Limitations of Implantable, Miniature Ultrasonic Transducers to Measure Wall Movement in the Canine Jejunum¹

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Background. We used implantable miniature ultrasonic transducers to measure longitudinal distance, circumference, and wall thickness dynamically *in vivo* in canine jejunum. We hoped to differentiate circular from longitudinal smooth muscle contractions and to correlate physical measurements of change in distance within the jejunal wall with intraluminal manometry.

Materials and methods. In acute experiments at the time of celiotomy, longitudinal distances, circumferences, and wall thickness were measured directly and by ultrasonic transducers sewn to serosa and mucosa. Measurements were obtained with the intestine empty and after distention with air, water, or semisolid slurry. In chronic in vivo experiments in conscious dogs with indwelling ultrasonic transducers and intraluminal manometers, sonometric dimensions were correlated with manometric recordings. In acute experiments, sonometric measurements were similar to direct measurements. In chronic experiments in vivo, smallest ultrasonometric measurements of circumferences of the jejunum correlated in a phase-locked temporal manner with both highest intraluminal pressures and greatest wall thickness.

Results. Longitudinal distances increased during decreases in circumference. Distances orad to the site maximal intraluminal pressure peaked at 0.58 ± 0.04 s ($\bar{x} \pm$ SEM) before, and those aborad to this point 0.42 ± 0.04 s after attaining minimum circumferences.

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³ To whom correspondence and reprint requests should be addressed at Gastroenterology Research Unit (AL 2-435), Mayo Clinic, 200 First Street SW, Rochester, MN 55905. E-mail: sarr.michael@mayo.edu. Conclusions. Ultrasonic crystals can monitor geometric changes in the bowel wall with certain limitations, especially when obtained *in vivo*. Contraction of circular and longitudinal muscles, although phaselocked, do not appear to occur exactly synchronously in canine jejunum. © 2004 Elsevier Inc. All rights reserved.

Key Words: intestinal smooth muscle; intestinal contractions; longitudinal muscle contractions; contractility; peristalsis; migrating motor complex; ultrasonic transducer.

INTRODUCTION

Propulsive peristalsis is characterized by an orad ring of circular muscle contraction proximal to the distending bolus and by an abroad relaxation which facilitates abroad movement of intraluminal contents [1]. While longitudinal muscle activity is probably important in the generation of these propulsive patterns, its role during peristalsis remains unclear [2–7], predominantly because it is difficult to measure contractile activities of the two muscle layers simultaneously *in vivo*. Raised intraluminal pressures measured conventionally by intraluminal manometry have been attributed to circular muscle contractions. On the other hand, a simple and reliable measure of longitudinal muscle contraction *in vivo* over longer distances than a strain gauge (*i.e.*, 1–3 mm) is not available.

Recently, miniature ultrasound crystal transducers (2.3 mm in diameter) have been used to measure changes in wall thickness and luminal diameter of the canine pylorus at baseline and in response to pharmacologic perturbations [8]. Previously, these ultrasound transducers were used extensively to assess *in vivo* changes in geometry of other tissues, especially in the heart and vascular system [9–13]. In principle, the





FIG. 1. Locations of implantable miniature ultrasonic crystal transducers along canine jejunum in acute studies. Crystals A, B, C, and F are sewn to serosal surface of bowel while crystal E is sewn to mucosal aspect just beneath mucosa.

sonic transit time between pairs of implanted transducers sutured with known separations at defined locations is recorded. These transducers are small and should not themselves have significant mechanical effects on the tissues [8]. Since ultrasound waves are believed to be conducted preferentially through tissue (e.g., the bowel wall), as opposed to direct radial pathways across the lumen, the use of multiple ultrasound microtransducers offer potentially unique features that might allow synchronous real-time measurements of circular and longitudinal muscular contractions and bowel wall geometry.

Our aims were twofold: first, to establish the ability and reliability of measurements made by implanted ultrasound transducers on the canine small intestine under acute and chronic conditions; and second, to evaluate the temporal interrelationship of longitudinal and circular contractile activity. In the acute model, we wanted to compare ultrasonic measurements to actual measurements in live tissues using different intraluminal content (air, liquid, semisolid slurry). Subsequently, we wanted to assess the ability and reliability of chronically implanted miniature ultrasonic transducers to measure changes in intestinal wall thickness, circumference, longitudinal distance, and wall geometry in a canine model. By these measurements, we hoped to be able to identify and differentiate circular and longitudinal smooth muscle contractions by changes in distances measured by the miniature ultrasonic transducers. One hypothesis was that circular and longitudinal muscle at the same site in the canine jejunum contract simultaneously in a phase-locked fashion to facilitate intraluminal transit.

MATERIALS AND METHODS

Sonomicrometers

We used ultrasonic crystal transducers (Sonometrics Corp., London, Ontario, Canada) to measure distances within the soft tissue of the canine jejunum by means of piezoelectric transducers of 2-mm width. The sonomicrometers consist of two parts, a transmitting and a receiving circuitry. The pulse duration of the emitting pulse is 343.8 ns at a frequency of 64 MHz. Receiving crystals oscillate with a resonance frequency of 2 MHz. The velocity of sound in soft tissue is well-defined (1530 m/s). A simple calculation (distance = velocity \times time) allows one to estimate the distance between crystals. The accuracy of the determination of distances *in vitro* was ± 0.2 mm. The sonometric system is capable of measuring up to 45 distances, between 10 sonometric crystals, every 0.0625 s. In connection with an IBM PC-compatible microcomputer, dimensional readings could be saved concurrent with manometric pressure readings.

Preparation of Animals

Surgical procedures and experiments were performed only after approval by our Institutional Animal Care and Use Committee and in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Acute Studies

Mongrel dogs weighing 20–25 kg were used after an overnight fast. After induction of anesthesia with intravenous methohexital sodium (12.5 mg/kg) and maintenance of anesthesia with inhaled halothane, the abdomen was opened and the jejunum isolated. The proximal 10 cm of jejunum was used; the orad and aborad portions were occluded with plastic ligatures. Five sonomicrometer, omnidirectional crystal transducers (crystal size, 2.3 mm) were sutured to the exterior of the jejunal wall. Four were spaced at equal distances around the circumference of the bowel at the same point and another 4 cm proximally. A sixth transducer was sutured to the mucosa directly under a serosal crystal via an enterotomy several centimeters away (Fig. 1). A tube was inserted orad to the test segment with its tip within the test segment to inject luminal contents.

Intercrystal distances were measured by two methods: first, directly by stretching a string between crystals and measuring the length of string with a ruler (we used a string because we could most easily adapt the string to the bowel wall, especially when measuring the circumference); and second, by recording the ultrasonic measurements of distance (see below) under the following conditions of luminal content:

^{1.} Baseline measurements with no luminal content, allowing the opposite walls of the intestine to move freely, *i.e.*, to collapse and touch one another.

^{2.} The lumen of the 10-cm segment was filled completely with air distending the jejunum to diameter (measured directly by the string) of at least 4 cm.

^{3.} The lumen was filled completely with distilled water to the same volume as that of the air, distending the jejunum to the same diameter of at least 4 cm.

^{4.} The lumen was filled completely with a blenderized solution of dog food as a semisolid slurry (semisolid; 1 can of Alpo food plus 30 mL of water) at the same volume as that of distilled water, distending the jejunum to a diameter of at least 4 cm.



FIG. 2. "Bent bowel" experiments in acute studies. (A) The bowel was bent upon itself such that the serosal surface of crystals A and F touched one another. (B) The bowel was bent in the opposite direction such that the serosal surfaces of the bowel opposite crystals A and F touched one another but A and F did not touch one another.

Thereafter, the jejunum between crystals A and F was bent by two means (Fig. 2, "bent bowel experiments"); one approximated the positions of the sonomicrometer transducers of A and F (left panel), while the other approximated the sides opposite the transducers (right panel). These bent bowel experiments were also performed with the four types of intraluminal content to assess if, when the adjacent bowel sides were touching, whether the sonometric signals would travel across the areas touching or longitudinally along the longitudinal length of the bowel wall between transducers.

Direct measurements of distance using string and indirect measurements using the sonometric measurements under these conditions were obtained once per dog as follows. First, we compared longitudinal distances along the jejunum from A to F (Fig. 1). Second, we compared half-circumference measurements by adding the distance measurements from A to C and C to B. Third, we evaluated wall thickness from A to E; these measurements were obtained with the lumen empty as well as distended with the different intestinal contents. Fourth, in the "bent bowel experiments" (Fig. 2), we compared direct and ultrasonic distances of A to F when the jejunum was bent such that A and F touched and when bent such that the bowel wall opposite to A and F touched.

Chronic Studies

The same four mongrel dogs used in the acute experiments were used. Prior to beginning experiments, they were trained to rest quietly in a Pavlov sling. Later, the dogs were anesthetized and operated as described above. Ten miniature ultrasonic transducers (crystal size, 2.3 mm) were implanted at each of three sites in the jejunum (Fig. 3). Six of these crystals were located at the same level, four of them spaced evenly around the serosa (A, B, C, D) and two in the submucosa (E and E'). Two other serosal crystals were sutured 4 cm orad (F, F'), and two others 4 cm aborad (G, G'); these were used to measure longitudinal distances and to reflect longitudinal muscle contractions. Six polyethylene manometry catheters (I.D. = 1.25 mm; O.D. = 2.25 mm) were sutured into the jejunum. Three (P_2, P_3, P_3) P_4) were fixed in place at the levels of the crystals (Fig. 3); another (\mathbf{P}_1) was located 10 cm orad to these crystals, and two more were positioned in the duodenum to monitor phasic contractions and to monitor the presence of the migrating motor complex (MMC).

Conduct of Chronic Studies

After a 2-week recovery from implantation of the crystals and the manometric catheters, manometric catheters were perfused with deionized water (0.1 mL/min) with low-compliance pneumohydraulic pump connected to strain gauge transducers, which transmitted

intraluminal pressures to a chart recorder. These measurements were collected by a computer at a sample rate of 4 Hz. Sonometric data were collected online simultaneously. On each of at least 4 days after an overnight fast, manometric data were evaluated to allow recognition of phase II and III for at least three complete cycles of the MMC.

Sonometric Measurements

Measurements were made during all contractions of phases I, II, and III. We recorded simultaneously manometric changes, bowel wall circumference (A to C + C to B + B to D + D to A; Fig. 3), bowel wall thickness (A to E), orad longitudinal distance (A to F), and aborad longitudinal distances (A to G). For comparisons of time lags between bowel circumference and either intraluminal pressure, wall thickness, or longitudinal measurements, only contractions during phase III were utilized; we specifically used all contractions during phase III, because these contractions propagated and we could be confident of the interrelationships of longitudinal and circular contractile activity [14]. At least 10 contractions from each of three phase IIIs per dog were evaluated randomly.

Data Analysis

In the acute experiments (Fig. 1), we plotted measurements of distances measured directly by the string against those obtained sonometrically for: 1) longitudinal distance (A to F); 2) half circumference (A to C and C to B); 3) wall thickness (A to E); and 4) the two bent bowel configurations (A to F) (see Fig. 2). In the chronic experiments (Fig. 3), the circumference was measured by summing (A to C + C to B + B to D + D to A); wall thickness was measured between the submucosal and serosal crystals (A to E). Orad longitudinal distances were measured from A to F; corresponding aborad distances were A to G.

Manometric data were collected online, and motor activity was analyzed visually and classified according to the criteria for phases of the MMC [3]. Statistical comparisons were made using Student's *t* tests. Data are presented as mean \pm standard error of the mean ($\bar{\mathbf{x}} \pm$ SEM) for four dogs.

RESULTS

Acute Studies

Longitudinal Measurements

Orad distances between transducers A and F, as measured by a string and by the sonometric technique, agreed quite closely whether the lumen was collapsed or filled with air, water, or semisolid slurry (Fig. 4A). Measurements (both string and sonometric) were greater when the intestinal segment was distended with intraluminal content; the type of intestinal content had no recognizable effect on longitudinal measurements.

Half Circumferential Measurements

When the half circumference was measured by sonometry or by measurement of the circumference with the string (Fig. 4B), larger values were obtained in each dog for (A to B) when the bowel was distended with air, fluid, or semisolid than when compared to measurements obtained when opposite aspects of the bowel wall touched one another in the collapsed site.



FIG. 3. Locations of implantable miniature ultrasonic transducers (A–F) and manometric catheters (P_1-P_4) for the chronic studies. The 10 miniature ultrasonic transducers were implanted at each of three sites in the jejunum. Four manometry catheters were inserted into the lumen of the jejunum (P_1, P_2, P_3, P_4) . Three manometry catheters (P_2, P_3, P_4) were inserted at the site of the serosal ultrasonic transducers; another manometry catheter (P1) was inserted 10 cm orad to P2. Two other manometric catheters (not shown) were positioned in the duodenum.

Indeed, agreements between sonometric distances and actual circumferential measurements by the string were similar, but measurements by the sonometric technique tended to be less than direct measurements with the string (slope was >1.0).

Wall Thickness

Actual wall thickness *in vivo* could not be measured directly with good accuracy, but rough estimates of the wall thickness of the canine jejunum were 2-4 mm (when the jejunum was opened at autopsy). When measured sonometrically (A to E), the values ranged from 2-2.5 mm. No sonometric measurements were made with the bowel distended by luminal content because direct measurements could not be obtained.

Bent Bowel Experiments

When the segment of bowel was bent upon itself such that the bowel walls at transducers A and F were touching (Fig. 2A), the sonometric measurements of the distance between A and F did not correlate well with the direct distances between transducers measured by the string (Fig. 5). The distances measured sonometrically were much less than the longitudinal distances between the transducers when the bowel was straightened (see above longitudinal measurements), suggesting that the ultrasonic signal passed directly across the opposing walls of the bent bowel and not longitudinally along the bowel wall (see *Discussion*). When the bowel was bent on itself such that the walls opposite to transducers A and F were touching (Fig. 2B), the sonometric distances between A and F were greater than in the other bent configuration (Fig. 2A). The sonometric distances tended to be greater than the direct string measurements. The measured distances were larger when the bowel was distended with any of the various luminal contents.



FIG. 4. Correlation of each measurement in each dog in the acute study of (A) longitudinal distances, and (B) half-circumference of the jejunum. We measured directly with a string and sonometrically. Dashed line has a slope of 1.0 and would denote an exact correlation between measurements; solid line is best fit.



FIG. 5. Bent bowel experiments. Direct and sonometric measurements of "longitudinal" distances in each dog when (A) the sides of bowel wall with crystals A and F were touching, and (B) the sides of bowel wall opposite crystals A and F were touching (see Fig. 2). The dashed lines represent a slope of 1.0 and would denote an exact correlation between measurements; solid line is best fit.

Chronic In Vivo Study

Rhythmic fluctuations in manometric and sonometric recordings of longitudinal lengths, circumferences, and bowel wall thickness were noted during the chronic experiments (Fig. 6). The manometric recordings were characteristic of the cyclic interdigestive fasting motor activity as recorded previously in our laboratory [15, 16]. Typical MMCs were noted regularly, and we were able to monitor cyclic manometric patterns from the duodenal catheters and from P_1 . Thus, we were able to accurately time the onset of phases II and III of the MMC at P_3 .

Fig. 6 illustrates the overall pattern in which minimal wall circumference corresponded approximately to maximal manometric pressure, elongation in both orad and aborad dimensions, and a thickening of the bowel wall.

Circumference Versus Pressure

When changes in circumference (measured as the summation of distances from A to C, C to B, B to D, and D to A; see Fig. 3) were correlated temporally with changes in manometric pressure at the same location (P₃), a close temporal and phase-locked relationship was noted (Fig. 6). When manometric pressure was reaching its maximum level, the circumference was reaching a minimum, and vice versa. However, the peak in the manometric pressures during phase III of the MMC was observed 0.41 ± 0.03 s after the minimum circumference was recorded.

Circumference and Manometric Pressure Versus Wall Thickness

Circumference and wall thickness (measured as sonometric distance between A and E; see Fig. 3) also



FIG. 6. Representative recording of sonometric and manometric parameters during phase III. Note the rhythmic phase-locked fluctuations in all parameters. Circumference, oral and anal longitudinal distances, and wall thickness varied from maximum to minimum in temporal relationship with the manometric changes. Line denotes minimum value of circumference.



FIG. 7. Time differences in minimum circumference compared to oral and anal longitudinal distances in a representative dog. The maximum oral longitudinal distances occur 0.6 before, while the greatest and longitudinal distances occurring 0.1 s at the minimum circumference.

varied indirectly in a temporal, phase-locked coordination. When the circumference was the smallest, the wall thickness was becoming the greatest (Fig. 6); however, wall thickness peaked 0.32 ± 0.25 sec before circumference reached its nadir. Similarly, when manometric pressure was reaching its peak, wall thickness was still reaching its nadir.

Circumference and Manometric Pressure Versus Longitudinal Distance

Orad longitudinal distances (A to F) and aborad longitudinal distances (A to G) also varied in a temporal, phase-locked coordination with the changes in circumference and manometric pressure (Fig. 6). When the circumference reached its nadir and manometric pressure was reaching its maximum, the orad and aborad longitudinal distances were reaching their peak values (Fig. 7). The greatest orad and aborad longitudinal distances were attained 0.58 ± 0.04 s before and $0.42 \pm$ 0.04 s after, respectively, the minimum circumference during phase III was reached.

Bowel Wall Measurements During the MMC

When measurements were compared across the phases of the MMC, the mean jejunal circumference was smaller, while wall thickness and longitudinal distances were greater during phase III (Table 1).

DISCUSSION

Miniature ultrasonometric transducers have been used extensively to define the geometry of the heart *in vivo* [9–13], but we are aware of only one published report [8] and one recent abstract [17] of the technique being applied to the gut. The report by Mandrek [8] suggested that the technique was reliable and a potentially attractive and useful approach to monitoring motility of the canine pylorus. As can be concluded from our acute studies, relative fixation of bowel segments being studied by this technique (to avoid kinking and transmission of the sonic wave across areas of serosal opposition) is necessary to maximize the reliability of the observations. Thus, the observations of Mandrek [8] at the canine pylorus and Adelson *et al.* [17] in the

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Phase of MMC	Circumference (A to $C + C$ to B + B to D + D to A)	Wall thickness (A to E)	Orad longitudinal (A to F)	Aborad longitudinal (A to G)
Phase I	$43.4 \pm 2.8^{*}$	$5.0\pm0.7^*$	25.2 ± 4.9	25.0 ± 3.1
Phase II	$43.6\pm3.0^*$	5.1 ± 0.6	25.7 ± 5.0	26.1 ± 3.4
Phase III	41.5 ± 2.6	5.4 ± 0.6	26.2 ± 4.9	26.1 ± 3.5

Note. Values are means \pm SEM over 1 min; N=4 dogs.

*P < 0.05 versus Phase III.

rat stomach measuring luminal diameter are likely to be robust, given the anatomic configuration and resistance of a short segment of pylorus and stomach to distention; longitudinal distortion in these areas would have minimal effects on luminal diameter.

Indeed, our measurements of longitudinal distances with the jejunal segment laid out longitudinally during the acute intraoperative experiments showed a very close correspondence between direct and sonometric measurements. Parity of these findings allow us to feel confident about the ability of this technology to measure overall patterns of longitudinal length and the timing of the changes in longitudinal length.

In contrast, when immediately adjacent aspects of the jejunal wall between the microtransducers were intentionally bent upon themselves during the acute experiments, measurements of longitudinal distances between the crystals by sonometry were unreliable compared to direct string measurements, especially when the bowel was distended. Under these conditions, the distances recorded by the ultrasonic transducers were spurious, probably because sound waves were conducted across the opposed segments, thereby "short circuiting" passage of sound waves between the transducers. For these reasons, we cannot be certain in the chronic experiments to what degree kinking or twisting of the study segments between the emitting and recording microtransducers may have interfered with the measurements of longitudinal distances with implanted microtransducers. However, because the study segment was only 8 cm in longitudinal distance, opposition of adjacent segments between the microtransducers was probably minimal. With longer distances, it is likely that intermittent opposition of adjacent bowel wall might very well short-circuit the sound waves leading to falsely short sonometric measurements of longitudinal distances. A model creating a straight segment of jejunum fixed to the abdominal wall at both ends, *i.e.*, a Thiry Vella loop, might overcome this potential limitation; however, this configuration might artificially affect normal contractility.

Similarly, our measurements of both circumference and diameter of the bowel lumen at any one point also had limitations that were problematic and potentially less reliable. On one hand, with the bowel segment distended by air, liquid, or semisolid slurry, measurements of circumferential distances were reliable; it appeared that the sound waves traveled within the bowel wall around the circumference and not directly across the lumen, because direct circumferential measurements and sonometric measurements agreed quite closely. In contrast, when the bowel lumen was empty and opposite sides of the lumen were opposed, circumferential measurements by sonometry were less than direct measurements of circumference, potentially limiting assessment of contractile activity under different states (e.g., postprandial versus during the interdigestive state) when different volumes of luminal content and fully luminal occluding contractions may be expected. In contrast, dynamic measurements of circumference over short time intervals (e.g., contraction to contraction) under the same physiologic state appear to be quite reliable.

When examining the interrelationships of manometry and sonometric distances, we chose to focus our analyses during phase III of the MMC, because it should be more predictable that contractions during phase III will begin at the orad end and be propagated to the aborad end of the segment [14]. However, we also compared wall dimensions during phases I, II, and III; circumference was least during phase III, and wall thickness tended to be greatest. However, other indices were not different between the phases. Thereafter, all other observations focused on phase III.

Fig. 6 shows reciprocity and phase-locking among the ultrasonic dimensions during contraction *in vivo*; clearly, the patterns are not identically reciprocal, and the significance of this partial separation of the time bases is unclear at this point. In this figure, maximal circumference was taken as an arbitrary starting point. There were mean time lags that could be calculated between minimal circumference, maximal orad and aborad dimensions, and wall thickness despite the constant phase-locked pattern. Similarly, there were time lags between minimal circumferences and maximal intraluminal pressures. Since our transducers and manometry catheters were sewn in place and confirmed to have not moved at autopsy, the timing of measurements should be quite precise. The lack of exact correlation of minimum wall thickness with maximum circumference is unexplained, but the lag might be related to viscoelastic properties of the bowel wall.

The peristaltic reflex is characterized by activation of ascending and descending neural reflex pathways [17]; moreover, ascending excitation and descending inhibition can be induced during peristalsis in vitro [5, 6]. However, whether longitudinal muscle and circular muscle contract simultaneously or not, or whether longitudinal muscle relaxes while circular muscle is contracting is still unclear [5-7, 16]. We interpret our experiments as showing that longitudinal distances along the bowel wall change during contraction. There are time lags, but orad and aborad distances do not change reciprocally, and although the changes seen are phase-locked, neither varies in complete reciprocity with wall thickness or circumferences of the bowel; how the viscoelastic properties of the bowel wall might affect this relationship is unknown.

In addition to the above considerations, conclusions about a direct relationship between changes in longitudinal distance and contraction/relaxation of longitudinal muscle are not absolute. Longitudinal distances will of necessity change to some extent, independent of longitudinal muscle contraction or relaxation, when circular muscle contracts, *i.e.*, law of mass and volume conservation. Although one might expect these changes to be small compared to changes induced by longitudinal muscle contraction, nevertheless, chronic measurements *in vivo* must be considered as only estimates of longitudinal muscle contraction and relaxation, again limiting the applicability of this technology to accurately reflect simultaneous activity of circular and longitudinal muscle activity.

In conclusion, pathways of ultrasound waves appear to pass through the jejunal wall and miniature ultrasonic crystals can monitor geometric changes in the canine jejunal wall under defined experimental conditions. Ultrasonically measured distances vary with changes in intraluminal pressure when the bowel wall contracts and relaxes. Circular and longitudinal muscles, although phase-locked in their contractile patterns, do not appear to contract at exactly the same time during phase III of the MMC in the canine jejunum. This methodology using ultrasonic crystals may be useful in further study of relative wall movements during peristalsis, but the absolute measurements of longitudinal distance and circumference have unpredictable limitations that may preclude their use in certain anatomic locations of the gut, limiting their usefulness.

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