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*Series Editors:* D. Neil Granger & Joey Granger

# Motor Function of the Pharynx, Esophagus, and its Sphincters

Ravinder Mittal

Morgan & Claypool Life Sciences

# Motor Function of the Pharynx, Esophagus, and its Sphincters

# Integrated Systems Physiology: from Molecule to Function to Disease

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**D. Neil Granger**, *Louisiana State University Health Sciences Center-Shreveport*

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# Motor Function of the Pharynx, Esophagus, and its Sphincters

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*INTEGRATED SYSTEMS PHYSIOLOGY:*
*FROM MOLECULE TO FUNCTION TO DISEASE #16*

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## ABSTRACT

Deglutition or a swallow begins as a voluntary act in the oral cavity but proceeds autonomously in the pharynx and esophagus. Bilateral sequenced activation and inhibition of more than 25 pairs of muscles of mouth, pharynx, larynx, and esophagus is required during a swallow. A single swallow elicits peristalsis in the pharynx and esophagus along with relaxation of upper and lower esophageal sphincters. Multiple swallows, at closely spaced time intervals, demonstrate deglutitive inhibition; sphincters remain relaxed during the entire period, but only the last swallow elicits peristalsis. Laryngeal inlet closure or airway protection is very important during swallow. Upper part of the esophagus that includes upper esophageal sphincter is composed of skeletal muscles, middle esophagus is composed of a mixture of skeletal and smooth muscles, and lower esophagus, including lower esophageal sphincter, is composed of smooth muscles. Peristalsis progresses in seamless fashion, despite separate control mechanism, from the skeletal to smooth muscle esophagus. The esophagus's circular and longitudinal muscle layers contract synchronously during peristalsis. Sphincters maintain continuous tone; neuromuscular mechanisms for tonic closure in the upper and lower esophageal sphincters are different. Lower esophageal sphincter transient relaxation, belching mechanism, regurgitation, vomiting, and reflux are mediated via the brain stem.



## KEYWORDS

esophageal peristalsis, lower esophageal sphincter, upper esophageal sphincter, neural control of peristalsis, circular and longitudinal muscle coordination, eneteric nervous system, high resolution manometry, transient sphincter relaxation, achalasia esophagus, deglutition center, swallow program generator

# Contents

# Introduction

Function of the esophagus is relatively straightforward: to transport swallowed bolus into the stomach and, infrequently, to allow retrograde flow of stomach contents into the esophagus and mouth during belching, regurgitation, and vomiting. In order to meet these functional needs, the design of the esophagus is simple, it is a relatively straight muscular tube that is guarded at the two ends by an upper and a lower esophageal sphincter. Following a voluntary act of swallow the two sphincters relax and open, and a wave of sequential inhibition followed by sequential contraction, i.e., peristalsis, sweeps behind the bolus autonomously through the entire length of the esophagus. Closure of upper and lower esophageal sphincter occurs following passage of peristaltic contraction at each site, respectively. Motor events during retrograde transport, i.e., regurgitation and vomiting, are distinct from swallowing; relaxation of the lower esophageal sphincter occurs first, followed by "retrograde peristalsis in the longitudinal muscle and relaxation of the upper esophageal sphincter." Unlike most of the gastrointestinal tract where motor events are completely autonomous, neuromuscular control mechanisms of the esophagus and its sphincters require seamless coordination between the volitional and autonomous components of the central nervous system and enteric nervous system located in the wall of the esophagus. Volitional component resides in the cerebral cortex and autonomous component in the brain stem. Muscles of the esophagus, skeletal in the upper part and transition into smooth muscles in the lower part, add complexity to the neural control mechanism. Disturbances of esophageal peristalsis and lower esophageal sphincter cause difficulty in swallowing (dysphagia) and esophageal pain. Detailed pathophysiology of esophageal motor disorders is beyond the scope of this book; however, observations that provide important insights into the physiological function will be discussed.

# Central Program Generator and Brain Stem

A swallow can be induced by mechanical stimulation of the pharynx or electrical stimulation of the superior laryngeal nerve (SLN) in decerebrate animals [1,2]. Events elicited by such stimulation show sequential contractions in the pharynx and esophagus along with relaxation of sphincters, similar to what one observes with a spontaneous swallow. Above implies that a programmed set of impulses that coordinate swallow-related events must emanate from the brain stem, often referred to as the central program generator (CPG) [3] or swallow program generator (SPG) [4]. It is not to say that the supramedullary sites cannot or do not influence the CPG [5,6]. Detailed discussion of the CPG can be found in several reviews [3,7]. Briefly, the critical elements of CPG are nucleus tractus solitarius (NTS), adjacent reticular formation, nucleus ambiguus and dorsomotor nucleus of vagus (Figure 1). All of these nuclei are not very well demarcated and have been studied in detail in mice, rat, cat, and sheep. The neurons of these nuclei, in addition to swallow function are also involved in respiration, cardiovascular reflexes, and possibly other functions. There is topographical representation of sensory input into the NTS such that the dense labeling of pharyngeal and laryngeal fibers is found in the intermediate and interstitial subnuclei and esophageal afferent labeling is observed primarily in the central subnucleus of NTS [8]. Local medullary and ascending supramedullary projections connect with the NTS. Local medullary connections either directly or through the reticular formation project to the premotor nucleus of vagus. In addition, NTS receives descending projections from the supramedullary and cortical centers. NTS is not a simple relay station of the vagus and sympathetic afferents; sequential activity in the NTS neurons can be recorded following stimulation of the SLN. Micro-injection of excitatory amino acid (EAA) agonist into the NTS induces peristaltic motor events in the pharynx and esophagus and these can be blocked by antagonist of EAA. *N*-methyl-D-aspirate (NMDA) receptor agonist and antagonist induce rhythmic "swallow like" contractions in the pharynx and esophagus. Micro-injection of GABA-A agonist and antagonist inhibits and facilitates motor events associated with swallowing and peristalsis, respectively, suggesting that the NTS must also exert tonic inhibitory control over

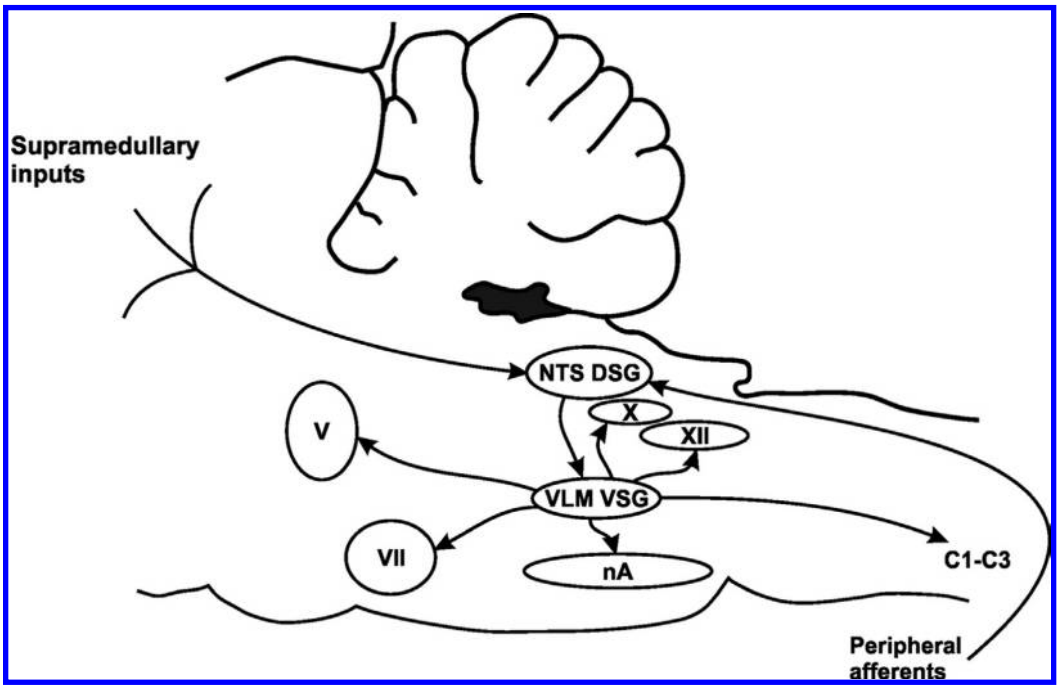



**FIGURE 1:** Central pattern generator (CPG). CPG includes two main groups of neurons located with the medulla oblongata: a dorsal group (DSG) located within the nucleus tractus solitarii (NTS) and the adjacent reticular formation and a ventral group (VSG) located in the ventrolateral medulla (VLM) adjacent to the nucleus ambiguus (nA). The DSG contains the generator neurons involved in triggering shaping and timing the sequential swallowing pattern. The VSG contains the switching neurons, which distribute the swallowing drive to the various pools of motor neurons involved in swallowing. It should be noted that the pathway including the peripheral afferent fibers neurons in the DSG and VSG, and motor neurons forms an oligosynaptic loop involved in swallowing (from Jean, *Physiol Rev* 2001;81:929–69).

premotor neurons of the DMV [9,10]. It is likely that the swallow reflex is a polysynaptic reflex at the level of brain stem and several neurotransmitters appear to be involved at these synaptic sites; i.e., acetylcholine (through nicotinic and muscrinic [11] receptors), epinephrine (alpha receptors), monoamines, serotonin, vasopressin, oxytocin, somatostatin, thyrotropine, nitric oxide [12,13], and possibly others [3].

# Pharynx—Anatomy, Neural Innervation, and Motor Pattern

Pharynx is 12–14 cm in its vertical length and extends from the base of skull to the upper border of upper esophageal sphincter (UES) [14]. It can be divided into three segments: nasopharynx that extends from the base of skull to the soft palate, oropharynx extending from soft palate to the pharyngoepiglottic fold, and hypopharynx from the pharyngoepiglottic fold to the UES (Figure 2). It is predominately a muscular structure although epiglottis, arytenoid, cuneiform, corniculate, and cricoid cartilage form part of the anterior wall. Furthermore, hyoid and thyroid bones provide attachment to some of its muscles. Muscles of pharynx can be broadly viewed as intrinsic and extrinsic; the former constitutes the superior, middle, and inferior pharyngeal constrictors along with thyropharyngeus (oblique) and cricopharyngeus (horizontal fibers). A triangular area of relatively scanty muscle fibers exists between the oblique and horizontal muscle fibers (Killian's triangle), which is the site of Zenker diverticulum. The latter is seen in patients with difficulty in swallowing, who have impaired relaxation or opening of the UES [15,16]. Extrinsic muscles of the pharynx can be categorized into three subgroups: Group 1—elevators and tensors of palate (levator veli palatini, tensor veli palatini, and palatoglossus); Group 2—geniohyoid, mylohyoid, stylohyoid, thyrohyoid, diagastric, stylopharyngeus, and palatopharyngeus, which cause superior and anterior displacement of the larynx during swallowing; Group 3—aryepiglottic, thyroarytenoid, and oblique arytenoids muscles, which close the laryngeal inlet. These muscles are supplied by the branches of cranial nerves V (trigeminal), VII (facial), IX (glossopharyngeal), X (vagus), ansa cervicalis, and XII (hypoglossal). Pharyngeal muscles are richly innervated, with a nerve–muscle fiber innervation ratio of 1:2 to 1:6, as compared to 1:2000 for human gastrocnemius muscle [14] and 1:9 for extraocular eye muscles, [17] which is important for the "fine" control required for its function.

A lateral radiograph of the oropharyngeal region shows an air column in the nasopharynx, oropharynx and upper part of the laryngopharynx (up to the lower border of laryngeal opening) [14]. While the posterior wall of pharynx is smooth, the anterior wall is irregular because it is formed by the posterior nasal aperture, soft palate, oral cavity, tongue, valleculae, epiglottis, laryngeal vestibule, and posterior surface of cricoids cartilage. Pharynx is in contact with four different cavities, nasal, oral, laryngeal, and esophagus and serves two major functions, breathing and swallowing.

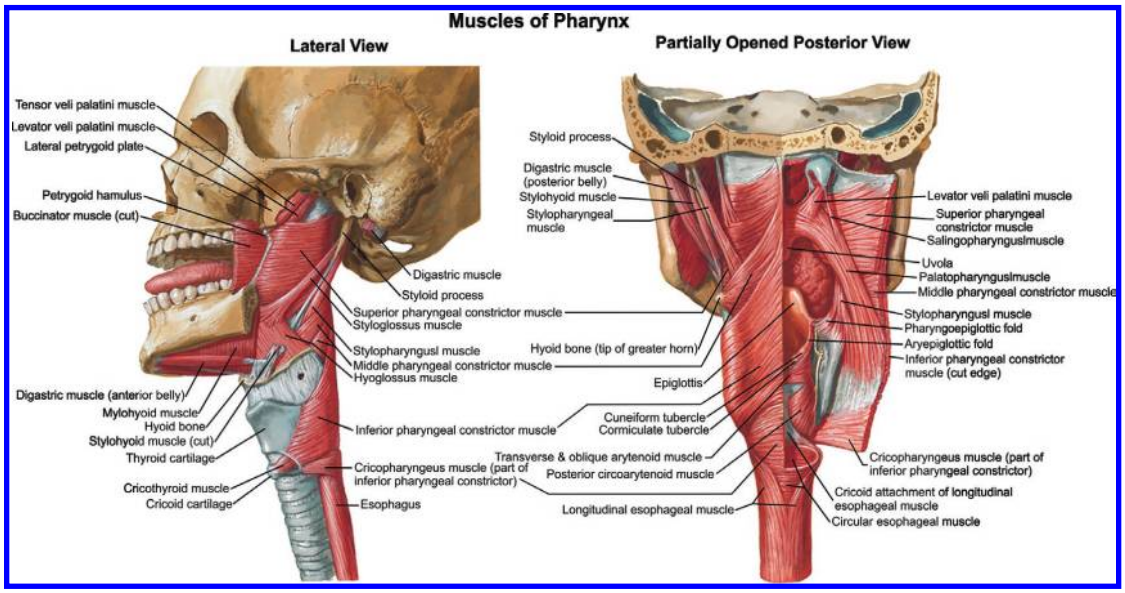
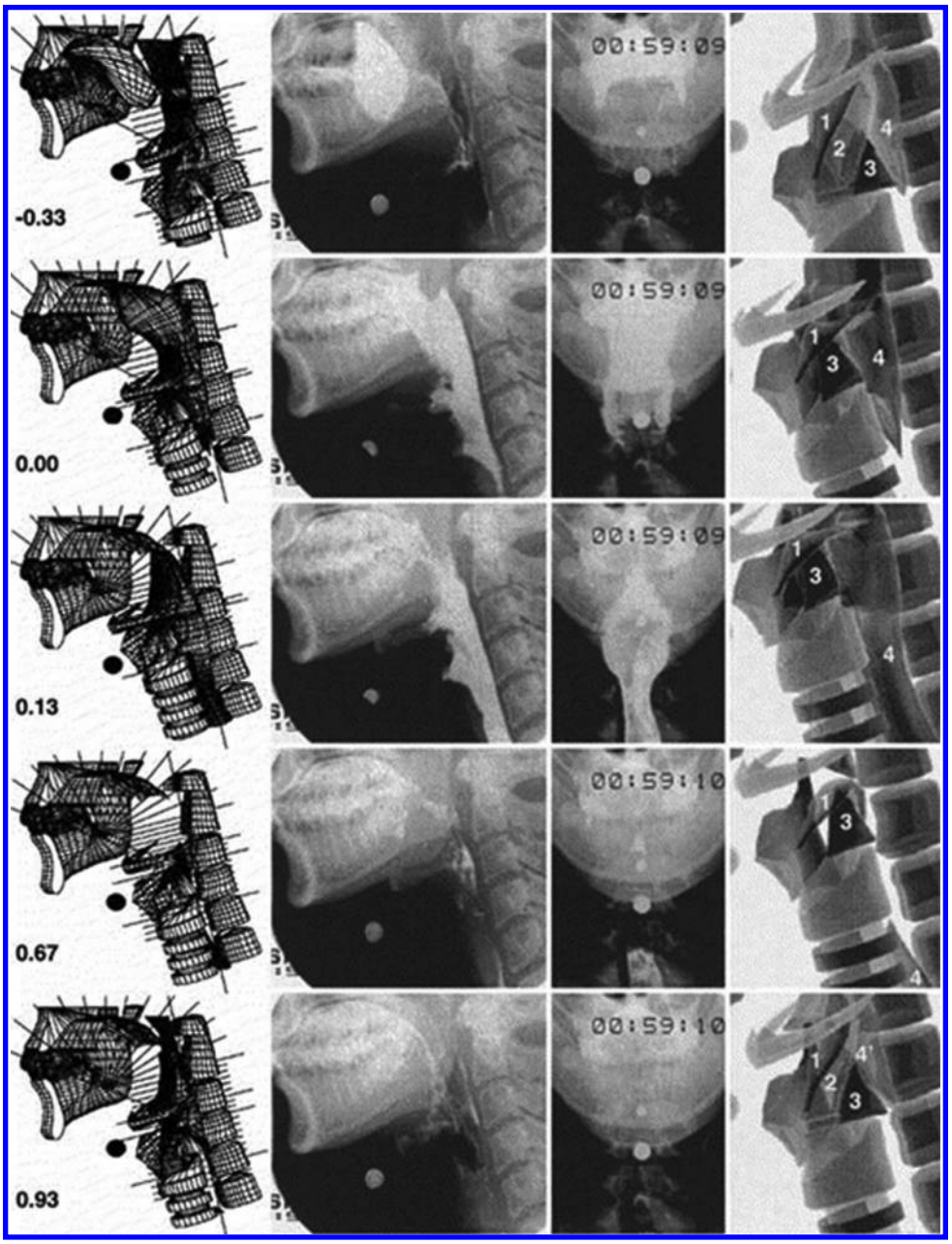



**FIGURE 2:** Musculature of pharynx and upper esophageal sphincter. (Source: Netter medical illustration with permission of Elsevier.)

Upon deglutition, pharynx becomes a swallow structure from a breathing structure that has distinct anatomy (Figure 3). The characteristics of pharynx during swallow are closure of palate along with elevation and closure of the laryngeal inlet. A swallow can be divided into four phases: (1) preparatory, (2) oral, (3) pharyngeal, and (4) esophageal. Oral and pharyngeal phase is also referred to as the oropharyngeal phase. During the preparatory phase, food bolus remains in the oral cavity, masticated and mixed with saliva, sized and shaped, and positioned on the dorsum of tongue. Sequential or peristaltic contraction of the tongue against the hard and soft palates generates peristaltic pressure wave that pushes the bolus into pharynx. With pharyngeal phase starts the involuntary phase of swallow. Elevation of soft palate during oropharyngeal phase seals the nasopharynx to prevent nasal regurgitation. Contraction of suprahyoid muscle causes elevation and forward movement of the larynx, pharynx, and UES that result in approximation of larynx against the epiglottis. At the same time, contraction of intrinsic laryngeal muscles results in closure of laryngeal inlet to provide additional airway protection mechanism [18]. Laryngeal closure is of paramount importance during swallowing. A three-tier system exists to protect the airway from swallowed contents. Three-tier system starts from the distal to the proximal end of larynx, (1) adduction of true vocal cords and arytenoids, (2) vertical approximation of closed arytenoids to the base of epiglottis, and (3) descent of epiglottis to cover the closed glottis thereby closing the laryngeal vestibule [5]. Larynx is elevated 2 to 3 cm during a swallow and it sits under the base of tongue at the height of its excursion, a key step in the airway protection mechanism. Temporal sequence of swallow related events in the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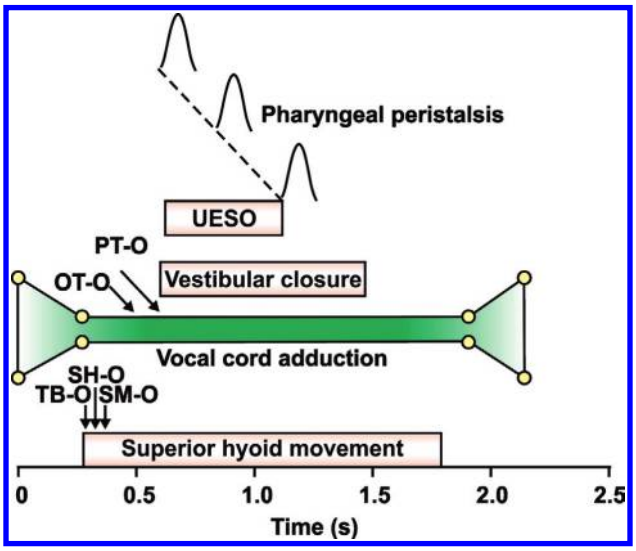


**FIGURE 4:** Bolus transit through the pharynx and across the upper esophageal sphincter (UES) begins and ends while the vocal cords are at maximal adduction. TB-O, onset of tongue base movement; SH-O, onset of superior hyoid movement; SM-O, onset of submental myoelectrical activity; UESO, UES opening; OT-O, onset of bolus movement from the mouth; PT-O, arrival of bolus into pharynx. (Source: Shaker R et al. Coordination of deglutitive glottic closure with oropharyngeal swallowing. *Gastroenterology* 1990;98:1478–84, with permission from American Gastroenterological Association.)

pharynx and larynx, crucial to the airway protection mechanism, is shown in Figure 4 [19]. A normal oropharyngeal phase consists of complete transfer of oral contents into the esophagus without any entry into the laryngeal inlet.

Movement of the head end of bolus in the pharynx and pharyngeal peristalsis are distinct and two separate events. Thrust caused by tongue peristalsis (and gravity in the upright position)

**FIGURE 3:** The oropharyngeal swallow as imaged by videofluoroscopy and reconstructed in three dimensions with computer graphics. From left to right, each horizontally arranged group of images contains the three-dimensional reconstruction of the pharyngeal cavity and surrounding structures, the lateral radiographic appearance of the pharynx during a 10-ml barium swallow, the corresponding posterior–anterior radiographic appearance, and a magnified view of the hypopharynx at the time are indicated at the left. Time 0.00 is the instant of UES opening; the entire sequence of events transpires within 1 second. The metal sphere under the chin is used to correlate among images. In the magnified hypopharyngeal reconstructions, 1 is the epiglottis, 2 is the laryngeal vestibule, 3 is the arytenoid cartilage, 4 is the esophagus, and 4′ is the pyriform sinus after closure of the UES. Note the importance of laryngeal elevation during the pharyngeal reconfiguration and synchrony of UES opening with laryngeal vestibule closure (from Cook and Kahrilas, *Gastroenterology* 1999;116:455–78).

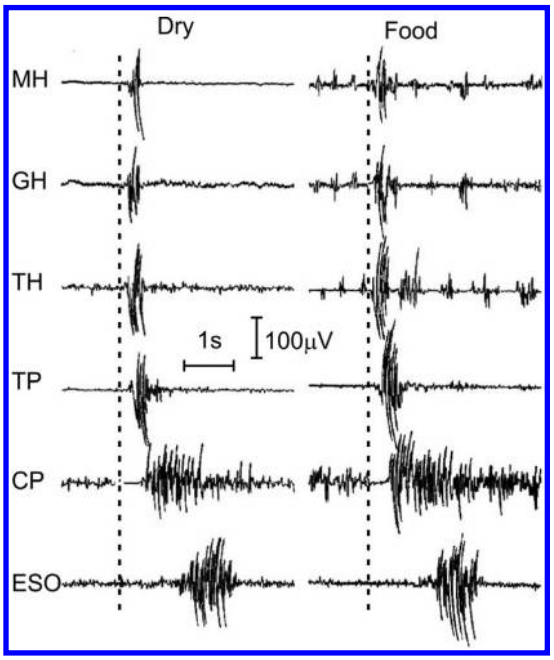


**FIGURE 5:** This figure illustrates the EMG responses of the three UES closure muscles and many of the superior hyoid UES opening muscles during a dry swallow ad lib feeding of canned food in a chronically instrumented dog. Note that the three UES closing muscles respond quite differently during swallowing but the superior opening muscles are activated almost simultaneously. MH, mylohyoideus; GH, geniohyoideus; TH, thyrohyoideus; TP, thyropharyngeus; CP, cricopharyngeus; ESO, esophagus 2 cm below CP (from Lange, *GI Motility Online* 2006; doi:10.1038/gimo12).

propels the head end of bolus rapidly into the pharynx. On the other hand, pharyngeal peristalsis that follows the tail end of bolus clears the bolus from the pharynx relatively slowly (approximately 1 second). Closure of nasopharynx and larynx has already occurred when the head end of the bolus enters pharynx. Mylohyoid is the first muscle to be activated when swallowing [2], and it is followed by contraction of other suprahyoid muscles [20,21] (Figure 5). Upper esophageal sphincter (UES) relaxes 0.3 seconds after the onset of mylohyoid muscle contraction and prior to the arrival of bolus head. Increase in bolus volume delays the onset of pharyngeal contraction without affecting timing of closure of nasopharynx and larynx, and UES relaxation. Certain aspects of swallow reflex are modifiable, such as the duration of UES opening and excursion of hyoid bone that increase with increase in the bolus volume [22,23]. Amplitude of pharyngeal peristalsis may also increase with bolus volume [19], suggesting that not all parts of swallow cascade are stereotypically programmed. Literature suggests that a negative pressure wave develops in the cervical esophagus with swallow-

ing that provides pressure gradient for the movement of bolus head into the esophagus [24–26]; however, it is not clear if it is a recording artifact. With the arrival of bolus in the pharynx, there is small increase in the pharyngeal pressure (bolus pressure), which likely provides the force that distends and opens the UES. A bolus volume increase from 1 to 20 ml increases bolus pressure from 5 to 17 mm Hg [27]. Increase in the bolus pressure is reflective of outflow resistance caused by a poor relaxation or low compliance of the UES and is seen in normal subjects with aging and patients with oropharyngeal dysphagia who have cricopharyngeal bar and Zenker's diverticulum [16].

Pharyngeal peristalsis follows the tail end of bolus and is the slower part of the bolus transport mechanism across the pharynx. The speed of peristalsis is approximately 15 cm/sec in the pharynx and it takes 1 second for the contraction wave to travel from the top of pharynx to the UES. Pharyngeal contraction waves are axially and circumferentially asymmetric, most likely related to the complex anatomy of the closed segment. Amplitude of pharyngeal contraction in normal subjects ranges from 100 to 150 mm Hg. Amplitude increases and duration decreases from proximal to the distal location in the pharynx [27]. Outflow resistance related to the UES dysfunction, as may occur with aging, cricopharyngeal bar, and Zenker's diverticulum are key modulators of pharyngeal peristalsis, pharyngeal contraction amplitude, and duration [18].

# Upper Esophageal Sphincter

Upper esophageal sphincter (UES) has also been referred to as the inferior pharyngeal sphincter because it is located at the lower end of pharynx and guards the entrance into the esophagus. It has two major functions: (1) to prevent air from entering into the esophagus during breathing and (2) to prevent reflux of esophageal contents into the pharynx to guard airway aspiration. It is best recognized functionally as a high-pressure zone that extends 3–4 cm in its vertical extent. Anatomically, it is located behind the cricoid cartilage but extends both above and below it. Even though it is generally agreed that cricopharyngeus is a major contributor to the UES high-pressure zone, thyropharyngeus (part of inferior pharyngeal constrictor) and cervical esophagus also contribute to it in its proximal and distal extents, respectively. Simultaneously, pressure and fluoroscopic imaging studies show that the peak pressure of the UES high-pressure zone is located above the cricopharyngeus muscle [28] (Figure 6). Furthermore, cricopharyngeus is only 1 cm in width but the UES pressure zone is 3–4 cm long. Accordingly, a surgical incision of 5–6 in length [29], which extends over inferior pharyngeal constrictor, cricopharyngeus, and cervical esophageal muscle, is required to completely ablate the UES pressure (as measured by the Sleeve sensor in the humans [30]). Fibers of thyropharyngeus are placed obliquely (pars obliques) and cricopharyngeus horizontally (pars profundus) to form the UES (Figure 7). Muscle fibers of the cricopharyngeus have both slow (oxidative) and fast (glycolytic) type fibers, even though slow ones predominate [31–33]. Slow fibers most likely contribute to the tonic and fast fibers to the phasic contractions that are involved in rapid reflex contractions of the UES high-pressure zone. Forty percent of the muscle mass is contributed by the collagen and elastic tissue (endomysial tissue) [31,32], and it is felt that the UES is functionally quite compliant even though noncompliant cricoid cartilage forms its anterior extent. Cricopharyngeus originates from the cricoid cartilage, loops around the pharynx in a C or "horse shoe shape manner," and is inserted back into the cricoid cartilage (unique muscle that has origin and insertion into the same structure). Most skeletal muscles generate maximal force at what is referred to as the optimal muscle length. However, the *in vivo* operational length of the UES muscle is significantly shorter than its optimal length (1.7 times) [34]. As a result, the greater the diameter of the manometry probe the greater the measured UES pressure. Muscle spindles are absent from the UES muscle but Golgi-tendon-like structure, through which motor neurons may monitor muscle tone, is present [35].

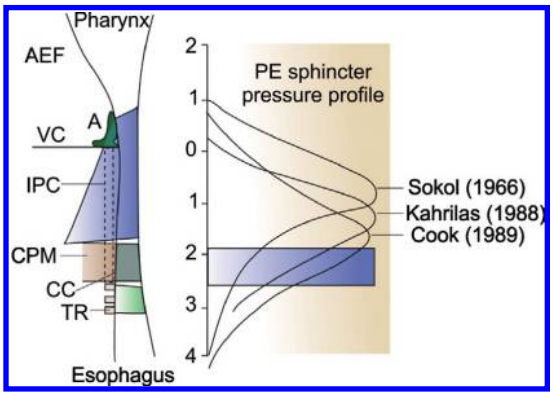


**FIGURE 6:** Data compiled from three different studies of the position of the UEHPZ with respect to individual pharyngeal muscles based on combined manometric and videofluoroscopic studies. Note that the peak pressures of the UEHPZ in humans at rest with the head fixed in all three studies coincide with the lower border of the IPC. A, arytenoid; AEF, aryepiglottic fold; CC, cricoid cartilage; ESO, esophagus; IPC, inferior pharyngeal constrictor; PE, pharyngoesophageal; TR, tracheal ring; VC, vocal cord. (Source: From Goyal RK, Martin SB, Shapiro J, Spechler, SJ. The role of cricopharyngeus muscle in pharyngoesophageal disorders, *Dysphagia* 1993;8:253–8, with permission of Springer Science and Business Media.)

UES is innervated by the glossopharyngeal, branches of vagus, ansa cervicalis, and sympathetic nerves (from cervical ganglion). The vagus nerve, through its pharyngeal, superior laryngeal and recurrent laryngeal nerve branches, is the major motor nerve of the UES. All these nerves form a pharyngeal plexus before penetrating into the muscle fibers. Nerve cell bodies of the vagal efferent fibers are located in the nucleus ambiguus. It is not clear if there are any special structures that form the sensory nerve ending in these regions, afferent nerves travel to nodose and jugular ganglion cells and from there go on to the nucleus tractus solitaries (NTS), which in turn communicates through the reticular formation of brain stem or directly to the motor neurons of the nucleus ambiguus. Sympathetic nerves supply the mucosal gland and blood vessels in the region and probably carry some sensory information. Acetylcholine acting through the nicotinic receptors located on the motor nerve plate is the major neurotransmitter of the UES muscles. However, other neuropeptides, calcitonin gene-related peptide, neuropeptide Y, substance P, vasoactive intestinal polypeptide, and galanin are present in the region, their function is probably related to the control of blood flow [28,36].

UES pressure is distributed predominantly in the anterior–posterior directions; lateral pressures are about 33% of the anterior–posterior ones. In addition to circumferential asymmetry, there is also axial asymmetry of the UES pressure. Peak anterior pressure is located more cranially than the peak posterior pressure. Laryngectomy decrease UES pressure asymmetry [37,38]. Normal range of

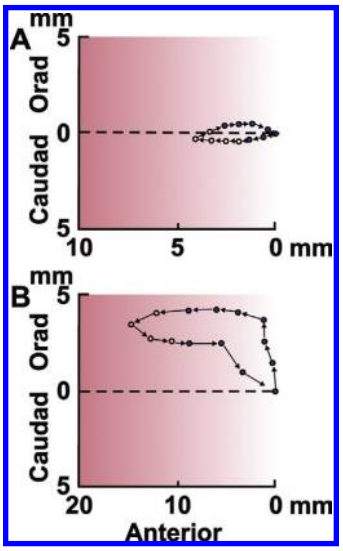


**FIGURE 7:** Trajectory of UES with swallow and belch. Open circles indicate UES opening observed by videofluoroscopy. Although hyoid bone movement during swallowing was invariably upward, forward, and counterclockwise, its movement during belching was mainly anterior and clockwise. The magnitude of hyoid bone movement during belching was significantly less than its movement during swallowing. Hyoid bone movement is an indication of the magnitude of the distraction forces that open the UES ($p < .02$). (Source: Shaker et al., *Am J Physiol* 1992;262:G621–8.)

UES pressure is quite large, 30–200 mm Hg (side-hole manometry or solid-state transducer) and 30–110 mm Hg (sleeve sensor). Therefore, measurement of resting pressure is not a useful parameter in the clinical studies. UES pressure is extremely labile; it is higher with rapid pull-through than station pull-through technique of pressure measurement, decreases with decrease in the wakeful state, and almost disappears with sleep [39] and anesthesia. Psychological stress and anxiety [40] also increase UES pressure significantly, and with aging, there is a decrease in UES pressure and its compliance [27]. Inspiration and phonation augment UES pressure [41]. A number of aerodigestive protective reflexes are operative in the UES. (1) The pharyngoglottal reflex, which is part of the gag reflex and results in increase in the UES pressure with pharyngeal stimulation. It can be elicited by injection of tiny amounts of water just above the UES [42]. (2) The esophago-UES reflexes can be either excitatory or inhibitory. Distension of the esophagus with a balloon or air causes reflex contraction of the UES (proximal distension greater than the distal). Rapid injection with air or a long cylindrical balloon causes UES relaxation which is important in belching [43]. It appears that rapidity of pressure change in the esophagus associated with gastroesophageal reflux is the major determinant whether UES relaxes or contracts in response to distension of the esophagus. Air

reflux into the esophagus, especially in the upright position is associated with UES relaxation. On the other hand, liquid reflux, associated with slow increase in the esophageal pressure induces reflex UES contraction [44]. Same may be true for regurgitation and vomiting where pressure increases quite rapidly in the esophagus to cause UES relaxation. Findings with regards to the effect of acid in the esophagus on UES have provided contradictory results [45,46]; even though latest word is that it has no significant effect [44].

Swallow-induced relaxation of the UES lasts for 0.32–0.5 seconds, depending upon the bolus volume, directly related to the bolus volume [47]. Two distinct events are responsible for the swallow-induced relaxation of UES: (1) cessation of tonic discharges of the motor neurons of nucleus ambiguus and (2) anterior and superior lift of the hyoid, cricoids, and UES by the contraction of suprahyoid muscles. Cessation of motor neuron discharges causes UES relaxation which is seen as the cessation of EMG activity in the cricopharyngeus and thyropharyngeus muscles. A residual UES pressure of 10–15 mm Hg [48], following cessation of the EMG activity in these muscles, is because of the viscoelastic properties of muscle and surrounding structures. The residual UES pressure is ablated by a forceful superior (2.5 cm) and anterior (0.75 cm) stretch exerted on the UES by contraction of suprahyoid muscles (geniohyoid and mylohyoid), which results in the UES opening. Extent of UES opening is related to bolus volume and bolus pressure. UES during a swallow is described as a grabber because it ascends to grab the bolus and then descends with it. Physical therapy used to strengthen suprahyoid muscles (Mendelsohn maneuver) improves UES relaxation and opening function in patients with dysphagia related to the UES relaxation and opening dysfunction [18]. UES relaxation and opening also occurs during belching but the trajectory of movement of cricoid cartilage and UES is different from the swallow. With belching, the UES moves mostly in the anterior direction (not in the oral direction) related to the contraction of infrahyoid muscles [49] (Figure 7), suggesting that different set of muscles are activated during these two events.

# Neuromuscular Anatomy of Esophagus and Lower Esophageal Sphincter

Esophagus extends between the lower edges of upper esophageal sphincter (UES) to the upper edge of lower esophageal sphincter (LES), and both of these edges are best defined functionally. They are not discernible anatomically, even on the autopsy specimen. Esophageal length varies between 20 and 25 cm and it is not related to an individual's height [50]. Esophagus traverses in the posterior mediastinum of the chest and comes in close anatomical relationship with the aorta, trachea, heart, and vertebral column. Esophagus sits to the right of the aorta in its upper extent and anterior to it in the lower extent, with the left atrium in close relationship in the anterior aspect. Even though relatively vertical in it courses, there are two gentle curvatures that are visible on the barium esophagogram. Similar to the rest of gastrointestinal tract, esophagus is made up of several layers, i.e., mucosa, muscularis mucosa, submucosa, and muscularis propria. Muscularis mucosa is thin, two- to three-cells thick, and oriented in the longitudinal axis. On the other hand, in the muscularis propria, also called as muscularis externa, the dominant muscle layer is organized into inner circular and outer longitudinal muscle layers, each of which is several muscle cells thick. Both circular and longitudinal muscle layers, based on the ultrasound images, are approximately 0.75 mm thick under baseline resting conditions in the live humans [51,52]. Circular muscle is continuous with the cricopharyngeus muscle and the inferior pharyngeal constrictor at the cranial end and with the muscles of the LES at the audal end. On the other hand, the longitudinal muscle layer originates from the dorsal, superior, and lateral margins of cricoid cartilage as two fascicles, cricoesophageal tendons, leaving a triangular gap in its most cranial and posterior aspect (Laimer's triangle) (Figure 2). As the fibers proceed caudally, they surround circular muscle completely and at the inferior end are inserted into the circular muscles of LES. Longitudinal muscle layer is thicker than the circular muscle in its proximal part, but the two layers are equal in their distal extent. Histological studies show that the muscle fibers are arranged spirally in the proximal part. Magnetic resonance diffusion tensor imaging, a relatively novel technique to decipher the orientation of muscle fibers, found helical arrangement in the proximal part of the bovine esophagus, but distally, fibers were arranged in the longitudinal and circular axis [53] (Figure 8). Inter-muscular septum that contains myenteric plexus resides in between the two muscle layers. Esophagus is unique, unlike any other organ in the

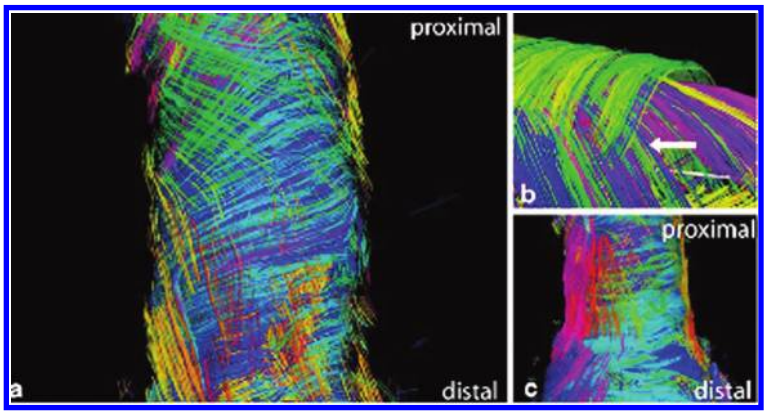


**FIGURE 8:** DSI tractography demonstrating the three-dimensional myoarchitecture of the esophagus. DSI with tractography was employed to image the mesoscopic fiber tract structure of the intact excised bovine esophagus. Fibers were color-coded according to the helix angle with respect to the central axis. (a) View of the mid-esophagus, focusing on the transition from helical to circumferentially aligned fiber tracts with increasingly distal locations (×10). (b) Higher magnification of the proximal esophagus showing the interwoven nature of the helically aligned tracts (white arrow) (×20). (c) The distal end of the esophagus is mainly circumferential with a thin superficial layer of longitudinally aligned tracts (×5) (from Gilbert et al., *Cell Tissue Res* 2008;332:461–8).

body, it is made up of partly skeletal and partly smooth muscles. Upper part is entirely skeletal (2–4 cm), the middle, a mixture of skeletal and smooth muscle (Figure 9), and the lower part, 11 cm or so in length is entirely smooth. Upper esophageal sphincter is composed of all skeletal muscles and lower esophageal sphincter of all smooth muscles. Based on the studies in mice embryo, esophagus is comprised of entirely smooth muscles at the beginning that slowly transdifferentiate into the skeletal muscles during later embryological age until few days after birth [54]. However, this issue has been debated by other investigators [55]. Transdifferentiation of fully differentiated phenotype cells is not commonly seen in the other body tissue types and has been of significant interest to the developmental biologists.

Neuromuscular anatomy of the lower esophageal sphincter has fascinated many because contrary to expected, no consistent thickening of the muscles at the gastroesophageal junction is found on autopsy specimens. However, *in vivo* intraluminal ultrasound imaging in the live humans clearly show a region of thick circular and longitudinal muscle layers in the LES region [56]. The muscle thickness increases and decreases with increase and decrease in the LES pressure, which suggests that the absence of muscle tone in the autopsy specimen account for the lack of muscle thickness. Liebermann–Meffert found an oblique gastroesophageal ring (GER) at the junction between the left side of the esophagus and the greater curvature of stomach, which was the site of greatest muscular thickness. It tapered toward cephalic and caudal direction for a length of approximately

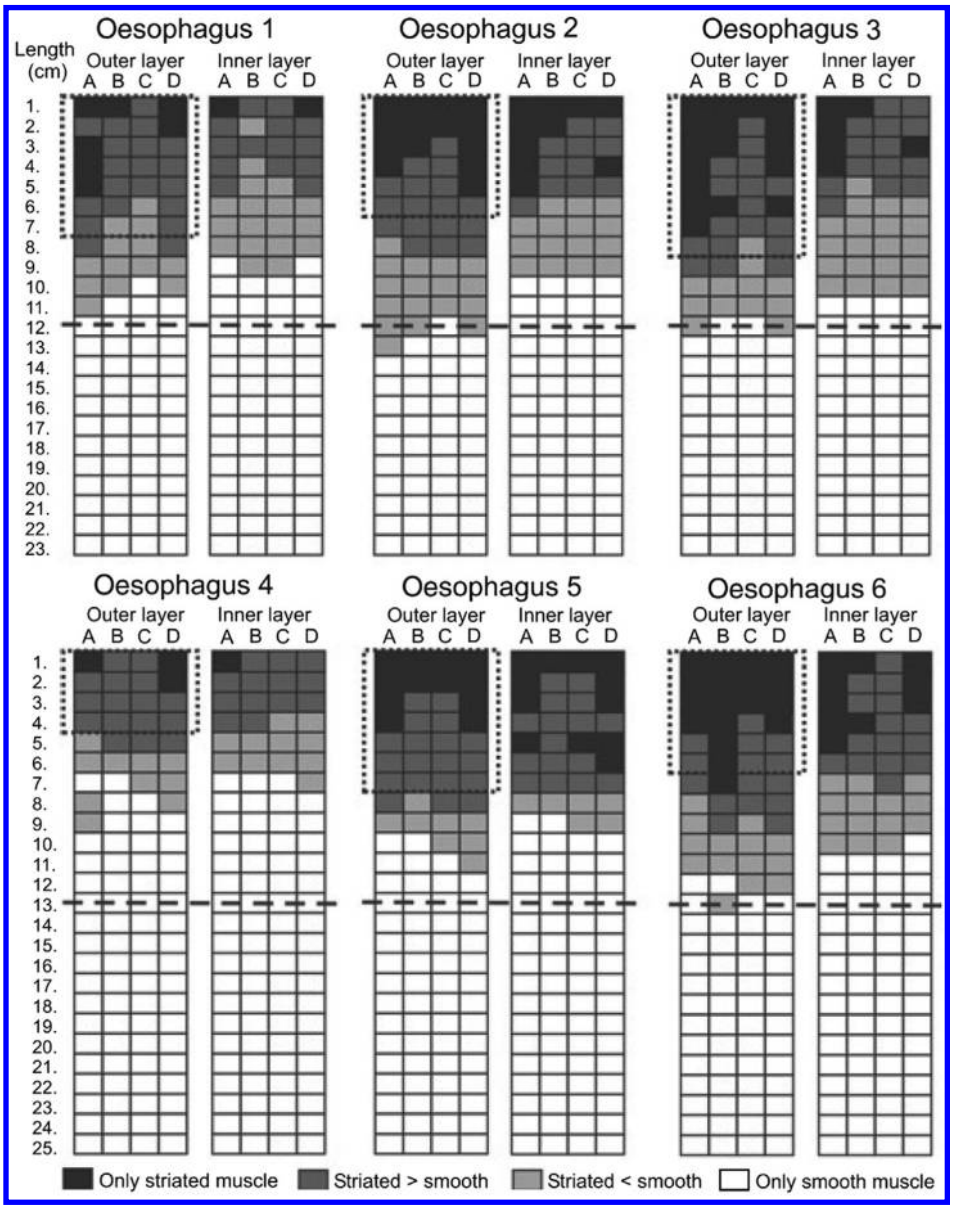


**FIGURE 9:** Smooth and striated muscle composition in human esophagus. The outer and inner layers of the tunica muscularis from each tissue block were investigated and demonstrated separately. Dotted rectangles indicate areas, in which the outer layer was 2- to 4-folds thicker than the inner layer. (Source: Kallmunzer et al., Enteric co-innervation of striated muscle fibres in human oesophagus. *Neurogastroenterol Motil* 2008;20:597–610.)

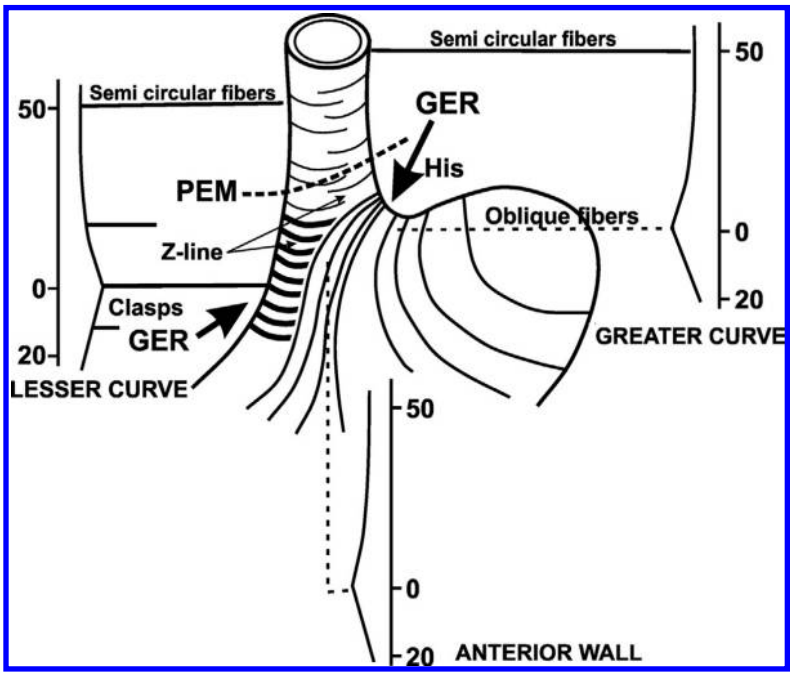


**FIGURE 10:** Anatomy of lower esophageal sphincter. Note that the muscle fibers of the LES are not circular, rather they are organized as clasp and sling fibers (from Liebermann-Meffert et al., *Gastroenterology* 1979;76:31–8).

31 mm (Figure 10) [57]. The muscle bundles split 10 mm above the GER and for a length of 25 mm to form short transverse muscle clasps on the right (lesser curvature of the stomach) and oblique fibers on the left (greater curvature of stomach). Oblique fibers form a collar around the left lower end of the esophagus that extends caudally and toward the lesser curvature. How do clasp and sling fibers result in a circumferential squeeze is not clear. As discussed later, clasp and sling fibers show marked differences in their physiological properties and have been referred to as two distinct lower esophageal sphincters [58]. Ultrastructural studies show that the LES muscle, unlike esophageal muscle, shows inward invaginations related to its state of tonic contraction [59]. The nerve varicosities in the LES are no different than that of the esophagus.

The crural diaphragm, which forms the hiatus for entry of esophagus from the chest into the abdomen is formed in the right crus of the diaphragm; its inner or medial fibers are oriented in the circumferential direction, and lateral fibers are directed in an oblique craniocaudal fashion [60]. Embryologically, crural diaphragm develops in the dorsal mesentery of the esophagus while the costal diaphragm develops from myoblasts originating in the lateral body wall [61]. Also referred as the pinch cock action of diaphragm, crural diaphragm provides a strong sphincter mechanism at

the lower end of the esophagus that has been appropriately called as the "external lower esophageal sphincter [63]." The LES and crural diaphragm are anchored to each other by the phrenoesophageal ligament, a condensation of the loose areolar tissue. It may form two leaves that extends from the under surface of diaphragm and attaches to the esophagus, approximately at the upper border of the LES. Because of the firm anchoring of LES and crural diaphragm by the phrenoesophageal ligament, the two structures move together with inspiration and expiration but can separate during longitudinal esophageal muscle contraction related to peristalsis [63] and transient LES relaxation [64].

# Extrinsic Innervation: Parasympathetic and Sympathetic

The dorsomotor nucleus of the vagus (DMV) and nucleus ambiguus (NA), located in the medullary region of the brain stem, contain cell bodies of neurons whose processes travel in the vagus nerve. Vagus, the major motor nerve of the esophagus, contains approximately 10,000–50,000 nerves fibers, 90% of which are afferents [65]. Vagal afferent nerve ending related to swallowing and esophageal motor activity originate in the mucosa and muscle layers of pharynx, larynx, esophagus, LES, and crural diaphragm. Intraganglionic laminar nerve endings (IGLE), "leaf-shaped" structure that overhang the myenteric plexus [66,67] (neurons and glia), are specialized terminals of the vagus nerve that carry mechanosensory information from the esophageal wall to the nodose and inferior jugular ganglia and then to the NTS. The other sensory vagal sensory endings located in the esophageal wall are intramuscular arrays that may connect with the interstitial cells of Cajal (ICCs). Some sensory endings located in the mucosa are free nerve ending. All vagal afferent terminals in the esophagus have their cell bodies in the nodose ganglion but some, especially from the cervical esophagus, are located in the jugular ganglion (first-order neurons) which communicates with the nerve cell bodies of NTS (second-order neurons).

## SYMPATHETIC/SPINAL/SPLANCHNIC NERVOUS SYSTEM

In contrast to the vagus nerve, most fibers present in the splanchnic nerves are motor or efferents (80–90%) [65]. These nerve fibers travel along with the branches of vagus nerve and blood vessels into the wall of the esophagus. The efferent fibers do not terminate on the end organs i.e., muscle; rather they are involved in the modulation of myenteric neurons. Afferents from the esophageal wall project to the cell bodies in the cervical and thoracic dorsal root ganglions (from C1–T9). Spinal afferent fibers innervating the cervical and thoracic esophagus and LES arise from a broad range of DRG but there is some craniocaudal representation. DRG contains various types of neurons (large, medium, and small) that are mostly pseudo-unipolar [65]. The DRG neurons project to the spinal cord via dorsal nerve roots and travel via spinothalamic tracts and relay visceral afferent input to the CNS. A high degree of convergence occurs in the termination of visceral and somatic afferent fibers in the spinal cord, which is the basis for the somatic referral of visceral pain.

## INTRINSIC INNERVATIONS OF THE LES AND ESOPHAGUS

Truncal portion of neural crest contributes to the formation of enteric nervous system of the esophagus, and *Mash-1* gene is essential in the migration of neural crest cells into the foregut (esophagus and cardiac stomach) [68,69]. Knockout or mutation of *Mash-1* genes causes aganglionosis in the esophagus, and these mice die soon after birth with no milk in their stomach. The cells proliferate in the neural crest prior to reaching the gut in the presence of appropriate growth factor and transcription factors. Uncommitted progenitors that exit from vagal cells obligatorily express Sox10 and respond to Notch and ET-3/ETB signals. Because Sox10 and Phox2b expression are required by early precursors, the entire gut becomes aganglionic when these transcription factors are deleted. The activation of Ret by GDNF/GFRa, which is essential to the formation of ganglions distal to the cardiac stomach, is not required for the ganglia to form in the esophagus and adjacent stomach. In contrast to Ret, esophageal gangliogenesis is Ascl1-dependent [69].

Enteric nervous system of esophagus is organized into myenteric (located in between the circular and longitudinal muscle) and Meissner's plexus (submucosal), both of which are not as developed in the esophagus as in the small and large intestines. Each plexus contains ganglia, collections of neurons (nodes) that are connected with each other by intermodal strands or fascicles. Ganglia are more numerous in the smooth muscles portion of the esophagus compared with the skeletal muscle esophagus [70,71]. Some of these ganglia lie outside the fascicular tracts (parafascicular). The density of neurons decreases 10 folds, from the cranial to the caudal end, reaching a nadir of 100–200 cells/$cm^2$ at the most distal end. Details, with regards to various types of cells in the myenteric plexus, are not as well known in the esophagus as is the case in small and large intestine where detailed mapping of all enteric neurons has already been accomplished. In the myenteric plexus of smooth muscle esophagus, there are two major types of neurons, excitatory and inhibitory. Excitatory ones contain acetylcholine and substance P, and the inhibitory one contains nitric oxide synthase and vasoactive intestinal peptide. Elegant studies using retrograde axonal dye (DiI) and organ culture technique show different patterns of innervations of the clasp and sling fibers of the LES, both of which are innervated by cholinergic (excitatory) and nitric oxide (inhibitory) nerves but the dominant ones in the case of clasp fibers are inhibitory neurons with their cell bodies in the esophagus, 2–12 mm above the LES. On the other hand, the cholinergic or excitatory neurons located in the stomach provide dominant innervations to the sling fibers of the LES [72,73]. This pattern or polarity of neural innervations is similar to the small and large intestine where inhibitory neurons always project in the aboral direction and excitatory neurons in the oral [74]. Inhibitory neurons are generally larger in size than the excitatory one. Large numbers of peptides neurotransmitters are present in the neurons and their processes of human esophagus but their function is not clear [75–77]. Unlike skeletal muscle, there are no identifiable motor end plates on the smooth muscle cells. Varicosities along the axonal process contain neurotransmitters, which are released

with the passage of action potential. Neurotransmitter diffuses in the spaces around the smooth muscles and act on the receptors present on the surface of smooth muscle cells.

Interestingly, myenteric neurons are present in the esophagus of animal species that have only skeletal muscle esophagus, as well as in the skeletal muscle portion of the human esophagus. Significant numbers, but not all of skeletal muscle fibers, have dual innervations; vagal nerve branches form the motor end plates on the muscles and enteric neurons that also innervate the motor end

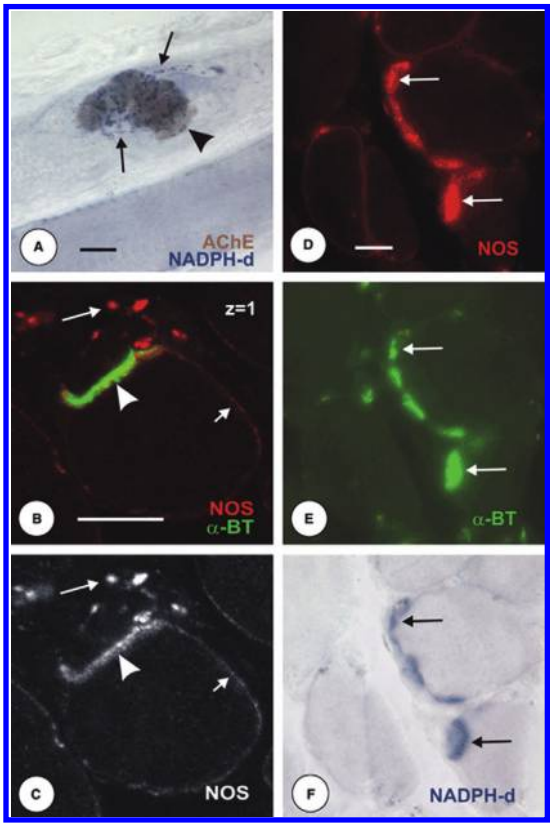


**FIGURE 11:** Innervations of skeletal muscle. Localization of NADPH-d and nNOS on motor end plates (MEPs) in the human esophagus: NADPH-d (A; arrows) and nNOS (B, C; long arrow) reactive varicose enteric nerve fibers were seen an AChE (A; arrowhead) and alpha BT (B; arrowhead) positive MEPs. In addition to the neuronal staining, muscle fibers show moderate nNOS immunoreactivity on the sarcolemma (B, C; short arrows) and the alpha BT positive postsynaptic side (B–E), which stained also for NADPH-d (D–F: arrows). Conventional (A, D–F) and confocal images (B, C single optical section demonstrated as double (B) and single channel image (C) of the same end plate) (bars = 10 μm) (from Kallmunzer et al., *Neurogastroenterol Motil* 2008;20:597–610).

plates of skeletal muscles (Figure 11). Dual innervations of skeletal muscle have been observed in mice, rat, and humans [78,79]. The enteric neurons that innervate the motor end plates contain nitric oxide synthase, CGRP, and several other peptides. They are thought to exert inhibitory influences on the skeletal muscle contraction, however, definite evidence is lacking. It is possible that the enteric neurons in the skeletal muscle esophagus are left over from the embryonic days, prior to the transdifferentiation of smooth muscle into the skeletal muscle.

# Interstitial Cells of Cajal

ICCs are present in the body of the esophagus as well as LES. They are dispersed in several different layers and are present in increasing numbers from the cranial to caudal end of the esophagus [80–82]. ICCs make close contacts with the smooth muscle cells and neurons. Axonal varicosities (site of storage of neurotransmitters) make closer contact with the ICCs than with the smooth muscle cells. Furthermore, ICCs make gap junctions with the smooth muscles. Based on the above, a large amount of literature during last 10 years suggests that the ICC serves as an intermediary role (between neurons and smooth muscles) in the neuromuscular transmission [83–86]. In addition, ICC contains receptors and signaling pathways for various neurotransmitters. Most direct evidence for the role of ICC in neurotransmission comes from observations that neurotransmission in the mutant animals lacking ICC (due to the c-Kit receptor deficiency) is impaired. Studies show that both cholinergic and nitrergic neurotransmissions in the c-Kit deficient animals are deficient in the LES [87] and stomach [88]. However, several recent studies suggest that the nitrergic neurotransmission in the LES is actually intact in the ICC deficient mice [89–91]. It may be that the impaired nitrergic neurotransmission is due to the smooth muscle defect associated with c-Kit receptor deficiency rather than the impaired neuromuscular transmission [92].

# Recording Techniques

Any investigator is as good as his recording technique and fortunately techniques to study esophageal motor patterns have improved tremendously over the years. From esophageal balloon and smoke paper kymographs to water-filled catheters, to infusion manometry and strip chart paper, to high-resolution manometry with computerized digital recording represent significant advance. In the case of LES, from side-hole sensor to Dent sleeve sensor to electronic sleeve sensor has made life simpler for the clinicians and researchers alike [93–97]. Catheter equipped with 36 solid-state transducers that are circumferentially sensitive and span the entire length of pharynx, esophagus, and proximal stomach has replaced infusion manometry recording technique during the last 5 years, and is currently the state of the art. Topographical visualization of pressure waves recorded by closely spaced sensors has come to age in the first decade of 21st century and is being used in most clinical laboratories across the country. High-resolution manometry (HRM), seamless color pressure plots using computer algorithm with linear interpolation of pressure between closely spaced transducer, along the entire region of esophagus represent significant advance. These plots beautifully show that during peristalsis, a segment rather than a focal point in the esophagus is contracted at any given time during peristalsis (Figure 12). Length of the contracted segment increases as peristalsis progress distally in the esophagus and may reach over 15 cm [93,94]. It is the contracted segment that traverses the length of the esophagus in a peristaltic fashion. The pressure in the contracted segment is distributed in the shape of a bell-shaped curve, with the peak pressure located several centimeters behind the tail end of the bolus. The transition between skeletal and smooth muscle esophagus is not seamless, as revealed by a trough in the contraction amplitude in the skeletal and smooth muscle transition zone [98,99]. Another pressure trough located distally may be related to the type of neural innervations of esophagus, proximal smooth muscle which is under greater cholinergic control and the distal smooth muscle which is under greater inhibitory control. However, hard evidence to support above claim is lacking.

Recording of longitudinal muscle contraction using catheter-based, intraluminal ultrasound imaging technique represents another technological advance [100–102]. US imaging can be used with manometry to record longitudinal and circular muscle contractions simultaneously for long periods of times and without radiation hazard inherent to the tracking of implanted radio-opaque markers technique of measuring longitudinal muscle contraction. Under physiological condition,

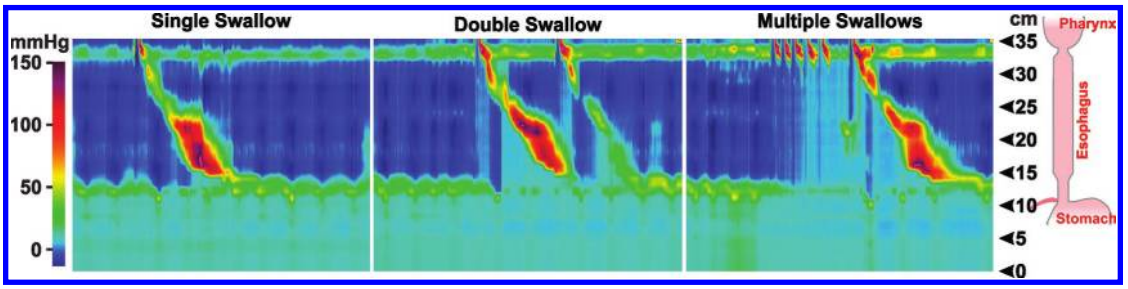


**FIGURE 12:** Deglutitive inhibition and esophageal muscle refractoriness recorded by high-resolution manometry. (A) Normal swallow-induced peristalsis and LES relaxation. (B) If the second swallow follows too soon after the first one, contraction amplitude of the second one is smaller than the first one, which is due to the esophageal muscle refractory period. (C) Multiple swallows at close intervals—note that only last swallow elicits peristaltic contraction of the esophagus. Each swallow following the first one inhibits the one preceding it, the so-called deglutitive inhibition.

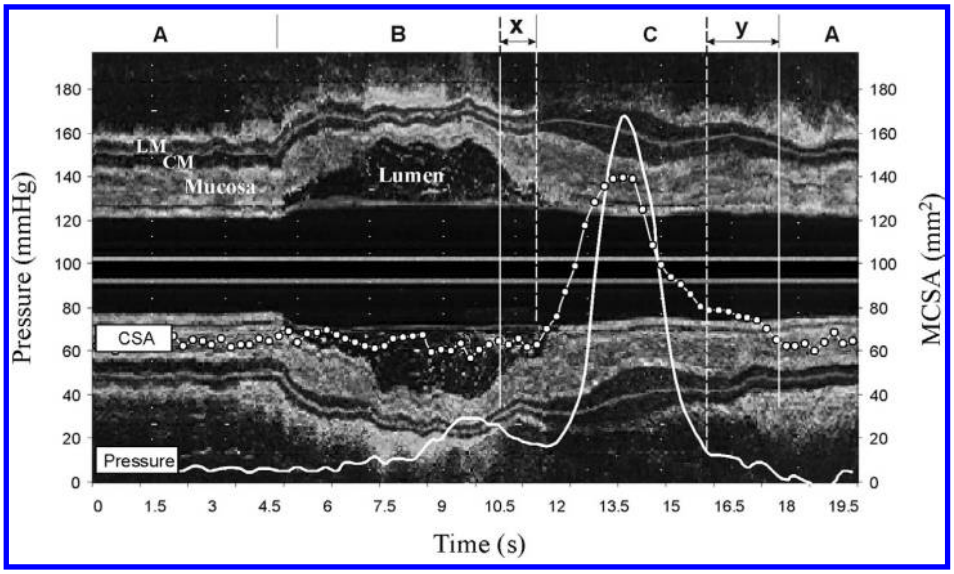


**FIGURE 13:** Coordination of esophageal circular and longitudinal muscle during peristalsis. M-mode echo-esophagraph with superimposed, intraesophageal pressure and muscle cross sectional area (MCSA) during 5 ml water swallow; ultrasound transducer positioned 2 cm above the lower esophageal sphincter. A: baseline esophagus, prior to swallow; B: bolus induced distension-bolus pressure, thinning of mucosa and muscle layers; C: esophageal contraction. Note the dissociation between increase in MCSA and increase in intraluminal pressure. The increase in MCSA (—o—) begins before and outlasts the intraluminal pressure wave (——). The difference between the onset of CSA and pressure is due to the delay in recording circular muscle contraction by manometry. LM: longitudinal muscle, CM: circular muscle. (Source: Mittal et al., *Am J Physiol Gastrointest Liver Physiol* 2006;290:G431–8.)

muscles contract in an isometric, isotonic, or a mixed fashion. Manometry is ideally suited to record isometric contraction of the circular muscle. Longitudinal muscle contraction occurs under isotonic conditions, and US imaging is ideally suited for such recording because it relies on measurement of changes in the muscle cross sectional area/thickness on tomographic US images. As esophagus shortens, related to longitudinal muscle contraction, there is a proportional increase in the muscle cross sectional area/thickness. Consequently, change in the muscle cross sectional area/thickness on the ultrasound images is a reliable marker of the longitudinal muscle contraction [103]. Tomographic ultrasound images can be displayed as m-mode images for the temporal display of changes in the muscle thickness along with the pressure recordings to display circular and longitudinal muscle contraction simultaneously and provide a complete picture of motor patterns of the esophagus (Figure 13).

# Motor Patterns of the Esophagus—Aboral and Oral Transport

Motor patterns of the esophagus can be divided in two main types: (1) one that carries the bolus toward the stomach, i.e., primary and secondary peristalsis, and (2) another that carries stomach contents toward the esophagus and mouth (oral transport). The latter is seen with belching, regurgitation, rumination, and vomiting. Transient LES relaxation is a key component of the second motor pattern. Swallowing (deglutition) or primary peristalsis begins with a voluntarily phase (oral phase) but once the bolus hits the tonsilar region and pharynx, it becomes autonomous or involuntary. Contraction of the mylohyoid muscle is the first recordable event of involuntary phase of a swallow. Sensory receptors for the involuntary phase are located on the base of tongue, tonsils, anterior and posterior pillars of the fauces, soft palate, uvula, and posterior pharyngeal wall [14]. In man, the tonsilar pillars and posterior pharyngeal wall are the optimal sites for initiation of deglutition reflex.

## CIRCULAR MUSCLE CONTRACTION

Each swallow induces a wave of inhibition that spreads along the entire length of the esophagus rapidly followed by a sequential contraction. Since esophagus does not have resting tone, inhibition and relaxation of the esophagus cannot be demonstrate with routine intraluminal pressure recordings. However, using the barostat technique, investigators have found tone in the circular muscle [104,105]. To demonstrate inhibition, one can create an artificial high-pressure zone in the esophagus by distending a small balloon, which shows a fall in the pressure with each swallow, as a marker of esophageal inhibition [106]. If one uses two high-pressure zones using two separate balloons at two different levels in the esophagus, there is simultaneous relaxation of both high-pressure zones with the swallow. Duration of relaxation is longer in the distal as compared to proximal esophagus. LES shows onset of relaxation soon after the onset of swallow (2 seconds before to 4 seconds after the onset of mylohyoid EMG activity). The LES remains relaxed for approximately 6 seconds, the entire time as the peristaltic wave traverses the esophagus. Once peristaltic wave passes the LES, it closes with a 5- to 10-second period of postrelaxation hypercontraction during which the LES pressures are significantly greater than the LES pressure prior to the onset of swallow.

LES relaxation may occur without pharyngeal or esophageal phase of swallow reflex. In the awake-state, normal subjects swallow once every minute, and the sequence of events of deglutition reflex repeats itself in a monotonous fashion. Peristalsis may not involve the entire swallowing apparatus as seen in the pharyngeal peristalsis and secondary peristalsis. If one infuses small amounts of water (rapidly or slowly) directly into the pharynx, contraction wave starts in the pharynx without the oral component and marches through the esophagus as in primary peristalsis. Secondary peristalsis under physiological conditions is seen with either retained bolus in the esophagus or distension associated with gastroesophageal reflux. In each of these conditions, wave of contraction starts above the bolus and proceeds distally propelling the bolus into the stomach without involving part of the esophagus above the bolus, pharynx or oral cavity. Under experimental condition one can study secondary peristalsis by distending a balloon in the esophagus. Contraction starts above the site of distension to propel the balloon toward the stomach. If one holds the balloon physically by a string attached to the balloon, the contraction above the balloon (esophageal propulsive force) may last several seconds. Contraction wave usually does not pass over the balloon, as long as the balloon remains distended. Distal to distended balloon, esophagus and LES remain inhibited or relaxed during the entire period of distension. Tertiary contractions of the esophagus are described by radiologists as the irregular contraction or indentations of the distal esophageal wall. Corkscrew esophagus, seen in diffuse esophageal spasm and achalasia esophagus, is also referred to as tertiary contraction. The counterpart of these contractions on manometry is not known but spontaneous simultaneous contractions seen on manometry have also been called as tertiary contractions.

# Deglutitive Inhibition and Muscle Refractoriness

Intraluminal pressure recordings show that each swallow induces a peristaltic contraction that traverses the entire length of the esophagus. It takes 6–10 seconds for the contraction wave to arrive at the distal esophageal end. However, if the subject swallows for the second time, before the contraction from the first swallow has a chance to complete its journey through the esophagus, i.e., within 4–6 seconds, the second swallow inhibits the contraction that would have been produced by the first swallow. Multiple swallows in rapid succession, i.e., at closely spaced intervals elicit only one peristaltic contraction that follows the last swallow [107–109] (Figure 12). In other words, esophagus remains silent during the period of rapid swallows, and LES remains relaxed. Above phenomenon, known as deglutitive inhibition, initially described by Dotty, allows fast drinking of fluids and beer guzzling [1]. During rapid swallows, larynx remains elevated and upper esophageal sphincter remains open, pharynx may or may not contract, esophageal and LES remain relaxed [14]. Esophagus becomes a simple conduit for the transfer of fluid pumped by the oropharynx into the stomach. If person swallows for the second time soon (within 10 seconds) after the completion of first peristaltic contraction, amplitude of contraction related to the second swallow is lower than the first swallow, a phenomenon related to the refractoriness of esophageal muscle [108,109]. As discussed later, central (brain stem) as well as peripheral mechanisms (within the smooth muscle esophagus) exist for the deglutitive inhibition. Initial inhibition and refractoriness form the basis for the clinical practice of spacing swallows at least 30 seconds apart during esophageal motility studies.

Motor events associated with the retrograde transport, i.e., belching, gastroesophageal reflux, regurgitation, rumination, and vomiting, are distinct from primary and secondary peristalsis. These events begin with spontaneous relaxation of the LES or transient LES relaxation, described in exquisite detail in the context of gastroesophageal reflux events. Transient LES relaxation was discovered in the context of gastroesophageal reflux, which is thought to be a "pathological event." Even though there is a major mechanism of reflux in patients with reflux disease, transient LES relaxation and physiological reflux occur in normal healthy subjects fairly frequently. Transient LES relaxation is also the key motor event during rumination and vomiting (physiological events).

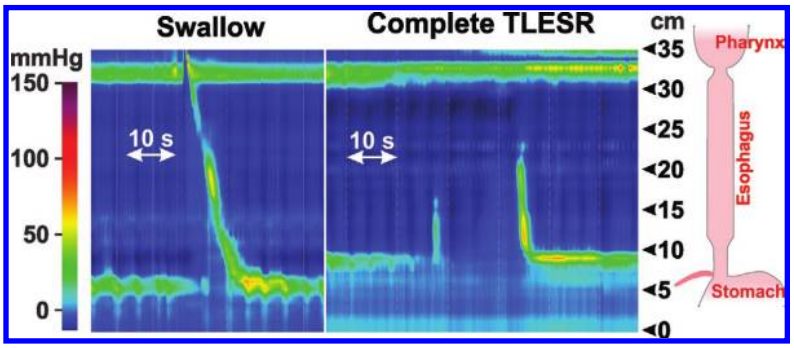


**FIGURE 14:** Swallow-induced and transient relaxation of the LES. Swallow-induced LES relaxation is a brief 6–8 seconds and follows swallow-induced pharyngeal contraction. On the other hand, transient LES relaxation is not preceded by a swallow and lasts for a longer duration than swallow (>10 seconds—can last up to 45 seconds or more).

Transient LES relaxation is unrelated to swallowing and is accompanied by simultaneous relaxation of the LES and crural diaphragm [110] along with the inhibition or contraction in the body of the esophagus. The hallmark of transient LES relaxation is that it is significantly longer (>10 seconds) than the swallow-induced LES relaxation (<10 seconds) [111] (Figure 14). Esophagus remains relatively quiescent during transient LES relaxation. However, a study that used a small distended balloon in the distal esophagus to record muscle activity found contraction of the distal esophagus [112]. During rumination and vomiting, retrograde contraction or reverse peristalsis of the esophagus has been observed using radiological studies [113]; however, pressure recordings generally do not show it. Contraction of the abdominal wall and costal diaphragm, by reducing size of abdomen cavity, increases intragastric pressure to provide propulsion force for the gastric contents to move into the esophagus and pharynx during vomiting and rumination. Generally, there is no increase in the gastric pressure in association with gastroesophageal reflux. Refluxed contents into the esophagus, whether they make it to the pharynx, depend on whether UES contracts or relaxes. It appears that the rapidity of esophageal pressure increase caused by reflux is the major determinant for UES relaxation, e.g., air reflux into the esophagus especially in the upright position that causes rapid increase in intraesophageal pressure is associated with UES relaxation [43]. On the other hand, liquid reflux, especially in the supine position is associated with slower increase in the esophageal pressure and UES contraction [44].

# Peristalsis in the Circular and Longitudinal Muscles of the Esophagus

Intraluminal pressure measurements, used extensively to record esophageal motor activity, monitor contraction of the circular muscles of the esophagus which comprises only 50% or less of the mass of muscularis propria. Longitudinal muscle contraction causes esophageal shortening. Primary and secondary peristalses associated esophageal shortening have been known to radiologists since 1950s. Using radio-opaque markers implanted on the esophageal wall, cineradiography studies show peristalsis in the longitudinal muscle layer of the esophagus [63,114–116]. Strain gauze recording of the opossum esophagus shows coordination between circular and longitudinal muscle layers [117,118]. These studies found that at a given site in the esophagus longitudinal muscles contract prior to and outlasts circular muscle contraction by 2–4 seconds. Ultrasound imaging and manometry studies show that, actually, the two layers of the esophagus contract in a precisely coordinated way; the onset, peak, and termination of contraction of the two muscle layers are precisely coordinated [103,119]. Similarly, contraction proximal to the site of balloon is also seen in the longitudinal muscle layer [120]. Caudal to the distension site, the two layers of esophagus demonstrate relaxation. Similar to circular muscles, deglutitive inhibition also occurs in the longitudinal muscles as demonstrated by the changes in distance of the radio-opaque markers implanted along the length of the esophagus (to measure longitudinal muscle contraction) [121]. All of the above observations suggest that ascending contraction and descending relaxation "law of intestine" described by Bayless and Starling [122] in 1899 holds true for the circular and longitudinal muscle layers of the esophagus (Figure 15), as is also the case with the colon [123,124]. There is strong correlation between the amplitude of circular and longitudinal muscle contraction during peristalsis [103,125].

If it is the milking action of circular muscle that is responsible for bolus propulsion, what is the function of longitudinal muscle layer of the esophagus? There are several advantages for the two layers to contract together during peristalsis? First, contraction of longitudinal muscle layers brings together the rings of circular muscles at the site of contraction which increases muscle mass and efficiency of circular muscle contraction [58,101]. Second, increase in muscle thickness at the site of

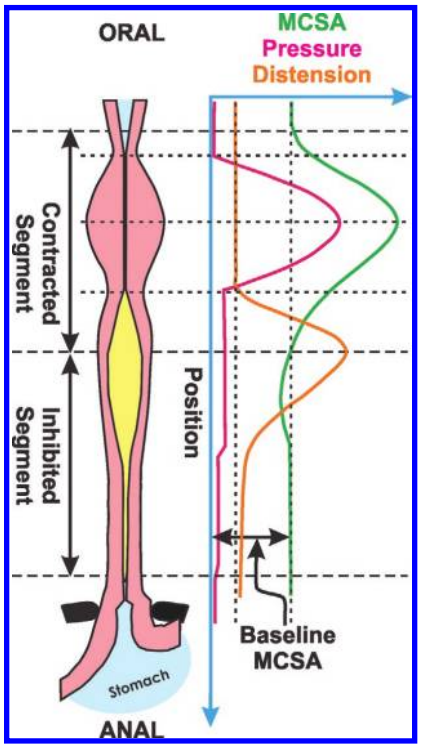


**FIGURE 15:** Schematic of contraction and distension during swallow-induced peristalsis. Note that the pressure and muscle cross-sectional area (MCSA), surrogate markers of circular and longitudinal muscle contractions, respectively, precede distension. The latter marches distally in front of the onset of contraction wave in a peristaltic fashion (from Abrahao et al., *Neurogastroenterol Motility* 2010 Nov 17; doi: 10.1111/j.1365-2982.2010.01624).

contraction reduces esophageal wall stress that prevents outward bulging or "aneurysm like effect." Discoordination with regards to the timing of contraction between the two muscle layers occurs in patients with nutcracker esophagus (hypertensive esophageal peristalsis) [126]. Interestingly, these patients also have a high incidence of diverticular (out pouch) formation. Discoordination between the two layers is related to a hypercholinergic state because cholinestrase inhibitor (edrophonium) induces discoordination between the two muscle layers in the normal subjects and an anticholinergic (atropine) ameliorates temporal discoordination between the two muscle layers in patients with nutcracker esophagus [127].

Retrograde transport and transient LES relaxation is associated with a unique and distinct profile of contraction in the two muscle layers [64,127]. Contraction of longitudinal muscle of the distal esophagus starts right before the onset of transient LES relaxation, and it gets getter stronger

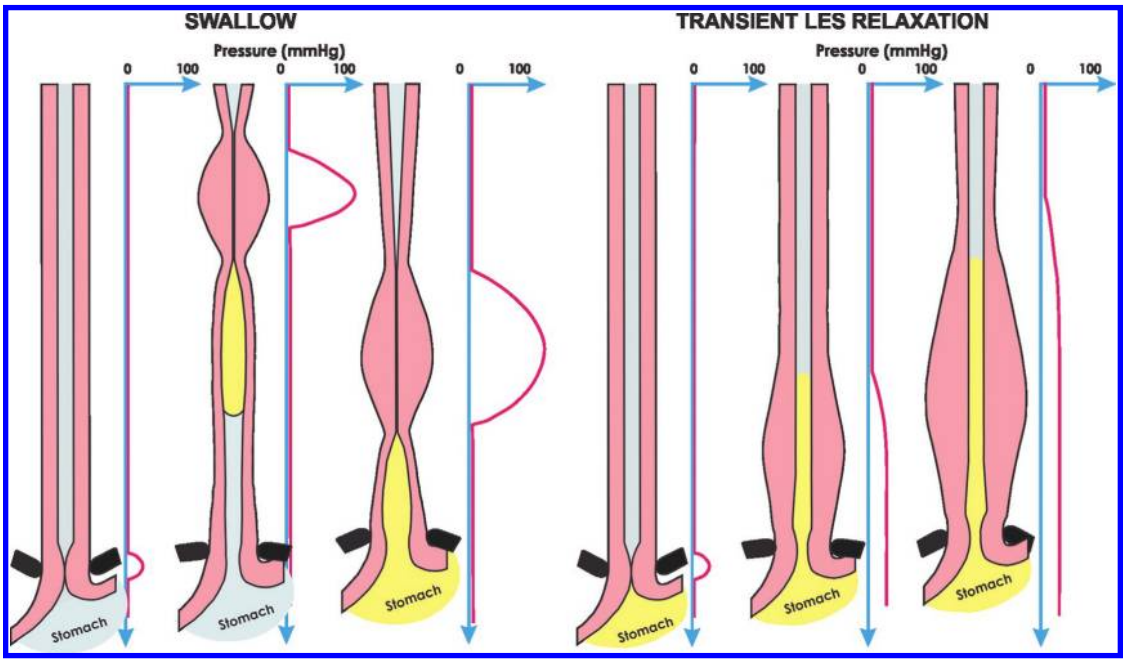


**FIGURE 16:** Patterns of longitudinal muscle contraction during peristalsis and transient LES relaxation. Peristaltic contraction is associated with an aborally traversing simultaneous contraction of the circular and longitudinal muscle of the esophagus. On the other hand, transient lower esophageal sphincter relaxation is associated with contraction of the longitudinal muscle of the distal esophagus, in the absence of circular muscle contraction. (Source: Babaei et al., *Gastroenterology* 2008;134:1322–31.)

and traverses in an antiperistaltic fashion toward the proximal esophagus during the entire duration of transient LES relaxation. Circular muscles do not contract during the entire period of TLESR (Figure 16). LES and crural diaphragm remain relaxed during the entire period of longitudinal muscle contraction, and with cessation of longitudinal muscle contraction, there is return of LES basal tone and crural diaphragm activity. Circular and longitudinal muscle layers of the esophagus contract together during peristalsis [119], and longitudinal muscle contracts independent of circular muscle during transient LES relaxation [127]. Since transient LES relaxation and swallow-mediated peristalsis are mediated via vagus nerve and brain stem, it is likely that the central program generator (CPG) can initiate two distinct motor programs, program 1, which is responsible for aboral transport with swallowing, and program 2, which is responsible for the retrograde transport of which transient LES relaxation is the key component. As will be discussed later, longitudinal muscle contraction of the distal esophagus is likely a key event that induces LES relaxation through the activation of stretch sensitive motor neurons of the LES.

# Neural and Myogenic Mechanism of Peristalsis

Major players in the peristalsis, as outlined in the previous paragraphs, are supramedullary (cortex), central program generator (NTS) and dorsomotor nucleus of the vagus nerve in the brain stem, vagus nerve, myenteric plexus with inhibitory and excitatory neurons, along with the skeletal and smooth muscles of the esophagus. In the esophagus, there are two major elements of peristaltic reflex, circular and longitudinal muscle layers both of which contract behind the bolus and relax in front of the bolus [128] (Figure 15). A recent study suggests that even the relaxed segment located just caudal to the contracted segment traverses in a peristaltic fashion along the length of the esophagus. Peristalsis of the contracted segment has been explained on the basis of central and peripheral mechanism.

# Central Mechanism of Peristalsis—Cortical and Brain Stem Control

Deglutition is a reflex mediated through brain stem, even though, both volition swallow and pharyngeal (reflexive) swallow are associated with activation of several cortical areas. These include sensory motor cortex, anterior cingulate gyrus, insular cortex, cuneus, and precuneus regions [5]. It is likely that these cortical regions are related to the oral phase/volitional or sensory aspect of swallow and not with the upper and lower esophageal sphincter relaxation and genesis of peristalsis.

Bilateral cervical vagotomy or cooling of the vagus nerve abolishes peristalsis in both the skeletal and smooth muscle esophagus, thus proving the crucial roles that brain stem, swallow center and central program generator play in the genesis of peristalsis [129,130]. Unilateral vagotomy does not prevent peristalsis completely because of the crossing over of the nerve fibers from the NTS to the motor nuclei [3]. Secondary peristalsis elicited by balloon distension of the skeletal but not the smooth muscle esophagus is also mediated through brain stem and vagus nerve [131]. In the proximal skeletal muscles of opossum esophagus and in all other animal species in which the entire esophagus is made up of skeletal muscles, there is general consensus that sequential activation of the muscles along the length of the esophagus is caused by sequential activation of motor neurons located in the nucleus ambiguus and some in DMV. Electrical stimulation of the peripheral end of transected cervical vagus nerve induces simultaneous contraction in the skeletal muscle esophagus. Therefore, in all those animal species, where the entire esophagus is made up of skeletal muscles, no peristaltic or sequential contractions are observed with electrical stimulation of the vagus nerve trunk. The ingenious nerve suture studies of Roman and Tiffenback [132–134] support sequential excitation of esophageal muscles by the brain stem neurons. In these experiments, the central end of a cut vagus nerve was anastomosed to the peripheral cut end of accessory spinal nerve (in the neck). Vagus nerve axons regenerated to activate the sternocleidomastoid and trapezius muscles in the neck and a swallow-activated EMG bursts in these muscles reflect discharges from the vagal preganglionic fibers. Similarly, vagus nerve can be sutured to the phrenic nerve in the chest and EMG activity recorded in the diaphragm muscle. These studies prove that the swallow induces sequential activation of preganglionic vagal efferent fibers, suggesting that the central program generator fires in a sequential

manner in both the skeletal and smooth muscles esophagus. It is suggested that both inhibitory and excitatory elements of deglutition reflex are mediated at the level of central nervous system. Swallow-evoked discharges in the preganglionic efferent of the vagus nerve reveal short (<1 second) and long latency fibers (1–5 seconds) [135]. Short latency fiber discharges have a lower threshold of activation, and their latency can be modulated by stimulation frequency of the superior laryngeal nerve and correspond to the inhibitory phase of esophagus. On the other hand, long latency fibers have a high threshold of activation, and these discharge patterns are not modulated by stimulation frequency, and they coincide with the esophageal contractions.

# Peripheral Mechanisms of Peristalsis

Several observations support that the mechanisms of peristalsis resides in the esophageal wall. Pattern of neural innervation and activation in the myenteric plexus are important determinants of peristalsis. Furthermore, pattern of spread of contraction in the muscles (myogenic) may play an important role in esophageal peristalsis. First, electrical stimulation of the peripheral end of cervical vagus nerve, which undoubtedly stimulates all vagal efferent fibers simultaneously and eliminates the possibility of sequential activation of vagal efferent fibers, can elicit peristaltic contraction depending upon the electrical stimulus parameters [136] (Figure 17A). Second, smooth muscle esophagus removed from the animal and placed in an organ bath (devoid of extrinsic innervations) demonstrates secondary peristalsis, with ascending contractions and descending relaxation [137]. Third, circular muscle strips studied in an organ bath show an increasing latency gradient from the cranial to caudal direction [138,139] (Figure 17B). In other words, when muscles from different levels in the esophagus are stimulated at the same time, they contract following a certain time period after the cessation of electrical stimulus, the so-called latency period. Muscle strips, devoid of extrinsic innervations, from the distal esophagus have longer latency period as compared to the proximal esophagus. Mechanical pinching and distension of the esophagus *in vitro* also evoke peristalsis in the smooth muscle esophagus, suggesting that the mechanism of peristalsis resides in the periphery, i.e., in the esophageal wall [140,141].

During the actual period of stimulation (electrical—vagus nerve/intramural or mechanical—balloon distension) or the latency period, intracellular recordings of the smooth muscles show hyperpolarization (decrease in the resting membrane potential) followed by depolarization and spike bursts [142,143]. Hyperpolarization is equivalent of inhibition or muscle relaxation and depolarization with spike bursts of muscle contraction. A swallow activates an immediate hyperpolarization along the length of esophagus that induces muscle relaxation, the duration of which corresponds to the latency of contraction, followed by depolarization and spike burst (contraction) [143]. The latency period as well as hyperpolarization is induced by activation of inhibitory nerves through the release of nitric oxide [144]. On the other hand, it is not totally clear if depolarization and spike discharges are due to passive rebound phenomenon from hyperpolarization or release of an excitatory neurotransmitter (acetylcholine). It is suggested that nerve-induced depolarization, following

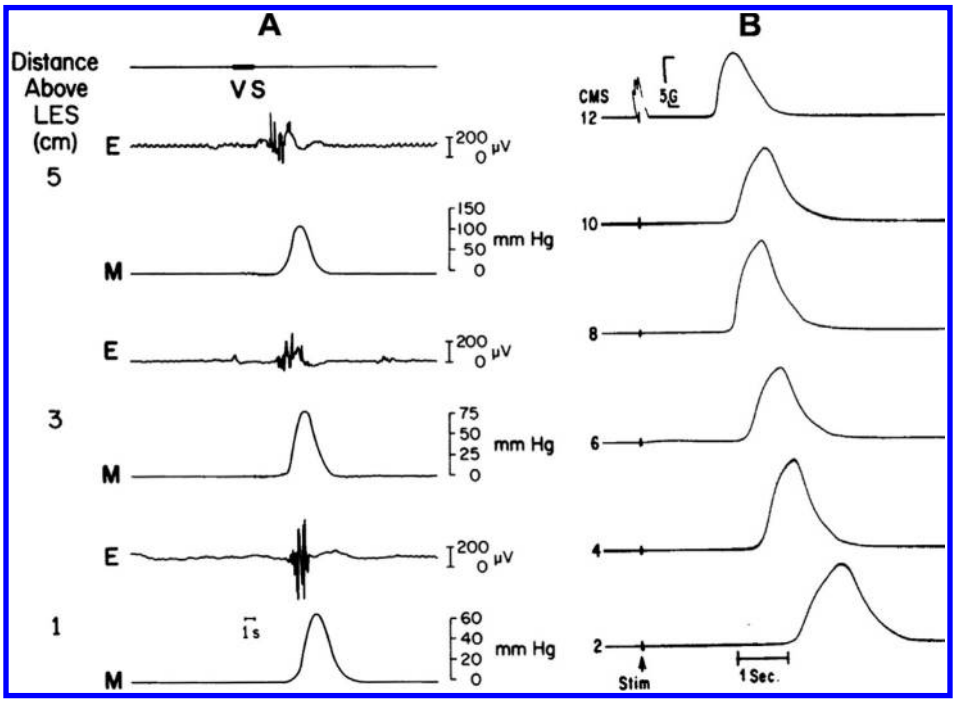


**FIGURE 17:** Mechanism of peristalsis in the smooth muscle esophagus. (A) Peristalsis elicited by electrical stimulation of the peripheral end of cervical vagus nerve in the opossum smooth muscle esophagus—note that even though all efferent fibers were stimulated simultaneously, it elicited a peristaltic wave of contraction. (B) Muscle strips from different levels of opossum esophagus were stimulated simultaneously using electrical field stimulation. Note latency of contraction is shorter in the muscle strips from the proximal as compared to distal esophagus. Latency of gradient resides in the wall of esophagus and constitutes peripheral mechanism of peristalsis. (Adapted from Weisbrodt, *Gastroenterology* 1972;62:1159–66; Gidda et al. *J Clin Invest* 1981;68:1411–9.)

hyperpolarization, depends upon the production of eicosanoids. This has been shown in the longitudinal muscles of esophagus [145] and colonic taenia coli [146]. With an increase in the frequency of electrical stimulus, the latency period decreases in the proximal esophagus but increases in the distal esophagus. Furthermore, latency period in the proximal esophagus is more susceptible to atropine (anticholinergic) than the distal esophagus [147]. Based on the above observation, gradients of cholinergic and nitrergic innervations are suggested along the length of the esophagus, with the former being greater in the proximal and the latter more in the distal esophagus [147]. Even though above observation would be consistent with the anatomical evidence of gradients in the density of myenteric plexus along the esophageal length, numbers of acetylcholinestrase positive neurons do not differ along the length of esophagus [148]. Furthermore, there are no anatomical data for

the differences in the nitrergic neurons along the length of the esophagus. Organ bath studies of the isolated esophagus suggest that descending hyperpolarization is mediated through a long descending neuron, at least more than 3 cm in size, and there are no synapses in this pathway [137].

Intrinsic differences in smooth muscle responses along the esophagus may also be the result of quantum or effects of released neurotransmitter on the muscles from different regions. Resting membrane potential along the esophagus is less negative distally, which may be related to the gradient of potassium content along the smooth muscle esophagus [149,150]. A number of regional myogenic differences along the length of the esophagus also exist [151–154]: (1) a more depolarized resting membrane potential due to sodium permeability and increase in the density of voltage-dependent potassium channels proximally [155]; (2) regional differences in the soluble *N*-ethylene-maleimide sensitive factors attachment protein receptor (SNARE) protein SNAP-25 [153], which regulate potassium channels [151]; (3) response to stretch and cholinergic stimulation, with strips from more proximal regions being more responsive [152]; (4) increased expression and current density of L-type calcium channels in the proximal versus the distal smooth muscle esophagus [153].

Studies in the humans highlight the significance of balance in the excitatory and inhibitory innervation in the genesis of spastic motor disorders [156–159]. Nitric oxide antagonist decreases latency of contraction in the distal esophagus and converts peristaltic contraction into a simultaneous one. Atropine delays the latency of contraction in the proximal esophagus to increase the velocity of peristalsis [160]. These observations support the concept that in patients with spastic motor disorders of the esophagus, there is an imbalance between the inhibitory and excitatory nerve activity.

There is also evidence to suggest myogenic mechanism of peristalsis [161–163]. Peristalsis can be recorded in live animals following administration of tetrodotoxin (TTX), which blocks all sodium channels' mediated action potential in the all neurons and its processes. Myogenic contractions and peristalsis can be elicited by the long pulse duration electrical current that activates muscle directly, by esophageal distention, by muscle membrane depolarization using high concentrations of $K^+$, and pharmacologic stimulation. It is suggested that the muscle-to-muscle communication such that depolarization of one smooth muscle cell will result in electrotonic spread of current to adjacent muscle cells in an aboral direction [164].

# Central Versus Peripheral Mechanism of Deglutitive Inhibition

Deglutitive inhibition in the skeletal muscle esophagus can be explained based on the inhibition of neuronal discharges in the brain stem. On the other hand, a peripheral mechanism of deglutitive inhibition is also present. Studies show that similar to closely spaced swallows at short intervals, high-frequency electrical stimulation of the peripheral end of vagus nerve at short intervals, and intramural stimulation of the smooth muscle strips *in vitro*, induce period of inhibition during the stimulation and contraction following the last stimulus. Low-frequency successive stimulation of the vagus induces contraction with the first and last stimuli (A and B waves or "on" and "off" contractions). Same is observed in the muscle strip experiments. The occurrence of "on" and "off" contractions is explained on the basis of inhibition and muscle refractoriness. High-frequency stimulation induces strong initial inhibition and therefore a second stimulus, immediately following the first, inhibits contraction induced by the first stimulus. On the other hand, refractoriness of the muscle following first stimulus prevents contraction from the low-frequency second stimulus. There are gradients of refractory period and inhibitory innervations along the length of esophagus with stronger refractory period and inhibitory innervations in the proximal and distal esophagus, respectively [165].

# Neural Control of Longitudinal Muscle Contraction

Control mechanisms are different in the longitudinal as compared to circular muscle. Stimulation of the cervical vagus nerve induces contraction of the longitudinal muscle esophagus that lasts for the duration of stimulus, as opposed to "on" and "off" responses described for the circular muscle earlier [166,167]. Stimulation of central end of superior laryngeal nerve induces swallow-induced peristaltic contraction of longitudinal and circular muscles [118]. Similarly, swallow-induced peristalsis in the humans induces peristaltic contraction in both layers [119], suggesting that peristalsis in the longitudinal muscle is mediated within the central program generator. Unlike circular muscles that show hyperpolarization with swallow, longitudinal muscles show depolarization, suggesting the absence of peripheral inhibitory mechanism in the longitudinal muscle [143]. However, studies do show relaxation of the longitudinal muscle distal to the site of esophageal distension site *in vivo* [120] in the organ bath studies and with repetitive swallows [121], raising the possibility that there indeed should be a peripheral mechanism of inhibition in the longitudinal muscles. May be it is the withdrawal of excitatory influence, rather than the active inhibition, that induces longitudinal muscle relaxation. Nitric oxide, which induces relaxation of the circular muscle, causes contraction of the longitudinal muscles through an excitation–contraction coupling mechanism, via a cGMP-dependent signaling pathway [145]. It requires extracellular $Ca^{2+}$ entry through the activation of L-type $Ca^{2+}$ channels [168]. Phosphorylation of a protein, possibly a 116-kDa protein, is a key step in the signaling pathway [169]. *In vivo* study in cats shows L-NAME (NO blocker) reduces longitudinal muscle contraction, but these effects were not as prominent as seen in the *in vitro* studies [170].

# Modulation of Primary and Secondary Peristalsis

It is clear that input from the central pattern generator (CPG) can modify the amplitude of contractions, speed of peristalsis, and even the polarity of esophageal contraction (peristalsis versus antiperistalsis) [136]. Inputs to the CPG may come from the supramedullary regions of brain, which are not well understood. On the other hand, inputs into the CPG from the periphery that modulate peristalsis have been well studied, e.g., wet swallows as compared to dry swallows elicit greater amplitude of contractions and lower speed of peristalsis [171,172]. Viscosity of the bolus reduces the speed of peristalsis [173]. The temperature of the bolus has significant effects as well; warm bolus increases and cold decreases the contraction amplitude and the occurrence of peristalsis [174–176]. Outflow obstruction has pronounced effects; peristalsis rarely traverses over the bolus trapped between an oncoming peristaltic contraction and closed distal end of the esophagus. In an obstructed esophagus, contraction dissipates when it can no longer propel the bolus because of resistance at the outlet, resulting in an escape of bolus toward the mouth [177]. In the skeletal muscle esophagus, these effects are mediated through vago-vagal pathway [129,178]. On the other hand, in the smooth muscle esophagus, controls may exist both in the periphery and brain stem.

# Neural Control of Lower Esophageal Sphincter and Crural Diaphragm

Smooth muscles of LES and skeletal muscle of crural diaphragm have also been referred to as the internal and external lower esophageal sphincters [179,180]. LES is under the control of autonomic nerves, myenteric plexus, and its own myogenic tone. On the other hand, crural diaphragm, like any other skeletal muscles, has no myogenic tone and contracts through neural discharges from the somatic nerves (phrenic nerves). Antireflux barrier function and transit (flow) across the esophagogastric junction (EGJ) require fine coordination between the two sphincters, which will be discussed later.

# Lower Esophageal Sphincter

LES is a unique muscle; it has its own myogenic tone that is modulated by neural, hormonal and paracrine factors [181,182]. Evidence for the myogenic stone comes from following *in vitro* and *in vivo* observations: (1) LES muscle strips, devoid of extrinsic innervations and studied *in vitro* (under no influence of hormonal factors) show steeper length tension characteristics than the muscle strips from the esophagus [183]. TTX which abolishes all intrinsic neural activity does not abolish tone in these muscle strips. In the presence of TTX, nitric oxide and other agents that act directly on the muscle reduce LES muscle tone [184]. (2) TTX does not abolish LES pressure in the *in vivo* studies [185]. Myogenic elements responsible for LES tone maintenance may be due to differences in the structural protein, LES has proportionally more α-actin and basic essential light chains LC17b, and less of a seven amino acid-inserted myosin isoform and caldesmon than the esophageal body circular muscle [186]. LES muscle utilizes more calcium from the intracellular than extracellular source as compared to the esophageal muscle [187]. There are also distinct intracellular signaling pathways in the LES as compared to the esophageal body [188]. From an electrophysiologic point of view, the LES muscle is in a state of greater depolarization than the esophageal muscle, as evidenced by a higher resting membrane potential than the esophagus [189]. The depolarized state of the sphincter muscle is suggested to be due to the resting chloride conductance [190]. Periodic spike bursts or increase in the depolarization results in an increase in the LES tonic activity. Tonic LES contraction is both spike dependent and spike independent [48]. Relative contribution of myogenic tone to the LES pressure differs in different species. In the opossum, myogenic tone dominates under basal resting condition. On the other hand, in cats [191], dogs [192], and humans [193], neural cholinergic drive contributes significantly to the basal LES tone. Atropine (15 μg/kg), which reduces excitatory neural cholinergic drive, reduces LES pressure by 50% to 70% in humans [194].

LES muscles are made up of clasp and sling fibers [57]—clasp fibers maintain stronger myogenic tone than the sling fibers [195] and sling fibers respond briskly to cholinergic agonist. Clasp fibers are predominantly innervated by inhibitory neurons located in the body of the esophagus and sling fibers by the excitatory neurons located in the stomach [73,196]. L-type calcium channels are predominantly seen in the clasp muscle fibers [197], and there are other differences as well in the

mechanisms by which sling and clasp muscles contract and relax [198]. Differences in the properties of sling and clasp muscles fibers may be responsible for the greater pressure and greater cholinergic responsiveness of the LES pressure on the left side. Sling fibers are likely to be responsible for the maintenance of angle of HIS and flap valve function, both of which are considered to be important in the prevention of reflux.

Myenteric plexus contains both excitatory and inhibitory neurons that have intrinsic activity and are also under the influence of extrinsic vagus and spinal nerves. Excitatory neurons contain acetylcholine and substance P; inhibitory neurons, on the other hand, contain VIP and NO. Electrical stimulation of the LES muscle strip that supposedly stimulates both excitatory and inhibitory neurons elicits relaxation, suggesting that inhibitory influence dominates over the excitatory one. Stimulation of the vagus and spinal nerve has opposite effects on the LES pressure. Vagus nerve, which contains fibers that are thought to innervate both excitatory and inhibitory nerves, elicits relaxation only when electrically stimulated [199]. The inhibitory effect is frequency (dose) dependent, and none of the stimulus parameters induces LES contraction. The above not mean that vagus does not innervate excitatory neurons to the LES, it may be that when all, i.e., both inhibitory and excitatory vagus nerves fibers, are stimulated, inhibitory influence dominates, just like *in vitro* muscle strip studies [200]. Motor neurons that supply LES show topographical localization in the DMV. Stimulation of the rostral neurons elicits LES contraction and caudal cause LES relaxation, both of these effects are blocked by bilateral vagotomy [201]. The above observation suggests that vagus nerve contains fibers that impinge specifically on either the excitatory or the inhibitory neurons. Electrical stimulation of the sympathetic nerves causes LES contraction that is mediated by α-adrenergic receptors [202,203]. It is likely that the sympathetic/spinal nerves innervate neurons rather than the muscles directly. β-adrenergic stimulation, on the other hand, leads to LES relaxation, an effect that could be mediated through $\beta_1$, $\beta_2$, or $\beta_3$ receptor [204,205]. $\beta_3$-receptor stimulation, unlike $\beta_1$ and $\beta_2$, does not cause any cardiovascular side effects, which could be relevant for the treatment of esophageal motor disorders and LES hypertension. A large number of neuropeptides, hormones, and paracrine substances modulate LES tone, either increase or decrease LES pressure, as shown in Table 1, but whether they play any physiological role is not clear. Studies in the past investigated the role of various different types of foods including alcohol, smoking, and caffeine on the basal LES pressure including their mechanism of action, in the hope of understanding how they may elicit gastroesophageal reflux.

Long-term recordings in the animals and humans show fluctuations or phasic pressure changes in the LES. Some of these are related and others unrelated to the gastric component of the migrating myoelectrical complex (MMC) [206–209]. During the first phase of MMC, the LES pressure is relatively stable, but during late phase II and throughout phase III, large-amplitude

**TABLE 1:** Effects of some hormones and putative neurotransmitters on the lower esophageal sphincter and the possible sites of action: From the article *Sphincter mechanisms at the lower end of the esophagus.* Ravinder K. Mittal and Raj K. Goyal; *GI Motility online* (2006) doi: 10.1038/gimo14

| | | SITE OF ACTION | | | |
|---|---|---|---|---|---|
| **AGENT** | **EFFECT** | **CIRCULAR SMOOTH MUSCLE** | **INHIBITORY NEURONS** | **EXCITATORY NEURONS** | **COMMENTS** |
| Bombesin | Contraction | ✓ | – | ✓ | Releases norepinephrine from adrenergic neurons |
| Calcitonin gene-related peptide | Relaxation | ✓ | ✓ | – | |
| Cholecystokinin | Biphasic | ✓ | ✓ | – | Inhibition overrides excitation, causes paradoxical excitation in achalasia patients |
| Dopamine | Relaxation ($D_2$)<br>Contraction ($D_1$) | ✓<br>✓ | –<br>– | –<br>– | |
| Galanin | Contraction | ✓ | – | – | |
| Gastric inhibitory polypeptide | Relaxation | ? | ? | ? | |
| Gastrin | Contraction | ✓ | – | – | |

| | | | | | |
|---|---|---|---|---|---|
| Glucagon | Relaxation | ✓ | – | – | Releases catecholamines from adrenal medulla |
| Histamine | Contraction | ✓($H_1$) | – | – | |
| Motilin | Contraction | ✓ | – | ✓ | |
| Neurotensin | Contraction | ✓ | – | – | |
| Nitric oxide | Relaxation | ✓ | – | – | |
| Pancreatic polypeptide | Contraction | ✓ | – | ✓ | |
| $PGF_{2\alpha}$ | Contraction | ✓ | – | – | |
| $PGE_{1,2}$ | Relaxation | ✓ | – | – | |
| Progesterone | Relaxation | – | – | | |
| Secretin | Relaxation | ✓ | – | – | |
| Serotonin | Contraction | ✓ | – | – | |
| Somatostatin | Contraction | ? | ? | ? | |
| Substance P | Contraction | ✓ | – | ✓ | |
| VIP | Relaxation | ✓ | – | – | |

PGE, prostaglandin E; PGF, prostaglandin F; VIP, vasoactive intestinal peptide; ✓, yes; –, no.

phasic contractions occur without a major change in the basal pressure. LES pressure increases before the increase in the gastric pressure. These MMC-related contractions are abolished by atropine and anesthesia [208,210]. Motilin, a neurohumoral agent released into circulation from the specialized cells in the wall of intestine, may be responsible for MMC-related phasic LES contractions [207]. LES also contracts in response to increases in intraabdominal pressure related to abdominal compression or straight leg raise, most likely through a vago-vagal reflex [194,211].

# Swallow-Induced LES Relaxation

LES relaxation dysfunction is a major finding in the motor disorders of the esophagus. In fact, it is suggested that the primary abnormality in all spastic motor disorders of the esophagus is impaired LES relaxation [52,101,130]. Therefore, the understanding of swallow-induced LES relaxation is crucial. Swallow-induced LES relaxation is mediated via vagus nerve because bilateral cervical vagotomy and cooling of cervical vagus nerve (that block traffic in the nerve) reduces LES relaxation [212,213]. Electrical stimulation of the vagus nerve causes LES relaxation in a dose-dependent fashion [214]. Since extrinsic nerves influence LES muscle through intrinsic or myenteric plexus, it is suggested that the vagal nerve fibers synapse with the inhibitory motor neurons. Acetylcholine is released at the presynaptic nerve endings and acts through the nicotinic (predominantly) and muscrinic (M1) receptors to activate inhibitory motor neurons [215]. What is released by the inhibitory motor neurons that causes LES relaxation was resolved in the 1990s to be nitric oxide. A series of studies *in vitro* and *in vivo* including some from the humans prove [157] beyond doubt that NO is the "noncholinergic nonadrenergic" inhibitory neurotransmitter [216–219]. While other inhibitory neurotransmitters, such as VIP, CO, and PCAP, have been suggested, they probably play a minor role in the LES [220]. Nitric oxide, a gas that diffuses quickly, is not stored in the nerve terminals, rather it is synthesized quickly by nitric oxide synthase upon neural stimulation. In addition to acting on the smooth muscle, NO may act on the presynaptic nerve terminals to stimulate VIP release [221] even though neutrally induced relaxation is associated with the increase in intracellular cyclic GMP-[222] and VIP-induced stimulation with cyclic AMP [223]. Nitric oxide increases intracellular cyclic GMP and other intracellular messenger system to cause LES muscle relaxation.

All types of LES relaxations *in vivo*, i.e., swallow-induced, esophageal distension-induced, vagus nerve stimulation-induced, and spontaneous transient LES relaxations, are associated with movement of the LES in the cranial direction [64,114,167]. Cranial movement with LES relaxation was a major issue in the 1970s because Dodds et al. recognized that it caused a relative movement between the recording pressure sensor and the LES [63]. In order to prevent this, animal studies used a technique to pin the catheter and LES together [185]. For the same reasons, Dent devised a sleeve sensor for continuous LES pressure recording in the humans [224]. Cranial movement of the LES is caused by one or the other patterns of longitudinal muscle contraction of the esophagus, as

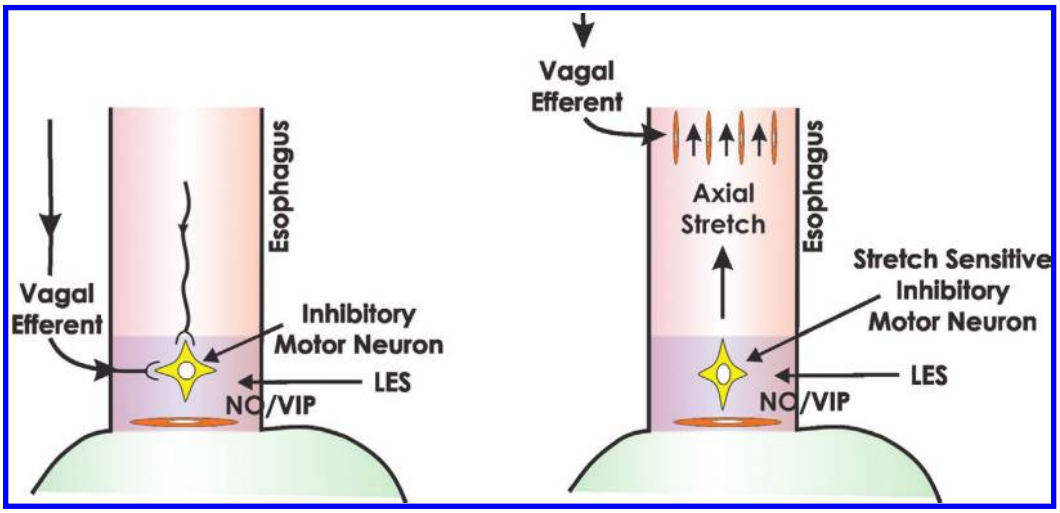


**FIGURE 18:** Vagus nerve induced relaxation of the lower esophageal sphincter. Traditional view is that vagal efferent fibers synapses with the inhibitory neuron of the LES, which in turn releases NO that causes LES relaxation. However, based on the recent studies it may be that vagus nerve activates longitudinal muscles of the esophagus, contraction of which in turn activates stretch sensitive motor inhibitory neuron of the LES (based on the study from Jiang et al., *Am J Physiol Gastrointest Liver Physiol*. 2009;297(2):G397–405).

described earlier. It turns out that a mechanical pull on the LES in the cranial direction activates LES relaxation through the activation of inhibitory motor neuron [225]. In mice, with a skeletal muscle esophagus and smooth muscle LES, vagus nerve-stimulated LES relaxation can be blocked by pancuronium (abolishes longitudinal muscles contraction) [91]. It is proposed that the vagus nerve fibers, instead of forming synapses with the inhibitory motor neuron, are actually destined toward the longitudinal muscles, contraction of which in turn activates the stretch-sensitive inhibitory motor neurons of the LES (Figure 18). In support of the above concept, surgical fundoplication (used to treat reflux) restricts cranial stretch as well as the LES relaxation in the rat (Jiang et al. *Gastroenterology*. 2011).

# Crural Diaphragm Contribution to EGJ and Neural Control

Crural and costal, even though part of the same respiratory diaphragms, are actually two separate muscles [226,227]. Costal diaphragm is primarily a ventilator muscle. On the other hand, crural diaphragm has two functions, ventilator and "sphincter-like action on the esophagus." Both parts are supplied by branches of the phrenic nerve, the motor neurons of which are located in the spinal cord at the level of C5–C7 (phrenic nerve nucleus). No topographical localization exists for the neurons of crural and costal diaphragm in the spinal cord [228,229]. Respiratory center located in the reticular formation of medulla innervates the phrenic nerve nucleus in the spinal cord. Recent studies show that vagus nerve also innervates (sensory and motor) crural but not the costal diaphragm [230]; however, its role in the EGJ physiology is not clear. Measuring the contribution of crural diaphragm to the EGJ pressure is challenging in the normal humans (in the absence of hiatal hernia) because LES and diaphragmatic sphincters are anatomically superimposed on each other. Simultaneous pressure and electromyogram (EMG) recording using a reverse perfuse sleeve sensor equipped with electrodes prove the following [194,231,232]: (1) under resting conditions and at end expiration, the EGJ pressure mostly comes from smooth muscle LES, and increase with each inspiration is related to the crural diaphragm contraction (Figure 19). Amplitude of EGJ pressure related to inspiration is directly related to the depth of inspiration. With maximal inspiration, the EGJ pressure increases from 20 mm Hg to more than 100 mm Hg. Crural diaphragm provides tonic or sustained increase in the EGJ pressure during periods of abdominal compression, straight leg raise, and valsalva maneuver. Best evidence for the tonic contraction of the diaphragmatic sphincter comes from a study in patients with a completely absent LES (resected due to cancer at the distal esophagus) [233]. In addition to the inspiratory pressure oscillation at the EGJ, a high-pressure zone also exists at end expiration in these patients. Significance of crural diaphragm to the antireflux barrier is as follows: contractions of the inspiratory muscles of respiration produces negative intraesophageal pressure, thus increasing the pressure gradient between the stomach and esophagus in favor of gastroesophageal reflux. Contraction of the abdominal wall also increases the pressure gradient between stomach and esophagus. Therefore, all involuntary/voluntary maneuvers, which are associated

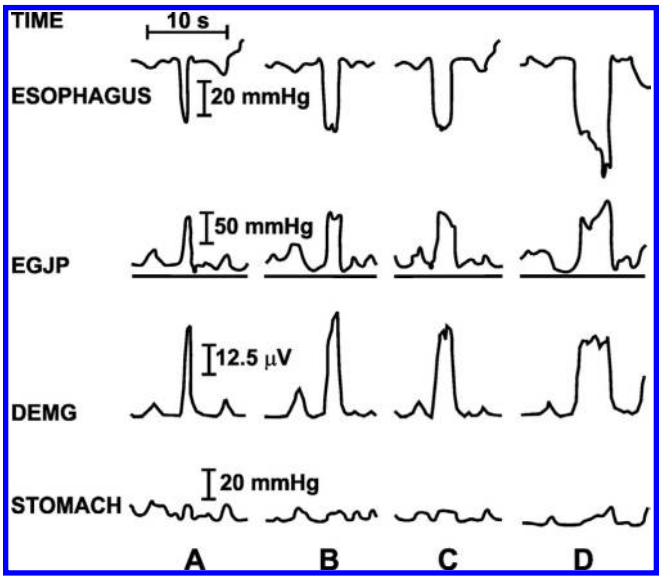


**FIGURE 19:** Diaphragmatic contraction and EGJ pressure. Standardized diaphragmatic contractions of 1, 2, 4 and 6 seconds duration were performed. Note that each diaphragmatic results in a negative esophageal pressure, an increase in the EGJ pressure and an increase in the integrated crural diaphragm EMG activity (DEMG) (from Sivri et al., *Gastroenterology* 1991;101:962–9).

with inspiratory and abdominal wall muscle contractions, and increase in gastroesophageal pressure gradients, are accompanied by augmentation of LES pressure by the crural diaphragm contraction, thus preventing gastroesophageal reflux.

Crural diaphragm relaxes along with the LES during swallow [234] and TLESR [110], less completely with the former than the latter. Fine coordination between the visceral (LES) and somatic (crural diaphragm) control mechanism is suggested to occur in the medullary region, like so many other cardiorespiratory reflexes. However, there was no inhibition of the spontaneously active inspiratory motor neurons with esophageal distension when clearly there was inhibition of the crural diaphragm [235] as demonstrated by the absence of EMG activity. A peripheral mechanism located at the level of crural diaphragm related to the stretch exerted by the esophageal longitudinal muscle contraction has been proposed, but the precise nature of such mechanism is not understood [126,236].

# Transient LES Relaxation and Pharmacological Inhibition

Spontaneous TLESRs occur only in the awake state [237,238], general anesthesia suppresses [239] them, and they are more common in the upright position [240,241]. In the experiment setting, gastric distension is the major stimulus to induce transient LES relaxation [242–244]. Distension-activated stretch on the gastric wall activates afferents in the vagus nerve that elicit TLESR through the central pattern generator, DMV, and efferent vagus nerve (vago-vagal reflex) [245]. Therefore, TLESR is blocked by cooling of the cervical vagus nerve [246]. TLESR frequency actually increases in the presence of a catheter in the pharynx, raising the possibility that pharyngeal receptors may be involved [247]. A subthreshold mechanical pharyngeal stimulus and low-frequency electrical stimulation of the superior laryngeal nerve can elicit isolated LES relaxation (without esophageal contractions) even though its phenotypic appearance is different from that of the TLESR [248]. In humans, a subthreshold pharyngeal stimulus, injections of small amounts of water, induces LES relaxation without crural diaphragm relaxation, a phenotype that also does not resemble TLESR [249,250]. Furthermore, unlike TLESRs, reflux rarely occurs during pharyngeal-stimulated-induced LES relaxations. Large numbers of neurotransmitters are involved in the sensory or afferent limb, motor pattern generator in the brain stem, and efferent motor limb of the TLESR reflex [251]. Therefore, it is possible to interrupt TLESR by many pharmacological agents. GABA b agonist has been consistently shown to inhibit TLESR frequency in the animal [252,253] and human studies [254]. Other pharmacological agents that inhibit TLESR are atropine [255,256], CCK-A receptor antagonist [257], morphine (mu receptor agonist) [258] nitric oxide antagonist, metabotropic glutamate receptor subtype 5 (mGluR5) antagonists [259], and cannabinoid receptor (CBR1) agonist [260]. Keep in mind that all of these agents reduce the frequency of TLESR and spontaneous swallows but do not completely block them. It is likely that these compounds increase the threshold of activation of CPG for swallow and TLESR. Site of the action of these agents is shown in Figure 20 [261]. To date, however, none of these agents have found a place in the treatment of reflux disease, partly because of their side effects profile.

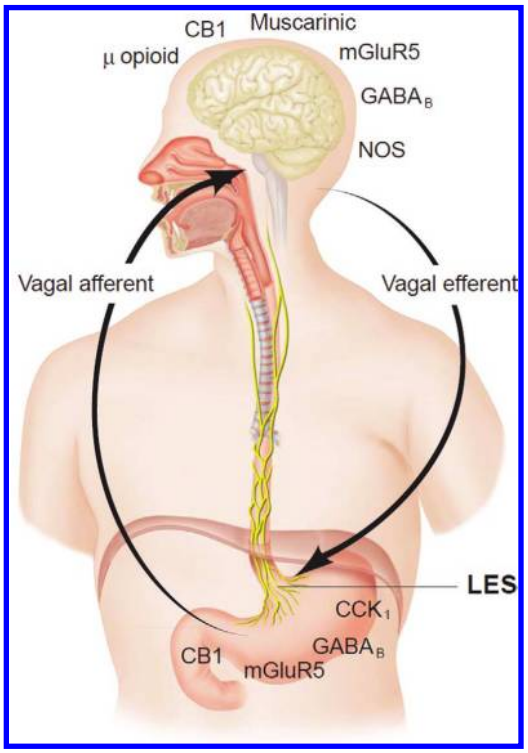


**FIGURE 20:** Site of action of various pharmacologic agents known to inhibit TLESR frequency (from Lehmann, *Esophageal Pain*. Plural Publishing, 2009).

# Compliance of the EGJ

Similar to upper esophageal sphincter, the LES also has distinct relaxation and opening function. X-ray studies with barium swallow show that the bolus arrives at the LES, soon after a swallow, but it does not flow across it in spite of the fully relaxed LES. Increase in esophageal pressure, even though relatively small (3–10 mm Hg), as peristaltic wave pushes the bolus into the distal esophagus, forces open the relaxed LES to the diameter of distal esophagus. Factors that determine EGJ compliance are likely to be different than relaxation because the latter is an active and nerve-mediated process, and the former is a passive viscoelastic property of the tissue-related issue. Opening/compliance function of the LES and EGJ can be studied by distending a balloon and imaging the constriction caused by the EGJ on the balloon using fluoroscopy imaging to determine the pressure–cross-sectional area relationship. Barostat [262], ultrasound imaging of the EGJ [263] and more recently functional luminal imaging probe (FLIP) have also been used [264,265] to study EGJ compliance. Studies show that in normal subjects, the hiatus (crural diaphragm), and not the LES, is the region of least compliance at the EGJ. Patients with reflux disease have a more compliant EGJ than normal subjects [266,267]. Some patients with achalasia esophagus have normal relaxation [268] but poor compliance of the LES [269]. Dysphagia following surgical fundoplication may also be related to poor EGJ compliance [270,271].

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