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Muscular Architecture and Manometric Image of Gastroesophageal Barrier in the Rat

SANDRA MONTEDONICO, MD, JORGE GODOY, MD, ALBERTO MATE, MD, ANNE K. POSSÖGEL, MD, JUAN A. DIEZ-PARDO, MD, PhD, and JUAN A. TOVAR, MD, PhD

The two components of the gastroesophageal barrier, the sphincter and the crural sling, closely overlap in humans, whereas they are widely separated in the rat. This investigation correlates the anatomical components of the barrier and their manometric counterparts in this animal. Sphincteric and crural sling pressures were measured in four quadrants in 23 rats. Muscle thickness was measured at nine levels of the gastroesophageal junction in the same quadrants in 12 rats and the muscular architecture of the region was studied in 10 fresh specimens. The manometric sphincteric component is stronger on the right side where the thickest muscle fibers anchor to the anterior and posterior borders of a mucosal ridge that almost surround the cardia. Conversely, the sling pressure is highest towards the left where the muscular bundles straddle the esophagus. In conclusion, there is a close correspondence between the manometric image and the muscular architecture of the components of the gastroesophageal barrier in the rat. The anatomical arrangement of U-shaped muscular bundles oriented in opposite directions creates a particularly powerful antireflux mechanism.

KEY WORDS: gastroesophageal barrier; manometry; anatomy; experimental model.

The two main components of the gastroesophageal barrier are the lower esophageal sphincter (LES) and the crural sling of the diaphragm. The sphincter has been well defined anatomically and manometrically (1), but, although there is increasing evidence of the participation of the contractions of the crural sling of the diaphragm, the extent of its contribution to the barrier is difficult to appreciate in humans because of the overlapping of the two structures (2). However, it is very apparent in the rat, in which both components are widely separated by a long intraabdominal segment of the esophagus (3). The investigation of the antireflux barrier at affordable costs in this laboratory animal opens unforeseen perspectives in this area in which many questions are still unanswered. The anatomy of this region in the rodent is so different from that of humans that a better definition of which muscular structures account for the pressure barrier effect in this animal was felt necessary prior to any further research. This study aims at defining the spatial arrangement of both the LES and the diaphragmatic crural sling in the rat in order to better understanding their respective manometric profiles.

MATERIALS AND METHODS

Adult male Wistar rats ($N = 23$) weighing an average of 398 g (range 300–500 g) (Criffa, Barcelona, Spain) were fasted overnight before the experiments but were allowed free access to water. For anatomical studies, 10 animals were killed and carefully dissected under an operating microscope (Wild M-650, Herrbrugg, Switzerland) aiming

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From the Department of Surgery and Experimental Laboratory, Hospital Infantil Universitario "La Paz," Madrid, Spain.
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Address for reprint requests: Prof. Dr. Juan A. Tovar, Department of Surgery, Hospital Infantil Universitario "La Paz," P. de la Castellana 261, 28046 Madrid, Spain.

at defining the anatomy of the gastroesophageal junction and that of the crural sling. The muscular architecture of the gastroesophageal junction was subsequently studied in fresh gastroesophageal specimens by opening their lumina from the pylorus to the gastric fundus along the greater curvature and removing the mucosa by microdissection with scissors to display the muscular layers, which were studied under direct light and by transillumination using a purposefully made light box. Another 12 animals were used to measure the thickness of the muscular walls of the gastroesophageal junction after removing the stomach and esophagus *en bloc*, fixing them with injection of 10% buffered formalin, and immersing them in the same solution for two days. They were subsequently sectioned with a razor blade either in the sagittal ($N = 6$) or frontal planes ($N = 6$) along the longitudinal axis of the esophagus and embedded in paraffin, stained with Masson trichrome, and studied with a conventional light microscope using a micrometric ruler. Muscular thickness was measured in four radial directions (anterior, posterior, right or lesser curvature, and left or greater curvature) every 100 μm including the gastroesophageal junction, which served as a reference point. Values are described as means ± SD, and the units are microns.

The rats allocated for manometric studies were anesthesized by intraperitoneal injection of ketamine hydrochloride (6.25 mg/100 g) and diazepam (0.5 mg/100 g) before the experiments that were performed with the animals in the supine position and under spontaneous breathing. The techniques of catheter placement and manometric recording were described in detail previously (3–8). Briefly, a tip-occluded single lumen catheter (outer diameter, 1 mm; internal diameter, 0.5 mm) with a distal side hole (1.0 × 0.5 mm) was positioned in the stomach and was perfused continuously with bubble-free distilled water at a rate of 0.4 ml/min by means of a pneumohydraulic pump and a capillary infusion system (Mui Scientific, Mississauga, Ontario, Canada). The catheter was connected to an external transducer (HP 1280; Hewlett Packard, Palo Alto, California) and the pressure was registered by a monitor with a screen display and on-line printout at a paper speed of 2.5 mm/sec (Schiller). The atmospheric pressure at the level of the atrium served as the zero reference. The catheter was pulled back through the esophagogastric junction into the esophagus with a specially made mechanical device at a constant speed (1 mm/sec). In an attempt to define the spatial arrangement of the gastroesophageal barrier, the measurements were successively repeated in the four quadrants around the longitudinal axis of the esophagus at 90° angles from each other by directing the side hole of the catheter strictly in the anterior, posterior, right (lesser curvature), and left (greater curvature) directions. The variables analyzed were: lower esophageal sphincter pressure (LESP) or difference between intragastric pressure and the highest pressure of the more distal component of the pressure profile and crural sling pressure (CSP) or difference between baseline pressure and the highest pressure of the more proximal component of the pressure profile. The values recorded are the average of three successive pull-through recordings.

Values are described as means ± SD and the units are millimeters of mercury. The normality of their distribution was assessed by comparing the actual values with the theoretical ones for the same means using Kolmogorov-Smirnov test. After ascertaining that parametric tests could be used, comparisons among the four different situations for both groups of variables were done by one-way analysis of variance (ANOVA) with Newman-Keuls *post-hoc* test. When the P value was <0.05, the null hypothesis was rejected and the difference was considered significant.

The experimental protocol was approved by the local Institutional Research Committee and met the requirements established by the current regulations for animal care and research in Europe (EC 86/L 609).

RESULTS

Anatomical Description

Gastroesophageal Junction. The anatomy of the esophagogastric junction in the rat is externally quite similar to that of humans. The intraabdominal segment of the esophagus is proportionally longer in the rat, in which it attains 15–20 mm and the cardial junction is located more distally in the lesser curvature, leaving a large fundus. The esophagus is lined by a squamous keratinized epithelium that extends to the proximal half of the gastric body, which is translucent and whitish while the remaining distal half, lined by gastric-type secretory mucosa, is opaque and reddish. The limit between both linings is well defined by a conspicuous mucosal ridge that divides the stomach into two clearly demarcated areas. The muscle fibers of the outer longitudinal and the inner circular layers of the esophagus are striated, and they become smooth only near the gastroesophageal junction where they attach tightly to the mucosa. After opening the stomach, the mucosal ridge is easily perceived as a clear-cut limit between the keratinized and secretory linings. Around the cardia the ridge is horseshoe-shaped when it is open and acquires an omega-shape that hides the cardia when it is closed. The lower bundles of the inner circular muscle layer that surrounds the esophagus at the level of the cardia diverge towards the anterior and posterior walls of the stomach in an oblique direction. These fibers are inserted in the mucosal ridge contributing to the cardial closure and some of them, departing from the right side of the cardia, extend downwards beyond the mucosal ridge along the anterior and posterior walls of the stomach (Figure 1).

Crural Sling. The esophageal hiatus of the diaphragm is very posterior and slightly displaced to the left in the rat. Its borders are formed by the diaphragmatic crura that are very muscular and attach to the anterior surface of the spine and to the retroperitoneal muscles along several vertebral bodies. The

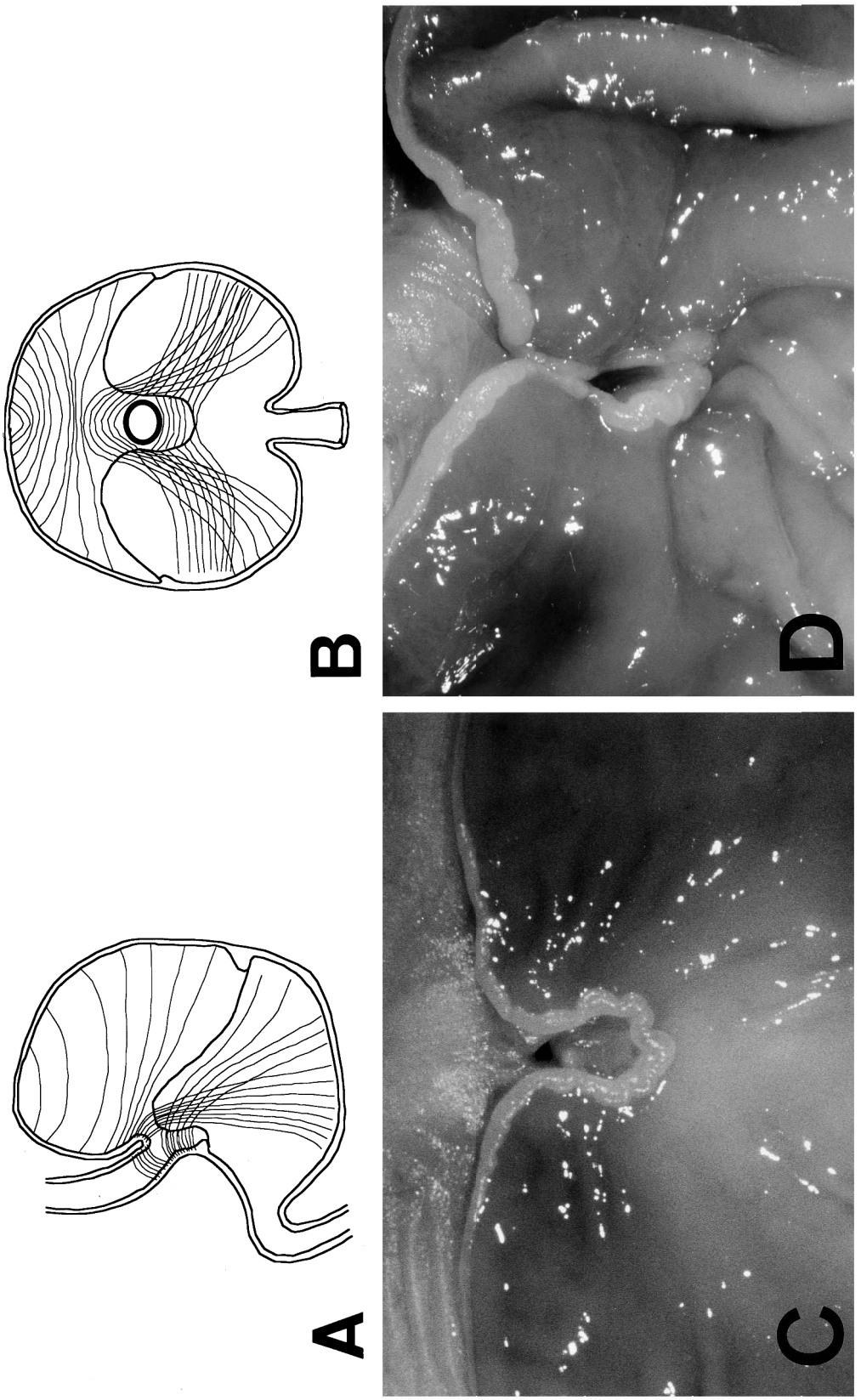

Fig 1. (A) Schematic drawing of the distal esophagus and the stomach in the rat showing the proximal half of the latter covered by esophageal mucosa and the distal part lined by secretory mucosa. Both portions are separated by a marked mucosal ridge. The main muscle bundles in the area are shown. (B) Schematic drawing of the same specimen longitudinally opened along the greater curvature. The duodenum is inferior and the fundus superior with the cardia in the middle. The muscle fibres are shown. (C) Picture of a fresh specimen of a rat stomach prepared as in B. The mucosal ridge is visible and the cardia is open. (D) When the fibers are contracted the cardia is hidden and tightly closed.

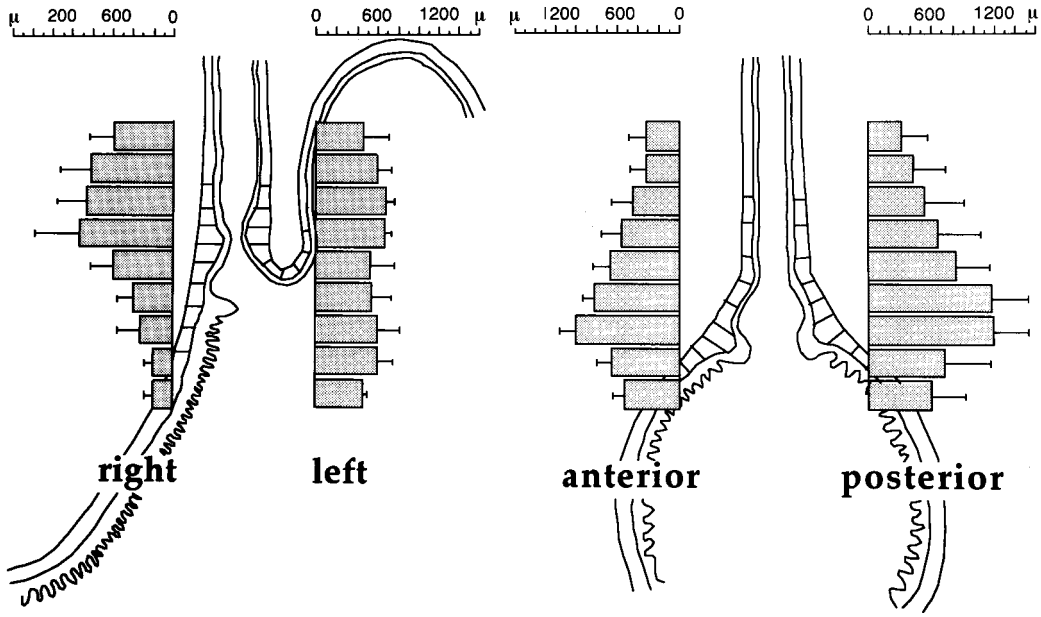

Fig 2. Schematic drawing of the gastroesophageal muscle wall thickness study. In the frontal plane (left), the measurements were taken every 100 μm along the right and left walls of the gastroesophageal junction. Values represent six specimens and are shown as means ± SD. It is obvious that the walls are thicker at the junction level. In the median plane (right) the greater thickness corresponds to the mucosal ridge level both in the anterior and the posterior walls.

outer contour of the sling is in continuity with the diaphragm. The hiatal orifice is, in fact, a short U-shaped tunnel opened downwards, posteriorly, and slightly to the right. The esophagus is surrounded by these muscle fibers above a long intraabdominal segment measuring up to 2 cm, and its attachments to the hiatus and to both the pleuræ and the peritoneum are quite similar to those seen in men except for a much looser arrangement that allows wide movement in either direction.

Muscular Thickness

Serial muscle coat thickness measurements on the right and left sides of the esophagus and stomach showed that the maximum width corresponded to the gastroesophageal junction itself. However, on the anterior and posterior walls the maximum width was located more distally and coincided with the squamocolumnar junction at the level of the mucosal ridge (Figure 2). At both points the increased thickness corresponded respectively to the bundles of circular fibers around the cardia and to the oblique fascicles that straddle the lesser curvature and extend to and beyond the mucosal ridge.

Manometric Image

The manometric profiles of the gastroesophageal barrier were basically similar to those described previously by us in the rat (3–8). Briefly, two clearly demarcated components can be identified: the more distal high-pressure zone was a wide tonic peak or plateau corresponding to the LES or ensemble of muscular structures located at or above the mucosal ridge. Cranially to this pressure event, the tracing returned to the intraabdominal pressure level for some millimeters, corresponding to the infradiaphragmatic segment of the esophagus, and immediately above it a group of high-pressure phasic oscillations was identified followed by intrathoracic esophageal pressures identified by negative inspiratory pressures. This second component corresponds to the crural sling contractions because the oscillatory frequency is identical to the respiratory one, because the respiratory reversal point can be identified at this point, and because it disappears after skeletal muscle relaxation (3).

Successive pull-through recordings of the esophagogastric junction in the four quadrants allowed further characterization of the origin of the two pressure components of the profile: the LESP was significantly

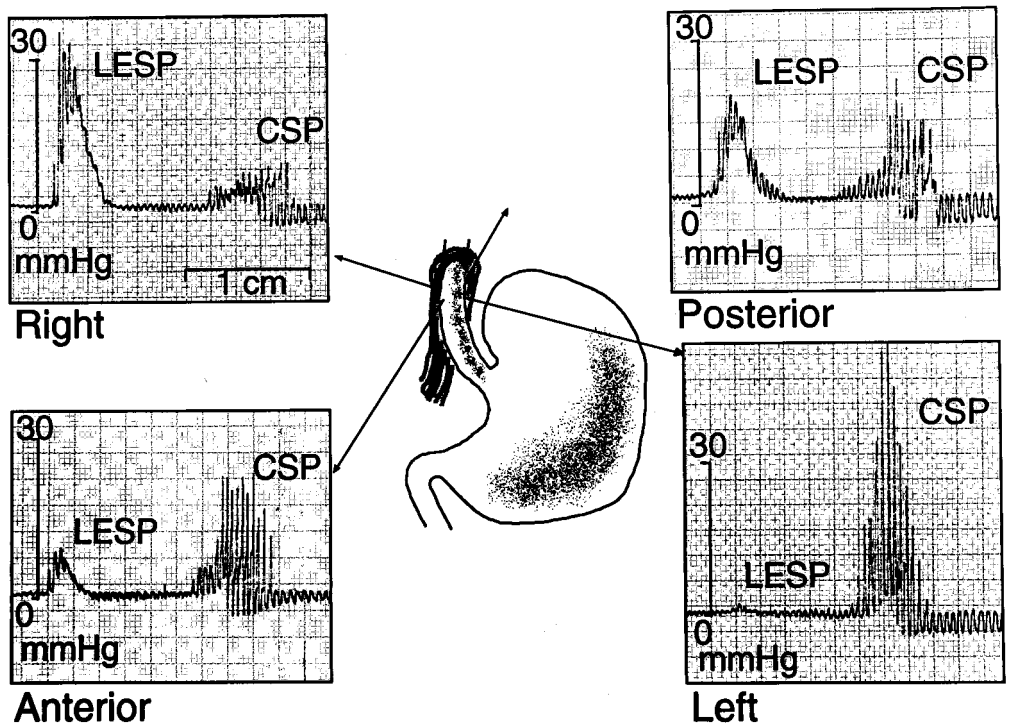

Fig 3. Pressure profiles obtained by pull-through manometry with the recording orifice successively oriented in the four quadrants. It can be appreciated that when it is open to the right, the predominant component is the sphincter, whereas the sling is unconspicuous. The opposite is observed on the left side and intermediate situations are apparent in the anterior and posterior positions. These manometric findings are in striking concordance with the muscular architecture of the region and show a powerful and well-organized barrier.

higher when the perfused orifice of the catheter was directed towards the right in coincidence with the thickest circular muscle. This component was somewhat less powerful but still very conspicuous when the orifice of the catheter was anteriorly or posteriorly oriented, coinciding with the oblique straddling fibers inserting in the mucosal ridge, and it was absent or almost inapparent when it was directed to the left where the fibers are less dense. Conversely, the pressures of the crural sling component were higher when the measuring orifice was oriented leftwards and anteriorly at the point where the U-shaped diaphragmatic muscular sling constricts the esophagus (Figure 3 and Table 1). Both components were recorded in all 23 rats.

DISCUSSION

It is currently accepted that the gastroesophageal barrier effect in most animals is mainly due to the concurrent action of the lower esophageal sphincter and the diaphragmatic crural sling, but the nature of the participation of these two components has not been disclosed simultaneously. In 1956 Fyke et al

Table 1. Sphincteric and Crural Sling Pressure Components of Gastroesophageal Barrier in Rats ($N = 23$) with Recording Orifice of Catheter in 4 Different Quadrants*

	Pressure (mm Hg)			
	Right (R)	*Anterior (A)*	*Left (L)*	*Posterior (P)*
LESP	42.2 ± 19.5a	13.5 ± 13.9b	4.4 ± 6.3	16.6 ± 18.6c
CSP	13.1 ± 16.3	39 ± 21.5d	40.7 ± 18.4e	18.4 ± 19.8

* LESP: Lower esophageal sphincter pressure, CSP: Crural sling pressure. Values are means ± SD. a, $P < 0.001$ vs LESP A, LESP L, and LESP P; b, $P < 0.05$ vs LESP L; c, $P < 0.01$ vs LESP L; d, $P < 0.001$ vs CSP R; e, $P < 0.001$ vs CSP R and $P < 0.01$ vs CSP P.

described a high-pressure zone at the human gastroesophageal junction (9) that was later confirmed by many authors (10, 11). Only later it was pointed out that the spatial orientation of the recording orifice had important effects upon the type of tracing obtained (12), and it was found that a significantly higher pressure could be detected when the catheter orifice was directed towards the left posterior quadrant (13, 14). However, this asymmetry of the high-pressure zone was interpreted as being due to compression of the esophagus by the lateral margin of the diaphragmatic hiatus since no muscular structure similar to a sphincter had been identified at the gastroesophageal junction. Only later did detailed studies on the human gastroesophageal junction lead Liebermann-Meffert et al to propose that the muscular equivalent of the LES corresponds to a thickened inner muscle layer straddling the lesser curvature and consisting of the "clasp" fibers and to the long oblique "sling-like" bundles straddling the greater curvature oriented almost perpendicularly to the former ones. These studies revealed an anatomical asymmetry of the LES (15) that was later confirmed when computerized 3-D manometry became available (1, 16, 17).

On the other hand, the participation of the crural diaphragm in the gastroesophageal barrier was not considered relevant until Boyle et al, in 1985, demonstrated its closing action by showing that in the cat the inspiratory increase of the LESP was due to its periodic contractions (18). Mittal, in a manometric study in which the artifacts produced by axial displacement of the probe during the respiratory cycle were minimized by using a sleeve pressure-measuring device, described two different pressure components in the gastroesophageal barrier: the smooth muscle LES and the crural diaphragm (19). Later on, a high-pressure zone located at the thoracoabdominal junction was demonstrated in patients who had undergone gastroesophageal junction resection, implying sphincter ablation (20), and it was also confirmed that crural pressure was abolished in patients with hiatal hernia and that crural repair reestablished normal levels (2). The main problem for accurately identifying this crural component in humans was the relatively short intraabdominal esophagus that causes anatomic overlapping of LES and the crural diaphragm in them (2).

Our previous work in the rat demonstrated a strong gastroesophageal barrier resulting from the concurrent effect of a powerful sphincter, a long intraabdominal segment of the esophagus, and a tight crural sling closure during inspiration (3–8). The pressures at both ends of the barrier were so much higher than the thoracoabdominal pressure gradient at any point of the respiratory cycle that reflux seems unlikely in this animal. We felt that such evidence necessitated the use of the rat, the most affordable and widely used laboratory animal, for research on the gastroesophageal barrier under various circumstances that facilitate gastroesophageal reflux in humans, like upper airway obstruction (4), abdominal closure under pressure (6), diaphragmatic hernia repair (7), or esophageal shortening (8). However, although our assumption was apparently correct and we proved that such manipulations actually weakened the barrier, it became evident that the anatomical differences between species were an obstacle for some interpretations and we concluded that an in-depth anatomical and manometric study of the barrier mechanism in the rat was needed before further investigation of the mechanisms of human dysfunctions should be undertaken on this model.

The present investigation contains detailed data on the anatomical basis of the gastroesophageal barrier in the rat, suggesting that the antireflux mechanism in this animal is also based on the concurrent action of sphincter and crural sling, although the architecture of both components and the length of esophagus interposed between them are considerably different from their human counterparts. The manometric part of this study demonstrates that the actions of both sphincter and sling are complementary since they are arranged in opposite directions: the U-shaped LES opens towards the greater curvature of the stomach while the U-shaped crural diaphragm is open posteriorly and to the right towards the lesser curvature, resulting their simultaneous action in a particularly powerful and effective sphincter mechanism. The striking correspondence of the anatomical arrangement with the manometric asymmetry of the gastroesophageal barrier in this study confirm our previous interpretations. Although there are no factual data on the presence or absence of reflux in this animal, we believe that it could hardly have a better gastroesophageal barrier.

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