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Determination of Biomechanical Properties in Guinea Pig Esophagus by Means of High Frequency Ultrasound and Impedance Planimetry

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Impedance planimetry and high-frequency ultrasound were used to determine circumferential stress and strain from measurements of luminal cross-sectional area and wall thickness during balloon distension of the guinea pig esophagus *in vitro* ($N = 30$). The excised esophagus was mounted on two plastic tubes in an organ bath containing oxygenated calcium-free Krebs-Ringer solution with 10^{-2} M $MgCl_2$ to abolish smooth muscle contractile activity. One of the plastic tubes was movable in order to stretch the esophagus longitudinally by 15% (elongated state). The impedance planimetry probe was placed with the balloon inside the lumen of the esophagus. A 20-MHz ultrasound transducer was mounted above the esophagus and provided scans in the transverse and longitudinal directions. The luminal cross-sectional area at the highest applied pressure of 2.9 kPa was 13.3 ± 0.3 mm^2 in the resting state. In the elongated state the luminal cross-sectional area at the highest pressure was 12.5 ± 0.1 mm^2 ($P < 0.02$). The wall thickness decreased from 990 ± 21 μm at 0 kPa to 640 ± 9 μm at 2.9 kPa at *in vitro* length. In the elongated state, the values were 940 ± 32 μm to 480 ± 13 μm ($P < 0.01$). The stress–strain relation was exponential ($\sigma = \alpha(e^{\beta\varepsilon} - 1)$, $r^2 > 0.98$, $P < 0.01$). The circumferential elastic modulus calculated at a Green strain of 0.95 was 44.5 ± 10.5 kPa in the *in vitro* state and 81.7 ± 13.1 kPa in the elongated state. The elastic modulus differed between the resting and elongated states ($P < 0.02$).

KEY WORDS: biomechanical properties; ultrasound; impedance planimetry; esophagus.

The esophagus is a distensible muscular tube that serves to transport food and liquids from the pharynx to the stomach (1). This function depends on active (contractile) as well as passive properties. A vast amount of literature exists on the contraction patterns of the esophagus (2). The entry of food into the hypopharynx elicits a swallow reflex. When a bolus of food or liquid passes through the esophagus, it distends. Since the function of the esophagus is mainly mechanical, it is necessary to understand and measure quantitatively the mechanical properties of esophagus before a complete description of the biological function can be provided (3). This work is focused on providing basic data on morphometric properties and elastic properties mainly in the circumferential direction.

Data in the literature pertaining to the elastic as-

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pects of esophageal function are obtained from uniaxial testing of esophageal muscle strips (2) and from distension studies using impedance planimetry. The latter was developed for measurement of luminal cross-sectional area (CSA) and pressure in tubular organs during luminal balloon distension (4–7). The impedance planimetry system provides an estimate of wall tension, compliance, and cross-sectional area distensibility (8, 9). Jørgensen et al (10) described a combined impedance planimetry–ultrasound system for measurement of wall thickness and luminal CSA during balloon distension of the porcine duodenum. Storkholm (11) used a similar set-up for studying the guinea pig small intestine. The combined approach made possible computation of circumferential stress and strain in tubular organs. Unfortunately, the previous work provided only sparse validation of the ultrasonic A-scan equipment, and the thickness measurements were done manually on the equipment's TV monitor using a built-in autocalibrated caliper (10, 11). In this work an ultrasound transducer placed outside the organ provided the measurement of wall thickness. We further developed and validated the ultrasonic thickness measurements by using the raw digitized RF echo signals. Hence, the purpose was to present a more accurate and precise thickness measurement. The technique was applied for measurement of pressure–CSA–wall thickness relations and computation of circumferential stress–strain of the guinea pig esophagus *in vitro* at resting and elongated conditions.

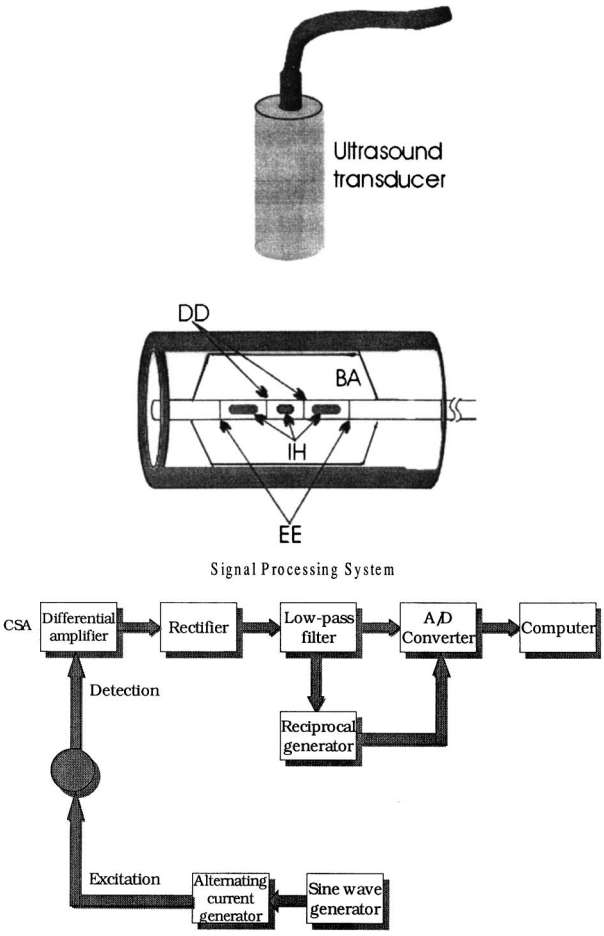

Fig 1. Top: Schematic drawing of the impedance planimetry probe and the ultrasound transducer. The excitation and detection electrodes are denoted by EE and DD, respectively. IH indicates side-holes connected to a fluid container for pressurizing the balloon (BA). Bottom: Block diagram over the impedance planimetry signal processing system.

MATERIALS AND METHODS

Specimen Preparation. Thirty female guinea pigs (700–950 g) were killed by pentobarbital and thoracotomized. The esophagus was dissected from the tongue to the stomach and separated from adjacent structures. A 4-cm-long segment with the proximal end beginning 2 mm from the root of the tongue was excised. It was placed immediately in an organ bath of 5°C oxygenated calcium-free Krebs-Ringer solution containing 6% dextran and 10^{-2} M magnesium chloride to obtain muscle relaxation and kept there until the time for measurement. The experiments were completed within 2 hr from the time the animals were killed. The experimental protocol was approved by the University of Illinois Laboratory Animal Care Advisory Committee and satisfied all campus and National Institutes of Health (NIH) rules for the humane use of laboratory animals.

Impedance Planimetry Probe Design. A four-electrode impedance measuring system located inside a balloon on a 3-cm-long 5-French probe was constructed for measurements of the luminal CSA of the esophagus (Figure 1). The two outer excitation electrodes have an interelectrode distance of 12 mm and are connected to a sinusoidal generator producing an alternating current of 100 μA RMS at 5 kHz. Two ring electrodes for detection of the potential difference are placed midway between the two excitation electrodes with an interelectrode distance of 3 mm. They are connected to the impedance measurement system. The electrode distances were chosen on the basis that CSA can be measured up to a diameter of 4–5 times the distance between the excitation and detection electrodes (4). The balloon is 20 mm long and made of 25-μm-thick nonconducting polyurethane. It could be inflated with electrically conducting fluid through an infusion channel (diameter = 1 mm) connected to a fluid column to a maximum CSA of 50 mm^2 without stretching the balloon wall. The size of the balloon was chosen on the basis of pilot experiments demonstrating that the CSA of the guinea pig esophagus did not exceed 50 mm^2 in the pressure range used in this study. The impedance planimeter was calibrated before measurement. Calibration was done in a PVC block with eight holes of known CSAs ranging from 3 to 50 mm^2. Multiple calibra-

tion points were used because of nonlinearity between the CSA of the calibration holes and the measured CSAs. Nonlinearity was corrected for by means of a software feature (Motan, Gatehouse, Aalborg, Denmark) up to a CSA of approximately 50 mm^2.

Measurements of CSA. The CSA of the balloon was measured from the impedance of the saline fluid inside the balloon (4). When a current I is induced in a conductor by two excitation electrodes, the potential difference V between the detection electrodes is $V = IZ$ (Ohm's law). Z is the electrical impedance of the fluid and is expressed as $d\ p/CSA$ where d is the distance between the detecting electrodes and p is the conductivity of the fluid. Thus, the voltage is inversely proportional to the CSA. Direct proportionality between the CSA and voltage was obtained by means of reciprocation software. The probe and signal processing system are illustrated in Figure 1. Sources of errors in the impedance planimetry system are due to temperature, salinity, and geometric factors (12–16). These errors were accounted for in this study. It is also known that dislocation of the probe from the center axis to a more radial position in the balloon gives erroneous result (16). However, the ultrasound B-scan imaging in this study demonstrated that the impedance probe was placed in the middle of the balloon during distension. It was also evident that the geometry of the cross section approached a circular shape at all pressures.

Data Acquisition System. The basic experimental set-up consists of a system to mount the esophagus, a temperature controlled water tank, a sampling oscilloscope, a system to move the ultrasound transducer, and a PC with an interface board (IEEE-488) to control movement and acquire data from the sampling oscilloscope.

We used an ultrasound system providing A- and B-scan images. The A-scan (amplitude demodulation) mode is a one-dimensional scan showing the echo amplitude versus depth along the path of the emitted ultrasound pulses. The B-scan (brightness) mode is a two-dimensional image composed of multiple A-scan lines. A 20-MHz focused ultrasound transducer (model V317, Panametrics, Inc. Waltman, Massachusetts) was used. The ultrasound transducer had a −6 dB pulse-echo bandwidth from 14 to 24 MHz and a focal distance of 12.4 mm (17). The transducer was excited by a −300-V pulse produced from a pulse/receiver (model 5800, Panametrics). The received RF echo signal was displayed on a Tektronix 11401K digital oscilloscope. The recorded 500-μsec/sec 10-bit, 1024-point waveform was transferred to and stored on the hard disk of the computer. The mathematical processing of the A-scan lines was done by MatLab (The Math Works, Inc., Natick, Massachusetts) on a Sun Sparc 20 workstation and a Pentium Pro 200 MHz PC. The position of the ultrasound transducer could be controlled in the x, y, and z directions by means of a computer-controlled precision motion system (Daedal Inc., Harrison City, Pennsylvania). The motion system was capable of making three linear and two rotational movements with the smallest movement of 1 μm (accuracy of ± 0.5 μm). The acquired A-scan lines (up to 1000 lines) from a single experiment were composed to a B-scan image. The user selected from this image the position where the A-scan line for the thickness calculation should be located. The selected A-scan line was the same in all ultrasound images obtained from an animal. The MatLab program semiautomatically located the same A-scan lines in the next image (at the next balloon distension step) and the program calculated the distance between the two A-scan lines based on the propagation speed in the tissue and Krebs-Ringer solution.

The thickness of the wall T_w was calculated from the echoes, one from the serosal surface and one from the mucosal surface using

$$T_w = 0.5(t_2c_2 + t_1c_1) \quad (1)$$

where c_1 was the propagation speed in the Krebs-Ringer solution, c_2 was the propagation speed in the tissue, t_2 was the sound-trip time between the transducer and the submucosal reflection, and t_1 was that for the serosal surface. The balloon wall could not be distinguished from the tissue on the images. Hence, the thickness of the balloon wall was ignored (approx. 30 μm). Figure 2 shows ultrasound signals obtained from a typical animal experiment.

Validation of Ultrasonic Measurements. The ultrasound measurements were evaluated using a thin-walled homogeneous rubber tube with uniform and known thickness (1.88 mm). The propagation speed in the rubber was 1550 m/sec. Measurements were done and the pulse-echo ultrasonic signals were saved for further statistical analysis. The accuracy for the first and second reflection wave form was 0.25% and 0.30% ($N = 40$, $P < 0.001$). The variation coefficient for the thickness measurements was less than 0.5%. The average value from the measurements were 1.86 mm. The effect of moving the transducer on the thickness measurement was also evaluated. First, the transducer's beam axis was placed perpendicular to the tube, and the A-scan line with the maximum amplitude was found by adjusting the transducer position in the z direction. The transducer was moved ± 120 lines away from the maximum point in the x direction. The resultant thickness change was found to be less than 5%.

Experimental Procedures. The organ bath was kept at a constant pH of 7.4 and a temperature of 37°C during the measurements. The esophagus was mounted in the organ bath on two small plastic cannulas. After calibration of the system, the impedance planimetry probe was inserted in the lumen of the esophagus. The zero point for the fluid column was set at the surface level of the fluid in the organ bath. Thus, the applied pressure corresponded to the esophageal transmural pressure. The balloon was inflated with NaCl solution by varying the level of the fluid column. The stepwise pressure loadings were carried out by increasing and decreasing the level of the fluid container corresponding to pressures of 0, 0.2, 0.5, 0.9, 1.5, 1.9, 2.4, and 2.9 kPa. Steady-state CSA was defined as a CSA increase of less than 0.1 mm^2 during a 30-sec period and was awaited at each pressure step. The CSA and wall thickness at steady-state conditions were used in the subsequent analysis. The esophagus was preconditioned six times between the minimum and maximum pressure before the actual measurements in order to obtain repeatable results. In 10 of the 30 guinea pigs, we elongated the esophagus by 15% and another distension test was made.

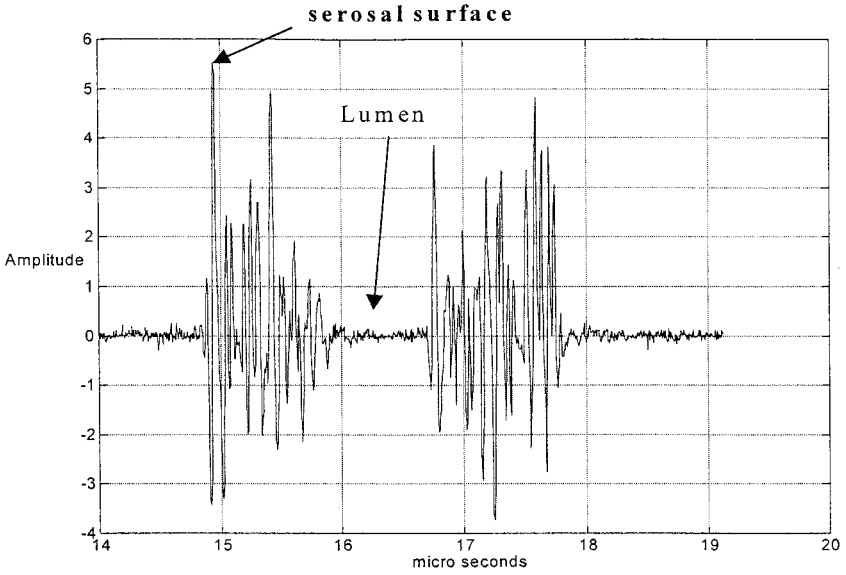

Fig 2. A-scan pulse-echo ultrasonic signal showing the esophageal wall with its serosal and luminal surfaces.

Biomechanical Data Analysis. The average circumferential wall stress was calculated as

$$\sigma_\theta = \frac{r_i p}{h} \tag{2}$$

where r_i is the inner radius, p is the transmural pressure, and h is the wall thickness. This equation is valid for thick-walled circular cylindrical tubes like the esophagus.

The circumferential Green strain was calculated as

$$\varepsilon = \frac{r^2 - r_0^2}{r_0^2} \tag{3}$$

where r is the midwall radius at any given balloon pressure over 0 kPa. r_0 is the midwall radius at 0 kPa derived by fitting the pressure–CSA curves using the polynomium $CSA = ap^2 + bp + c$. The elastic modulus was calculated as the first derivative of the stress–strain relation.

In order to evaluate the longitudinal strain during balloon distension, we assumed that the tissue was incompressible. The radial and circumferential stretch ratios were calculated as

$$\lambda_r = \frac{h}{h_0} \text{ and } \lambda_c = \frac{r}{r_0} \tag{4}$$

where h is the wall thickness and r the midwall radius. The subscript zero indicates the nonpressurized state. The stretch ratio in the longitudinal direction was calculated as

$$\lambda_l = \frac{1}{\lambda_r \lambda_c} \tag{5}$$

Data are presented as mean ± SD. Two-way analysis of variance was used for statistical analysis using SigmaStat (Jandel Scientific). $P < 0.05$ was considered significant.

RESULTS

The pressure–CSA data are shown in Figure 3a. At the resting length, the luminal CSA increased from 5.0 ± 0.3 mm^2 at zero pressure to 13.3 ± 0.3 mm^2 at 2.9 kPa. The curve showed a good fit to the second-order polynomium $y = ax^2 + bx + c$ ($r^2 = 0.998$). The CSA increase in the elongated state was from 4.7 ± 0.8 mm^2 at zero pressure to 12.5 ± 0.1 mm^2 at 2.9 kPa. The curves obtained for resting and elongated states differed ($P < 0.02$).

Representative ultrasound signals from the esophagus are shown in Figure 2. The wall thicknesses in the resting and elongated states are shown in Figure 3b as function of pressure. The wall thickness at the resting length decreased from 990 ± 21 μm at 0 kPa to 640 ± 9 μm at 2.9 kPa. The figures for the elongated state were 940 ± 32 μm at 0 kPa and 480 ± 13 μm at 2.9 kPa. The thickness differed between the resting and elongated states ($P < 0.01$).

The circumferential stress–strain relations at *in vitro* length and in the elongated state are shown in Figure 3c. The stress–strain relations showed a good fit to the exponential function $\sigma_\theta = \alpha(e^{\beta\varepsilon} - 1)$ ($r^2 >$

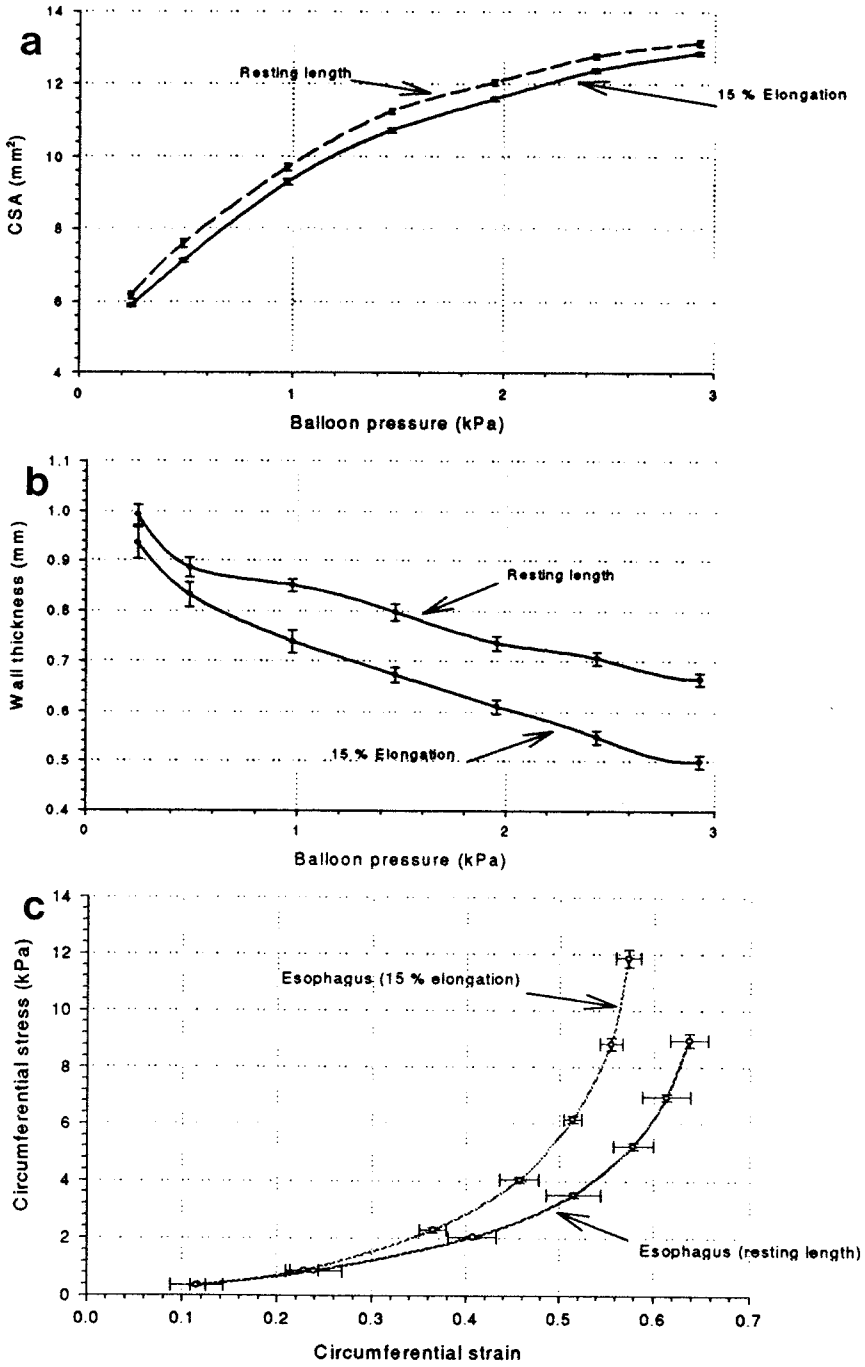

Fig 3. Luminal cross-sectional area (a) and wall thickness (b) as function of distension pressure for resting and elongated states. (c) Stress–strain relations for resting and elongated states are shown.

0.98 for both resting and elongated states). The circumferential elastic modulus calculated at a Green strain of 0.95 was 44.5 ± 10.5 kPa at resting state and 81.7 ± 13.1 kPa in elongated state. The elastic modulus differed between resting and elongated states ($P < 0.02$).

The circumferential, radial and longitudinal stretch ratios in resting state are shown in Figure 4. The

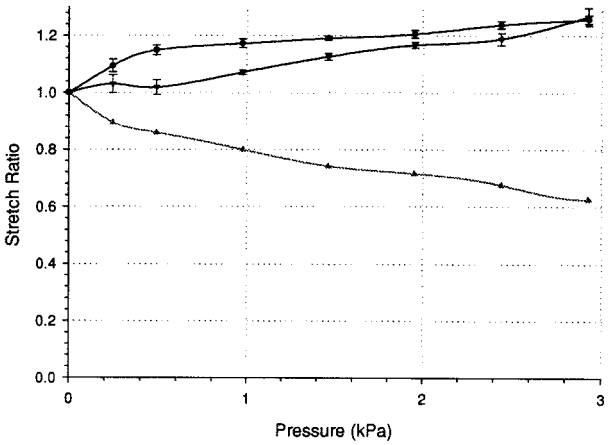

Fig 4. Illustration of stretch ratio in circumferential (◆), longitudinal (▼), and radial (▲) direction as function of the distension pressure. See text for definition of stretch ratio. Incompressibility was assumed. The balloon distension caused axial elongation to a lesser degree than the circumferential stretch.

longitudinal strain was tensile but less than the circumferential strain. The radial deformation was compressive.

DISCUSSION

In this study we have devised a method for the determination of circumferential stress–strain properties in the esophagus based on impedance planimetry and high-frequency ultrasound. Impedance planimetry provided the luminal pressure–CSA data and has previously been used in gastroenterology to determine biomechanical wall parameters such as compliance, pressure elastic modulus, and hysteresis (5–7). The method has been thoroughly validated previously and possible sources of error were described in detail (15, 16). These aspects were taken into account in this study by controlling factors such as temperature and probe position. Dislocation of the impedance probe from the center of the longitudinal axis to an eccentric position in the balloon can be an important source of error (14). However, in this study ultrasonic measurements demonstrated that the impedance probe was located in the central longitudinal axis of the balloon. Therefore, this source of error was insignificant in these studies.

The wall thickness was measured by means of ultrasonic A-scans that also proved highly accurate. It was assumed that the stress induced by balloon inflation did not itself alter the biomechanical properties of the wall and thus did not alter ultrasonic characteristics such as the ultrasound propagation speed. This would have introduced an error in determining the wall thickness, but it is unlikely to happen with the low pressures applied in this study. Furthermore, we have investigated the propagation speed of the balloon material under different circumstances with a scanning laser acoustic microscope in resting and elongated states. The balloon velocity was 1999 m/sec. The change of length in the balloon in our investigation was so small that its propagation speed change would not be significant. In this study it was not possible from the measured A-scan lines to separate the tissue layers. The ultrasound propagation speed could therefore not be determined for the individual layers of the esophageal wall. Rather a mean value of propagation speed was obtained in this investigation.

The balloon distensions increased the CSA and decreased the thickness of the esophageal wall. Using the principle of incompressibility, we demonstrate that the esophagus elongates at the site of distension, although to a lesser degree than the tensile deformation in the circumferential direction. This adds to yet unpublished data from our group where microbeads on the surface were used to track the deformation, and it indicates anisotropic mechanical behavior. Such data are likely important for the interpretation of distension-evoked mechanical responses of the esophagus, since mechanoreceptors are located in the esophageal wall and may respond to different mechanical stimuli.

Previous studies of esophageal elasticity have mainly been conducted on isolated smooth muscle strips to provide data on length–tension relations (2) and in various animal experimental models to provide data on compliance and tension–strain relations (5, 8, 9). However, to the best of our knowledge, no data have been published on the stress–strain relation obtained in the intact esophagus. In this respect the guinea pig esophagus serves as a model for the human esophagus, and the applied pressures were in the physiological range. The CSA and thickness data were used for the calculation of the circumferential stress–strain relation under resting and elongated conditions, assuming cylindrical geometry and isotropic mechanical properties. The circumferential stress–strain relation of the esophagus followed an exponential law as in most other tissues (18). Thus, the mechanical properties are nonlinear. At low pressures, the esophagus yields readily to allow boli to pass, whereas at higher pressures it resists forces to avoid damage to the tissue. Furthermore, the stress–strain curve for the elongated state was shifted to the left, indicating that the stiffness of the esophageal wall increases after elongation (Figure 3c). Stress was

calculated according to Cauchy's definition rather than as Kirchhoff stress. This does not significantly affect the curve shape and the differences reported.

The current paper presents data on the passive circumferential stress–strain relation of the esophagus. Analysis of swallowing and pathological conditions taking stress–strain data into account is needed to establish the physiological influence of these stresses and strains. We are currently collecting data and developing studies that will be reported in subsequent papers.

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