparison between instruments, at each age

respectively, representing a significant, albeit small, difference between the two eyes (0.9 mmHg, $P = .03$). A similar but nonsignificant trend is evident in the TonoLab data, with mean IOP of 28.28 ± 6.31 and 28.04 ± 6.69 mmHg recorded for right and left eyes ($P = .23$). Because right and left eye IOPs were found to be highly correlated (Tono-Pen ICC 0.7, TonoLab ICC 0.9), only right eyes were included in further analysis. IOP data collected with the Tono-Pen and TonoLab are summarized in Table 1. Analysis of the repeatability of measurements at each age for each instrument are also included in Table 1. For both tonometers, the coefficient of variation was highest for the youngest chicks (Tono-Pen: 19.7%, TonoLab: 8.3%) and decreased with age thereafter. However, of the two instruments, the TonoLab consistently recorded the lowest variability across all ages.

Both age and the instrument used to measure IOPs significantly influenced readings (two-factor ANOVA, $P < .001$ for both). While for the youngest (3 day-old) chicks, both instruments gave similar readings (Tono-Pen: 17.0 ± 5.6 mmHg, TonoLab: 15.2 ± 3.7 mmHg, $P = .27$), those taken from older chicks with the TonoLab were consistently and significantly higher than TonoLab-measured IOPs at 3 days of age ($P < .001$ for all ages, Figure 1). Except for the youngest age tested, readings from the TonoLab were higher by 7.2 to 17.1 mmHg compared to those from the Tono-Pen. The results of Bland-Altman analysis of these age-dependent biases, along with limits of agreement data, are included in Table 1 and shown graphically in Figure 2.

In situ manometry

IOP recordings made with the Tono-Pen and TonoLab on cannulated eyes of different ages are shown as a function of manometric pressure setting in Figure 3. Here also, the profiles of the two tonometers differed significantly. While in both cases, readings increased linearly within the 8 to 40 mmHg range of manometric pressure settings, those from the Tono-Pen were consistently lower than manometric pressures across all ages, while those from the TonoLab showed the opposite trend. For the TonoLab, the disparity also increased with increasing manometric pressure, reaching an approximately 20 mmHg disparity for the 60 mmHg setting, and plateauing thereafter.

In situ data from all eyes were used in linear regression analyses to calculate calibration functions for each instrument and age combination. Calibration functions for the Tono-Pen and TonoLab are presented in Tables 2 and 3, respectively.

Discussion

In the study reported here, we compared IOP readings in young chicks, a commonly used model for myopia research, obtained with two different tonometers, a Tonolab and a Tono-Pen, both of which have seen use extensively in animal-based studies. Previous calibration studies using manometry in porcine, cat, and chinchilla eyes have shown that the Tono-Pen underestimates true IOP

Figure 1. (A) Intraocular pressures (mean $\pm$ standard error) measured with Tono-Pen (filled symbols) and TonoLab (open symbols) from chicks aged 3 to 45 days; * significance at Bonferroni corrected level of <.007.

Figure 2. Bland-Altman analysis of intraocular pressure (IOP) readings taken with Tono-Pen TonoLab; dashed line represents zero bias.

Figure 3. Intraocular pressure (IOP) measured *in situ* with A) Tono-Pen and B) TonoLab versus manometric pressure settings of 0 to 100 mmHg for eyes of chicks ages 10 days (darkest symbols) to 45 days (lightest symbols); line represents 1:1 relationship.

and shows more variability than the TonoVet.^{43–45} We also found that the Tono-Pen, an applanation tonometer, generally underestimated IOPs in chicks, as measured by manometry, while being relatively consistent across age, while on the other hand, the TonoLab, a rebound tonometer, overestimated IOPs, more so with increasing age from 3 to 45 days. These findings highlight

the need for calibration functions for tonometry instruments designed and calibrated for different species, with potentially different corneal dimensions and biomechanics.

While the Tono-Pen was designed and calibrated for human eyes, it has been used on a wide range of species, including chicks,^{7,9,35} rodents,⁴⁶ and monkeys.⁴⁷ The current study

Table 2. Linear regression equations for the Tono-Pen measured IOP as a function of chick age, calibrated with manometric IOPs.

Age (days)	N	Tono-Pen	Regression Statistics
10	3	$0.774x + 6.39$	$F_{1, 18} = 16.15, R^2 = 0.47 P < .001^*$
17	3	$0.683x + 1.74$	$F_{1, 18} = 176.05, R^2 = 0.91 P < .001^*$
24	4	$0.489x + 6.14$	$F_{1, 24} = 34.49, R^2 = 0.59 P < .001^*$
31	4	$0.722x - 1.75$	$F_{1, 23} = 123.51, R^2 = 0.84 P < .001^*$
38	4	$0.866x - 0.11$	$F_{1, 25} = 88.53, R^2 = 0.78 P < .001^*$
45	4	$0.685x - 2.14$	$F_{1, 23} = 220.40, R^2 = 0.91 P < .001^*$

*significant at Bonferroni corrected level of 0.007

Table 3. Linear regression equations for the TonoLab measured IOP as a function of chick age, calibrated with manometric IOPs.

Age (days)	N	TonoLab	Regression Statistics
10	3	$0.632x + 5.36$	$F_{1, 18} = 123.40, R^2 = 0.87 P < .001^*$
17	3	$0.535x + 6.93$	$F_{1, 18} = 66.1, R^2 = 0.79 P < .001^*$
24	4	$0.545x + 3.64$	$F_{1, 25} = 177.85, R^2 = 0.88 P < .001^*$
31	4	$0.608x + 6.96$	$F_{1, 24} = 483.83, R^2 = 0.95 P < .001^*$
38	4	$0.508x + 6.04$	$F_{1, 25} = 177.45, R^2 = 0.88 P < .001^*$
45	4	$0.536x + 6.55$	$F_{1, 28} = 399.99, R^2 = 0.93 P < .001^*$

*significant at Bonferroni corrected level of 0.007

represents a further and significant extension of three previous studies involving chicks, providing IOP data for a wider range of ages and calibration functions for the Tono-Pen (Table 2). In the three previous studies, readings were also compared with manometric data, with both similarities and differences from the trends reported here. For example, in a study of 1-week-old chicks, the Tono-Pen was found to underestimate manometric readings,⁹ similar to our findings. In another study involving chicks aged between 7 to 19 days, Tono-Pen readings were reported to correlated well with manometry.⁷ In the third study involving 3-week-old chicks, the authors reported readings to be unreliable for manometry settings of less than 35 mmHg.³⁵ The latter observation contrasts with our findings; while readings were only obtained from approximately half of the eyes for manometry settings of less than 8 mmHg, IOP readings were obtained for all eyes at all pressure settings greater than 8 mmHg. In another study involving 275 eyes of 39 avian species, Korbel reported Tono-Pen readings to be reproducible on eyes with a minimum corneal diameter of 9 mm, with readings being less reproducible for corneal diameters between 5–9 mm, and not reliable for corneal diameters less than 5 mm.⁴⁸ Although corneal diameters were not recorded in the current study, based on data from another study reporting a mean corneal diameter of 6.55 mm for chicks ages 20–55 days,⁴⁹ it is plausible that the relatively small corneal diameters of the younger chicks used in this study may have contributed to the observed greater variability of Tono-Pen readings for this group. Interestingly, we also observed a small but significant difference between right and left eyes in IOPs measured with the Tono-Pen; potential explanations including a reduction over time in the stress level of the animal or position and operator effects in measurement technique.

The TonoLab used in the current study was designed for use with mice and rats, with two corresponding calibration settings. All measurements here were performed with the rat calibration setting, because the youngest chicks have eyes more similar in size to the rat eye. Adult rat eyes are reported

to be approximately 7 mm in length,⁵⁰ compared to approximately 8 mm in newly hatched chicks. Nonetheless, significant age-dependent variations in IOP were observed with the TonoLab under both *in vivo* and *in situ* (manometry) measurement conditions, necessitating calibration functions to be derived for each age (Table 3). The likely contributions of corneal dimensions, including thickness, and axial length to these age-dependent differences are considered below.

The elastic and viscous properties of the cornea, and thus corneal thickness, are believed to significantly influence the interaction between the rebound tonometer probe and the ocular surface.⁵¹ For example, a previous study in humans reported a 0.3 mmHg deviation in IOP per 10 μ m difference in central corneal thickness.⁵² Another study reported even larger deviations of up to 0.7 mmHg per 10 μ m difference in central corneal thickness.⁵³ Rats have a central corneal thickness of approximately 159 μ m,⁵⁴ substantially thinner than that of chicks of approximately 240 μ m, although there are also age-related changes in the chick central corneal thickness.⁵⁵ A previous study utilizing rebound tonometry in 3 week-old chicks found corneal thickness to be one of two significant sources of variation in IOP in a multivariate stepwise regression analysis, with body weight being the other.³⁵ To assess the contribution of age-dependent differences in central corneal thickness to the age-related increases in IOP recorded with the TonoLab here, we modelled central corneal thickness over the age range of our chicks using equations reported by Montiani-Ferreira, et al.⁵⁵ This modelling yielded a bimodal distribution for the central corneal thickness of the chick, with both very young (3-day-old) and older (45-day-old) chicks have relatively thicker corneas (240.7 and 241.4 μ m, respectively), with corneal thickness reaching a minimum around 10 days (238.9 μ m). Nonetheless, these age-dependent differences in central corneal thickness are of the order of only 2–3 μ m, arguing against corneal thickness differences as an explanation for the age-related over-estimation in IOPs recorded with the TonoLab.

That age-related increases in eye length may contribute to the observed over-estimation of IOPs taken with the TonoLab is an alternative possibility that is indirectly supported by a study in humans. Specifically, in a comparison of IOPs recorded using rebound compared to Goldmann applanation tonometry,⁵⁶ rebound tonometry readings were found to be higher than applanation tonometry readings, with myopic eyes showing the highest overestimation. The eyes of young chicks enlarge rapidly over the first few months of life, from approximately 8.5 mm at hatch to 11 mm in length by 6 weeks of age.⁵⁷ There are also important structural differences between the avian and mammalian eyes of potential relevance. Of particular note, the chick eye has a ring of scleral ossicles surrounding and stabilizing the periphery of its cornea, which contributes to ocular accommodation by corneal steepening.⁵⁸ These structural differences and unique biomechanical properties of the chick cornea compared to the mammalian eye, for which the TonoLab is calibrated, may contribute to the observed over-estimations of IOPs recorded with rebound tonometry.⁵⁹

A potential limitation of the manometry component of this study is that the vitreous chamber was cannulated rather than the anterior chamber, as commonly targeted in such studies.

However, while vitreous chamber pressures were found to be significantly greater than anterior chamber pressures in a direct comparison of IOP readings from simultaneously cannulated anterior and vitreous chambers of enucleated porcine eyes, the difference was small, being 0.4 mmHg different in the range of 5–13 mmHg and 0.28 mmHg different from 13 to 20.5 mmHg.⁶⁰ Our choice of the vitreous chamber for cannulation took advantage of its relatively large size, compared to the very shallow anterior chambers in young chicks. Additionally, in the current manometry study, pressure changes were limited to increases, ruling out the possibility of hysteresis as a major confounding factor, although a related study in 3-week-old chicks reported no differences in rebound tonometry readings for inflation versus deflation.³⁵

In conclusion, findings from this study revealed important differences in performance between the Tono-Pen and TonoLab, both of which have been widely used in animal studies, including myopia-related studies. The small probe diameter of the TonoLab is an advantage with small eyes, such as encountered in young chicks; it was also well tolerated by without topical anesthesia and readings showed less variance with repeated measurements than Tono-Pen readings. The significant disparities between manometric pressures and tonometry readings observed with both instruments urge caution in interpreting uncalibrated readings.

Disclosure statement

The authors have no conflicts of interest related to this work.

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ORCID

Christine F. Wildsoet <http://orcid.org/0000-0003-2671-2391>

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