Glaucoma

Corneal Modulus and IOP Measurements in Canine Eyes Using Goldmann Applanation Tonometry and Tono-pen

Junhua Tang,1 Xueliang Pan,2 Paul A. Weber,3 and Jun Liu1,3

PURPOSE. To experimentally examine the effect of corneal modulus on Goldmann applanation tonometry (GAT) and Tono-pen (Tono-pen XL, Reichert, Inc., Depew, NY) measurements of intraocular pressure (IOP) in a canine eye model.

METHODS. Twenty-one canine globes were recovered from healthy animals. IOP was controlled at 10, 15, 20, 30, and 40 mm Hg and measured by GAT and Tono-pen following standard protocols. The corneas were dissected and uniaxial tensile tests were performed on corneal strips. The correlation between GAT and Tono-pen errors and corneal secant modulus was evaluated using Pearson correlation coefficients. The influence of corneal thickness and the true pressure was also examined.

RESULTS. At a true IOP of 10, 15, 20, 30, and 40 mm Hg, the GAT readings were 1.1 ± 1.0, 5.1 ± 1.5, 9.5 ± 2.0, 17.3 ± 1.6, and 25.3 ± 1.8 mm Hg, respectively. The corresponding Tono-pen readings were 7.8 ± 1.7, 12.4 ± 1.7, 16.1 ± 1.9, 22.5 ± 2.1, and 28.1 ± 2.2 mm Hg, respectively. The mean secant modulus at 1% strain of the canine corneal strips was 1.54 ± 0.45 megapascal (MPa). Corneal secant modulus was significantly correlated with GAT errors when the true IOP was 30 mm Hg (R = 0.49; P < 0.05). No significant correlation was observed between tonometric errors and corneal thickness. Both GAT and Tono-pen errors increased significantly at higher pressures (P < 0.001).

CONCLUSIONS. Both GAT and Tono-pen underestimated IOP in canine eyes. There was preliminary experimental evidence for a correlation between corneal modulus and GAT in the canine eyes and a higher corneal modulus was associated with higher GAT readings at a certain pressure level. The tonometric errors appeared to be pressure-dependent. (Invest Ophthalmol Vis Sci. 2011;52:7866–7871) DOI:10.1167/iovs.11-7407

The cornea is not an ideal thin membrane and exerts some resistance to the application. In addition, the surface tear film may also exert some forces on the tonometer tip. Thus the measured pressure could deviate from the prediction of the Imbert-Fick law.4,5 Goldmann et al. proposed an applanation area of 3.06 mm in diameter and believed that this applanation area would allow a fairly accurate measurement of IOP for human corneas with average dimensions and properties.3,4 It was believed that the corneal resistance was canceled out by the tear film traction at this applanation area.

Due to the natural variances in corneal thickness, radius of curvature, and biomechanical properties, not all corneas satisfy the calibration conditions of GAT. Central corneal thickness (CCT) and corneal curvature have long been suspected to be factors affecting the accuracy of GAT measurements. Numerous studies have reported a significant effect of CCT on the accuracy of GAT with the measurement error ranging from 0.11 to 0.71 mm Hg for each 10-μm deviation from the population mean CCT.5–8 The effect of corneal curvature has also been reported and the GAT errors ranging from 0.57 to 1.14 mm Hg per 1 mm change in radius of curvature.10–12

There has been an increasing interest in the effect of corneal stiffness on GAT measurements because theoretical modeling suggested that corneal stiffness could potentially play a larger role than CCT or curvature.10,12–14 For example, an analytical model assuming isotropic linear elasticity showed that a variation in corneal Young’s modulus from 0.1 to 0.9 MPa could introduce a difference greater than 10 mm Hg in GAT measurements.10 Numerical models assuming nonlinear material properties generally produced similar results.13,14 These modeling results have provided valuable insight into the GAT measurement accuracy; however, little experimental data have been acquired to directly examine the effect of corneal stiffness.

Tono-pen is a hand-held instrument that measures IOP based on the MacKay-Marg principle. In one study, Tono-pen was found to be accurate for corneas with surface abnormality.15 Other studies showed that Tono-pen was capable of producing comparable measurements with GAT on normal corneas.16–19 The effect of CCT was reported to be less significant for Tono-pen measurements compared with GAT.20 The effect of corneal stiffness on Tono-pen measurements has not been fully determined.

Tensile tests have been widely adopted in characterizing the mechanical properties of soft tissue including the cornea.21–24 The purpose of this study was to experimentally examine the effect of corneal modulus, as obtained from tensile tests, on GAT and Tono-pen measurements using a canine eye model. We also explored whether the IOP measurement errors were dependent on true IOP. Canine eyes were used in this study because their corneal thickness was close to that of human eyes. Other frequently used animal models including porcine or bovine eyes have corneal thickness much greater than that of human’s. In addition, canine eyes can be obtained immediately after death and tested within hours to minimize

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swelling and other postmortem changes, which is important for preserving corneal properties close to the in vivo conditions.

**METHODS**

**Sample Preparation**

Twenty-one fresh canine globes were collected immediately after death from healthy dogs that were humanely euthanized for population control purposes at a local animal shelter. CCT was measured in the whole globes using an ultrasound pachymeter (DGH-550 Pachette 2; DGH Technology, Inc., Exton, PA) before all experiments and also after tonometric measurements. Three readings were recorded and the average was used for further analysis. The speed of sound setting was 1640 m/s, which has been used for canine eyes.25 All measurements were completed within 4 hours postmortem. The corneas were non-swollen (indicated by their normal thickness and transparency) and the epithelia were intact throughout the experiments.

**Goldmann Applanation Tonometry and Tono-pen Measurements**

GAT measurements were performed on enucleated globes using the experimental setup shown in Figure 1. The globe was placed in a holder padded with moistened gauze. The holder was affixed to a plastic plate that was vertically mounted on the headset of a standard slit lamp (Topcon, Oakland, NJ). A 22-gauge needle was inserted into the anterior chamber of the globe from the limbus. Through the needle and a tubing system, the anterior chamber was connected to a saline column which controlled the intraocular pressure and also a pressure sensor (Omega Px154; Omega Engineering Inc., Stamford, CT) which was monitored in real time.

The pressure was first set to 10 mm Hg by adjusting the height of the saline column and confirmed by the pressure sensor readings. A drop of fluorescein solution (Fluorox; Altaire Pharmaceuticals, Inc., Aquebogue, NY) was gently spread onto the cornea surface using a cotton tip. Fluorescein was used to visualize the Goldmann mires for slit lamp examination, as in the clinical measurements. IOP was measured by using a Goldmann tonometer (AT900; Haag Streit, Switzerland) and the slit lamp according to the standard protocol. The Goldmann tonometer tip was then temporarily moved away from the cornea for Tono-pen measurements (Tono-pen XL; Reichert, Inc., Depew, NY) under the same pressure setting. Both IOP measurement devices were calibrated before experiments and rechecked at the completion of the experiments. The intraocular pressure was adjusted to the levels of 15 mm Hg, 20 mm Hg, 30 mm Hg, and 40 mm Hg. The corresponding pressure sensor readings and the GAT and Tono-pen measurements were recorded. Each pressure measurement was repeated three times and the average was used for further analysis.

**Uniaxial Tensile Test of Corneal Strips**

After completing the pressure measurements, the globes were dissected and cornea strips (3.5 mm by 18 mm) were prepared along the nasal-temporal direction. Standard uniaxial tensile tests were performed on the corneal strips using a rheometer (Rheometrics System Analyzer III [RSA-III]; TA Instruments, New Castle, DE) with a displacement resolution of 0.05 µm and a force resolution of 20 µN. The sample was coupled between a motor and a transducer that measures the resultant force generated by sample deformation. The initial sample length between the two gripping jaws was approximately 10 mm. Sample geometric information, including width and thickness, was input into the rheometer control panel. The sample width and thickness were measured by using a high resolution ultrasound imaging system (Vevo660; VisualSonics Inc., Toronto, Canada). A 55-MHz transducer was used with an axial resolution of 30 µm and a lateral resolution of 62.5 µm.

The stress and strain were computed by the rheometer using the initial sample geometry including sample width and thickness. A preload of 20 mN was applied to each sample to flatten the curvature and ensure full contact between sample and grips. After preloading, the sample length was recorded automatically. The sample was then subject to a constant strain rate of 0.1% per second until strain reached approximately 6%. The data were stored on the hard disc of the computer for further processing. The strain rate was selected from the typical values used in the literature.26–28

**Statistical Analysis**

A computer program (SAS, version 9.12; SAS Institute Inc., Cary, NC) was used for all data analysis. The GAT and Tono-pen measurements were summarized as mean ± SD at each IOP level (10, 15, 20, 30, and 40 mm Hg). The correlation between GAT and/or Tono-pen measurement errors and the secant modulus was evaluated at each IOP level using Pearson correlation coefficients, R. The influence of CCT on the measurement errors was also explored using Pearson correlation at different IOP levels. The influence of the true IOP on measurement errors was tested using linear mixed models for repeated measures to account for the correlation among errors at different IOP levels for the same eye. According to power analysis, a sample size of 21 was chosen to provide at least 80% power to detect a Pearson correlation of 0.6 between IOP measurement errors and corneal modulus at the significance level of 0.05.

**RESULTS**

The mean CCT of the 21 canine corneas was 611.9 ± 55.3 µm before all measurements and 623.6 ± 51.6 µm after the tonometric measurements. The small change in CCT suggested a typical values used in the literature.26–28

**TABLE 1.** GAT and Tono-pen Readings at Various True IOP Levels in Canine Eyes (n = 21)

<table>
<thead>
<tr>
<th>True IOP (mm Hg)</th>
<th>GAT (mm Hg)</th>
<th>Tono-pen (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.1 ± 1.0</td>
<td>7.8 ± 1.7</td>
</tr>
<tr>
<td>15</td>
<td>5.1 ± 1.5</td>
<td>12.4 ± 1.7</td>
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<td>20</td>
<td>9.5 ± 2.0</td>
<td>16.1 ± 1.9</td>
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<tr>
<td>30</td>
<td>17.3 ± 1.6</td>
<td>22.5 ± 2.1</td>
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<tr>
<td>40</td>
<td>25.3 ± 1.8</td>
<td>28.1 ± 2.2</td>
</tr>
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Data are given as mean ± SD.
Both GAT and Tono-pen underestimated IOP at all pressure levels. Tono-pen had a significantly higher reading than GAT at all pressure levels (the differences were 6.7, 7.3, 6.6, 5.2, and 2.7 mm Hg for IOPMan at 10, 15, 20, 30, and 40 mm Hg, respectively). In the same eye, the GAT and Tono-pen readings were significantly correlated for certain pressure levels but not others. When IOPMan was 15 mm Hg and 20 mm Hg, the two tonometric readings were significantly correlated (Pearson correlation coefficient $R^2 = 0.44$, $P = 0.05$; and $R^2 = 0.54$, $P = 0.05$, respectively). No significant correlation was found at higher pressures (when IOPMan was 30 or 40 mm Hg; $R^2 = 0.04$ or 0.07, $P = 0.86$ or 0.61). There was no correlation between GAT and Tono-pen readings when IOPMan was 10 mm Hg ($R^2 = 0.06$, $P = 0.80$), likely because GAT readings were close to zero and thus not reliable at this pressure level in the canine eyes. For an overall correlation analysis including the readings at all pressure levels, the Pearson correlation coefficient between GAT and Tono-pen measurements was 0.96 ($P = 0.0001$) indicating a strong influence of the range of data in comparing these two tonometric devices.

The measurement errors for GAT (difference between measured IOP and IOPMan) were shown in Figure 3. At each level of IOPMan, there was significant spread across different eyes in both GAT errors (with an average range of 6.2 mm Hg) and Tono-pen errors (with an average range of 6.8 mm Hg). In addition, both GAT and Tono-pen errors increased significantly as the true IOP increased ($P < 0.001$, based on the mixed models).

Based on the stress-strain curves obtained from uniaxial tensile tests, secant modulus was calculated as the ratio of stress and strain at a given level of strain. The mean secant modulus was $1.54 \pm 0.43$ MPa at 1% strain and increased to $3.93 \pm 1.35$ MPa at 5% strain, demonstrating typical nonlinearity (Fig. 4). According to the estimation of corneal in-plane tensile stress based on Laplace law and also considering the prestress introduced in tensile tests, the secant modulus at 1% strain seemed a reasonable representation of the tensile stiffness in the range of the loadings relevant to the IOP measurements in this study and thus was chosen as the primary modulus measure for further analysis. It is noted that Laplace law does not predict well the exact stress distribution in the cornea due to inhomogeneous corneal thickness, curvature, and material properties.

A statistically significant Pearson correlation was found between the GAT errors at 30 mm Hg IOPMan and the 1% secant moduli ($R = 0.49$, $P < 0.05$; Fig. 5). At this level of true pressure, the GAT readings appeared to be higher in corneas with higher modulus. No significant correlation was found between GAT errors and the 1% secant moduli at other pressure levels ($R = 0.33, 0.31, 0.26, 0.09$; and $P = 0.14, 0.17, 0.26, 0.71$, for IOPMan at 10, 15, 20, and 40 mm Hg, respectively). No statistically significant correlation was detected between GAT errors and the initial CCT (and also the CCT measured after experiments) at all pressure levels ($R = -0.08, -0.15, 0.05, 0.15, 0.28$; and $P = 0.72, 0.52, 0.82, 0.53, 0.22$, for IOPMan at 10, 15, 20, 30, and 40 mm Hg, respectively).

No significant correlation was observed between Tono-pen errors at any levels of true IOP and the secant moduli at 1%
strain \((R = 0.29, 0.16, 0.04, -0.25, -0.04; \text{ and } P = 0.20, 0.49, 0.85, 0.27, 0.88, \text{ for IOP}_{\text{Man}} \text{ at } 10, 15, 20, 30, \text{ and } 40 \text{ mm Hg, respectively})\). No significant correlation was observed between Tono-pen error and initial CCT (and also the CCT measured after experiments) at all pressure levels \((R = 0.19, 0.07, 0.13, 0.24, 0.27; \text{ and } P = 0.41, 0.76, 0.57, 0.29, 0.25, \text{ for IOP}_{\text{Man}} \text{ at } 10, 15, 20, 30, \text{ and } 40 \text{ mm Hg, respectively})\).

CCT (initial or measured after experiments) was not correlated to the secant modulus at 1% strain \((R = -0.006 \text{ or } -0.038, P = 0.98 \text{ or } 0.86)\).

**DISCUSSION**

To our best knowledge, this study is among the first that examined the relationship between tonometric measurement errors and experimentally determined corneal modulus. The primary findings include: a substantial underestimation of IOP by both GAT and Tono-pen in canine eyes and a significant correlation between corneal modulus and GAT errors at a certain pressure level. The tonometric errors also appeared to be pressure-dependent. These findings are elaborated in the following paragraphs.

We found a substantial underestimation of IOP by GAT in canine eyes although the dimensions of the canine corneas were quite close to those of human corneas. For example, GAT readings were around zero or even slightly negative in some eyes at the true pressure of 10 mm Hg. The average GAT error was 9.9 mm Hg at a true pressure of 15 mm Hg and the error further increased at higher pressures. This level of underestimation was comparable to what has been reported on porcine eyes where GAT measurements were approximately 12.5 mm Hg or more lower than true pressures. As discussed before, GAT is calibrated for human eyes with normal corneal dimensions and properties. The large underestimation in normal canine eyes may likely result from certain biomechanical or geometrical characteristics that are distinct between canine and human eyes. The pachymetry readings of the canine corneal thickness \((611.9 \pm 55.3 \mu m)\) was comparable to those reported for in vivo canine eyes indicating insignificant postmortem thickness changes. This thickness was slightly higher than that in normal human corneas. Because a thicker cornea would be more likely to be associated with a higher IOP reading rather than a lower reading by GAT, the difference in thickness should not account for the underestimation we observed. Interestingly, an even larger CCT \((842 \mu m)\) in the porcine corneas did not compensate for the GAT underestimation in porcine eyes, as reported in the previous study. Radius of curvature in canine corneas has been reported to be approximately 8.5 mm, which was slightly larger than that in human corneas. However, this difference in radius of curvature would likely only produce an underestimation no greater than 2 mm Hg. Previous theoretical simulations have suggested over 10 mm Hg GAT errors associated with the difference in corneal modulus. It is thus possible that the GAT underestimation seen in canine eyes were mostly caused by the difference in the material properties between canine and human corneas.

Our results showed a correlation between GAT error (at 30 mm Hg) and corneal tensile modulus (secant modulus at 1% strain). This outcome supports the hypothesis that GAT errors are at least in part explained by the variation in corneal mechanical properties, consistent with the predictions of the theoretical models. However, we did not find a consistent correlation between corneal tensile modulus and GAT errors at pressure levels other than 30 mm Hg. It is possible that the current sample size \((n = 21)\) was not enough to detect the correlations, given that other factors including the true pressure and corneal thickness could also affect GAT accuracy. A sample size of 21 provides 80% power to detect a correlation of 0.6, and only achieves 45% or 67% power to detect a correlation of 0.4 or 0.5, even without the significance adjustment for multiple comparisons. In addition, the tensile modulus is likely not the sole determinant of corneal resistance to applanation. The ability to resist applanation (i.e., the bending rigidity of the cornea) may be influenced by multiple factors including corneal thickness (and its regional variance) and corneal collagen microstructure such as the circumferential arrangement of collagen fibers around the limbal area, in addition to corneal tensile modulus. The correlation between corneal modulus and GAT errors observed in the present study indicated that corneal tensile modulus may serve as a good indicator for the overall corneal resistance to applanation. Future studies are needed to investigate which and what combinatory biomechanical and structural factors can best predict the accuracy of GAT.

From our observations during experimental handling of both canine and human corneas, it was often found that the canine corneas tended to wrinkle or collapse and it was difficult for them to maintain a curved shape after dissection. Conversely, human corneas from adult donors can usually maintain the curvature even after dissection. These observations indicated that these two species may have different capabilities in maintaining corneal curvature and the human eyes appear to have a higher resistance to shape change. We acquired several human corneal strips and performed uniaxial tensile tests following the same protocol as described earlier for the canine corneas. We found that the human corneal tensile modulus was significantly higher than that of the canine corneas. The average secant modulus in the human corneas \((n = 8)\) was 2.44 MPa at 1% strain compared with 1.54 MPa in the canine corneas. These data indicated that the lower corneal modulus in canine eyes was likely responsible for the large underestimation of GAT.

Our results showed that Tono-pen also underestimated IOP in canine eyes but appeared to be more accurate than GAT when measuring IOP in canine eyes (a smaller deviation from the unit line in Fig. 2) especially for normal pressures. For example, at a true IOP of 15 mm Hg, the average underestimation was 2.6 mm Hg. This was consistent with literature reports that Tono-pen was relatively reliable for canines at normal pressure levels. It was also reported that the calibration curve for canine eyes had a smaller slope than that for human eyes, consistent with the findings in the present study. Tono-pen has been shown to be accurate in human cadaver eyes also. We did not detect a significant correlation between Tono-pen errors and corneal modulus. These results suggested a lesser dependence of Tono-pen on corneal biomechanical properties compared with GAT. This may be explained by the design motivation of Tono-pen. As stated by the inventors, Tono-pen was designed to minimize the influence of corneal resistance by using a two-step flattening procedure and a much smaller area of applanation. Both GAT and Tono-pen revealed a substantial range (approximately 6 mm Hg) in IOP measurement errors across different eyes. This is of clinical interest because these devices are calibrated for the average eye. If such intersubject variance also exists when measuring human eyes, there would be significant clinical consequences in terms of glaucoma diagnosis and management due to tonometric inaccuracy.

We did not observe statistically significant correlations between CCT and GAT and/or Tono-pen errors in the measured eyes, although there was generally a trend of weak positive associations. This was possibly due to the small sample size. It is also noted that the dependence of GAT on CCT was predicted to be smaller for lower corneal modulus according to...
the previous theoretical model. As discussed above, canine corneal modulus was significantly smaller than human corneal modulus. This would predict a lesser effect of CCT on GAT measurements, which was consistent with the current experimental results.

We did not find a significant correlation between CCT and corneal tensile modulus in the measured eyes. Because the present study was not designed to test the correlation between CCT and corneal modulus, the sample size may not be sufficient to detect the correlation even if it existed. Nonetheless, corneal modulus and CCT may not be regulated by the same factors and it is possible that CCT does not correlate with corneal stiffness. Future studies with larger sample size are needed to further examine the relationship between corneal modulus and thickness.

Our study confirmed the dependence of the tonometric measurement errors on true IOPs. In the canine eyes, GAT errors increased with pressure (Fig. 3). A similar trend was observed in porcine eyes. Using finite element analysis, Elsheikh et al. showed a pressure-dependent GAT error in human corneas. Srodkiewicz also showed a pressure-dependent GAT error using finite element analysis and proposed that the corneal resistance to applanation was pressure-dependent because of the structure and measurement configuration.

Tono-pen appeared to have a larger underestimation of IOP at higher pressures than lower pressures (Fig. 2). This result was consistent with the reported greater underestimation by Tono-pen at high pressures in live canine eyes. Similar results about Tono-pen have been reported on cat, cow, sheep, and horse. The underlying reasons for this tendency are not well understood.

The limitations of the present study include the following. First, the 1% strain secant modulus was used to represent corneal stiffness during IOP measurement. At a given IOP, different corneas experience different levels of strains due to the difference in thickness, radius of curvature, and material properties. In addition, the same cornea experiences different strains at different IOPs. Because of nonlinearity, corneal modulus varies with strain. To examine whether nonlinearity affects the correlations seen in this study, we further analyzed the data and estimated the IOP-corresponding secant modulus (i.e., using Laplace law to estimate IOP-corresponding stress and then using the experimental stress and/or strain curves to find the corresponding modulus). We also performed exponential curve-fitting (i.e., $\sigma = A\left(e^{B\sigma} - 1\right)$) to obtain 1% strain tangent modulus, IOP-corresponding tangent modulus, and the coefficients $A$ and $B$. These analyses yielded the same result in that GAT errors at 50 mm Hg were significantly correlated with the stiffness parameter (i.e., IOP-corresponding secant modulus, 1% strain tangent modulus, IOP-corresponding tangent modulus, or $A \cdot B$) and other correlations were not significant. This outcome indicated that the nonlinearity of corneal properties may not have a strong influence on the correlations found in this study. This result however needs to be interpreted with the understanding of the significant limitations of using Laplace law to estimate IOP-generated stresses. Laplace law is an oversimplification and does not take into account of the heterogeneous configuration of the cornea (i.e., nonuniform thickness and radius of curvature). Future studies, likely in combination with computational models, are needed to accurately determine corneal stresses and nonlinear properties.

Second, we only measured corneal tensile modulus while other biomechanical or geometrical factors may also influence corneal resistance during applanation and thus GAT errors. It is likely that the “correction” factor may include a number of biomechanical and geometrical parameters in a weighted manner validated through a statistical model. This should be considered in future studies. It is also of interest to note that it may not be meaningful to compare the absolute values of tensile modulus across different studies at different laboratories because of the highly nonlinear nature of corneal properties. Factors such as the level of prestress and the strain rate all have a significant effect on the reported modulus.

Third, the experimental setup in the present study adopted an “open stock” system so that the true IOP did not change during the application of the tonometer tip while in the real eye the applanation procedure may induce a significant IOP rise. The “open stock” system was helpful in studying the relationship between equilibrium pressures and their tonometric readings. The results of this study therefore should be interpreted with the understanding of potentially more complicated processes in actual clinical situations.

Fourth, the present study was performed in canine eyes. The canine cornea has a multilayered morphology and includes epithelium, stroma, Descemet’s membrane, and endothelium. Bowman’s membrane, a thin collagenous layer situated between the epithelium and the stroma in the human cornea, is absent in the canine cornea. The Bowman’s membrane was found to contribute little to corneal tensile properties, but it is unclear whether the resistance to applanation is also unaffected by this layer. Future studies are needed to investigate the correlation between corneal modulus and GAT errors as well as the variance in GAT errors in human eyes.

In summary, the present study found a significant underestimate of both GAT and Tono-pen measurements of IOP in canine eyes. The errors increased at higher pressure levels. The GAT errors appeared to have a correlation with the tensile modulus of the cornea and higher corneal stiffness was associated with higher GAT readings at a certain pressure level.

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