

ORIGINAL PAPER

Contributions to the micro-anatomical evaluation of the ratio between the muscular tunic structures of the oesophagus. Biomechanical, anatomic and clinical implications

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Abstract

The aim of our research is to make a micronatomical evaluation of the ratios of the structural elements of the oesophagus muscular tunic and suggest a new perspective in the general knowledge about this tubular organ. The visualisation of the structural elements was done using classical microanatomical methods Hematoxylin–Eosin for general orientation, Van Gieson for picrofuxinophile collagen fibers and Gömöri for reticuline fibers. The neurofilaments were identified using silver impregnation on Cajal block. The examination of the sections was made with the Nikon Eclipse 600 photomicroscope. The images were taken and processed using a computer with LuciaNet licensed Microsoft. The co-existence in the architecture of the oesophagus of two microcinematic systems structurally divergent, but functionally convergent represents microanatomical arguments for the nomination of a new type of muscles – the oesophageal muscle – anatomo-functionally different from the skeletal striate muscle, from the smooth visceral muscle, from the cardiac muscle or from the embryonic type muscle of the excitocductor system. The extra cellular matrix of the muscular tunic of the oesophagus contributes to the organization of the endo- and peri-misium, as well as to the three structures involved in the biomechanics of the oesophageal muscle: the connective-vascular septa, the microtendons that have in their structure receptors of the peristaltic reflex and, finally, the lamina propria mienterica strewed with numerous neuronal structures. The co-existence of the stereo-distribution of the monostructured fascicles (skeletal muscular fibers or smooth muscular cells) with bistructured fascicles (skeletal-smooth) gives the oesophagus a heterogeneous architecture. Lamina propria mienterica, which realizes the border between the circular and longitudinal strata, contains two neuronal structures of its own, but anatomically inserted the neural mienteric plexus which is equivalent to the Auerbach plexus and the neuroganglion of the mienteric plexus, equivalent to the visceral neuroganglion Cajal–Retzius.

Keywords: oesophagus, muscular tunic structures, implications.

☐ Objectives

The structural anatomy of the oesophagus has always been under the scrutiny of clinicians, radiologists, physiologists, anatomists and pathologists due to the problems it raised in identifying the factors responsible for the reflexes that push the alimentary bolus. It still presents many unknown and controversial sides [1–3].

We aim to make a micronatomical evaluation of the ratios of the structural elements of the oesophagus muscular tunic and suggest a new perspective in the general knowledge about this tubular organ.

☐ Material and methods

Our study comprised the analyses of 600 seriate sections made on three oesophagi of newly born children and four of adults (30–55 years).

They were treated with Lille solution for the oesophagus sections in the case of the evaluation of the extracellular matrix and 50% chloralhydrate–ethanol solution for the neuronal elements using silver impregnation on Cajal block.

The visualization of the structural elements was done using classical microanatomical methods Hematoxylin–Eosin for general orientation, Van Gieson for picrofuxinophile collagen fibers and Gömöri for reticuline fibers.

The neurofilaments were identified using silver impregnation on Cajal block. The examination of the sections was made with the *Nikon Eclipse 600* photomicroscope in the labs of The Academy Research Center of Medical Sciences, the branch of Craiova.

The images were taken and processed using a computer with LuciaNet licensed Microsoft.

☐ Results

The microanatomical evaluation of the ratios between the oesophagus muscular tunic structures was based on five anatomical functional criteria: the criterion of the appurtenance to the subsystems which integrate the oesophagus with the reflexes of deglutition; the criterion of the microstereotaxic assembling of the structures in the fundamental tunics; the criterion of the ratios and interactions among the

structural elements according to “the axioma of bonds”; the geometric criterion of microstereotaxic analysis and the criterion of “reciprocal induction” in the ortogenesis of the structures.

The microanatomical analysis of the ratio among the structural elements of the oesophagus muscular tunic was made on seriate sections and took into consideration the following parameters: the stereotopographic location of the extracellular matrix collagen, the identification of the connective structures dividing the oesophagus wall, the neuronal elements topography and the evaluation of the miostromal, neuromuscular and neurotendinous relations in the layers of the muscular tunic.

The microanatomical analysis of the muscular tunic integration into the oesophagus architecture

The study of the oesophagus architecture depends primarily on the general stereo-distribution of the subsystems in the oesophagus wall.

From the microanatomical analysis of the seriate sections and the images of reconstruction in plane, we could easily differentiate the circumferential topography of the tunics of the oesophagus wall: mucosa tunic, tela submucosa, muscularis tunic and adventitia.

In *mucosa tunic* we identified three structural units: epithelium, lamina propria mucosae, lamina muscularis mucosae under which is tela submucosa. In *muscularis tunic* the circular and longitudinal microcinematic layers can be very well seen.

This micronatomical stereotaxic analysis was necessary to identify the constitutive elements of the oesophagus wall and make their structural analysis.

The micro-anatomic analysis of the conjunctive structures that delimit the compartments of the oesophagus

We identified and nominated four structures that participate in the construction of the borders of the oesophageal wall: the subepithelial basal membrane, the propria mucosae lamina, tela sub mucosa and the mienteric lamina.

The subepithelial basal membrane appears like an obvious line visible on the sections colored with picrofuxine according to the Van Gieson method and located between the epithelium and the lamina propria mucosae.

In the profound part, the subepithelial basal membrane appears unified by the subadjacent connective tissue through an ample net of reticulum fibers.

The second connective formation taken into consideration for the construction of the compartments of the oesophageal wall is lamina propria mucosae. It appears located between the epithelium and lamina muscularis mucosae and it is made of picrofuxinofile collagen fibres distributed under the form of a net containing meta-arterioles and capillaries with longitudinal trajectories.

Lamina propria mucosae penetrate deep in the papilla delimited by the basal stratum of the mucosa

epithelium and is crossed by thick fascicules of reticulin fibers, and in some places, are intersected under variable angles.

The third connective formation used for the delimitation of the compartments of the oesophagian wall is tela submucosa that separates two miocinematic structures – lamina muscularis mucosae and tunica muscularis.

The fourth connective formation was identified as a connective structure located between the structures of the muscular tunic (circular and longitudinal) and nominated as lamina propria muscularis (syn. lamina mienterica).

The micro-anatomic analysis of the ratios between the structures of the muscular tunic

We've noticed the presence at this level of a great quantity of neuro-vascular-connective tissue that realizes the separation of the muscular strata within the muscular tunic. We nominated this formation as “lamina propria muscularis”.

Inside each stratum we identified stripes of collagen fibers that circumcise the muscular fascicles (Figure 1 A).

On the entire inferior half of the muscular tunica, we've noticed the alternation of skeletal muscular fascicles with smooth muscular fascicles (Figure 1 B).

At the intersection of several muscular fascicles we have identified condensations of collagen fibers that produce genuine micro anatomic tendons containing neuro-receptors of the neurotendinous unit.

We took into consideration two types of relations and interactions: miostromal and neuromuscular. We have analyzed micro anatomically the miostromal relations and interactions on the seriate sections made in a transverse plane through the medium third and the inferior third of the oesophagus.

In the medium third of the oesophagus, we have noticed the simultaneous existence of the fascicles of the skeletal muscle and of the smooth muscle, delimited by the connective-vascular tissue.

In the proximity of the skeletal muscular fascicles we have easily identified the presence of the neuromuscular unit (Figure 1 C).

Numerous nervous structures of variable sizes and trajectories cross the territories of the muscular tunica strata (Figure 1 D).

The spaces between the muscular fascicles contain arterioles sectioned under variable angles determined by the helicoid trajectory of the vessels. These are accompanied by fascicles of collagen fibers that realize the connection between the vascular adventitia with the miocinematic elements. In the inferior third of the oesophagus we have remarked the numeric and volumetric increase of the fascicles of the smooth muscle. At a micro anatomic level, the stereotaxic replacement of the skeletal muscle is realized (Figure 2).

The relations and neuromuscular interactions have been studied through the synchronic analysis of the neuronal elements: nervous branches, fascicles of neurofibrils and fibrils.

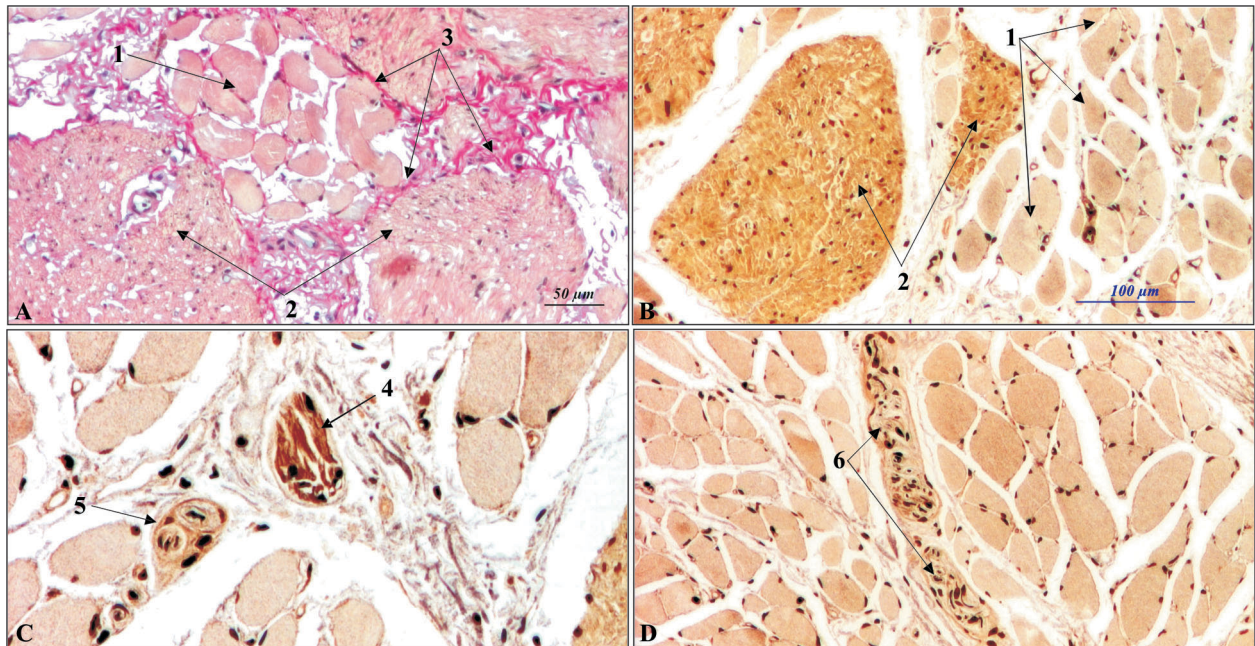


Figure 1 – A. The muscular tunic of the oesophagus contains pencils of muscular fibers (skeletal) (1) and pencils of muscular smooth cells (smooth) (2), separate by an extracellular matrix rich in picrofuchsinophilic collagen fibers (3); B. In the lower half of the oesophagus there is the coexistence of both skeletal (1) and smooth (2) fibers; C. The connective pencils achieve in intermuscular spaces microtendons with neurotendinous spindle (4), and in the proximity of a muscular pencil the presence of neuromuscular spindle (5); D. There are numerous nervous branches longitudinally crossed in muscular interstitia (6) (Van Gieson – A; silver impregnation on block Cajal – B, C, D; oc. $\times 7$, ob. $\times 10$ – A, B, D, and $\times 20$ – C; ob. $\times 70$ – A, B, D, and $\times 140$ – C)

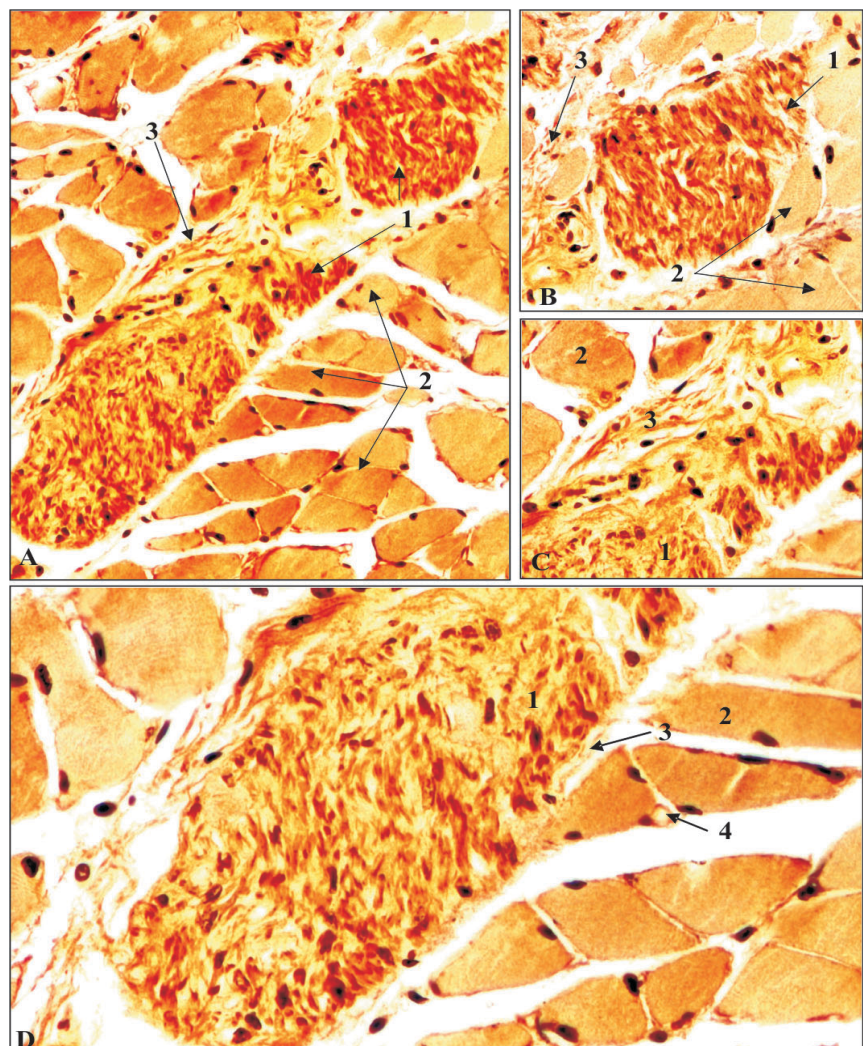


Figure 2 – Implementation of smooth muscular fascicles (1) in the interstitium delimited by skeletal muscular fascicles (2). There are contiguity rapports of both muscular structures by thin bands of connective tissue (3). Skeletal muscular fibers has rapports by sarcolemma with muscular sinusoidal capillaries (4) (Silver impregnation on block Cajal; oc. $\times 7$, ob. $\times 10$ – A, B, C, and $\times 20$ – D; $\times 70$ – A, B, C, and $\times 140$ – D)

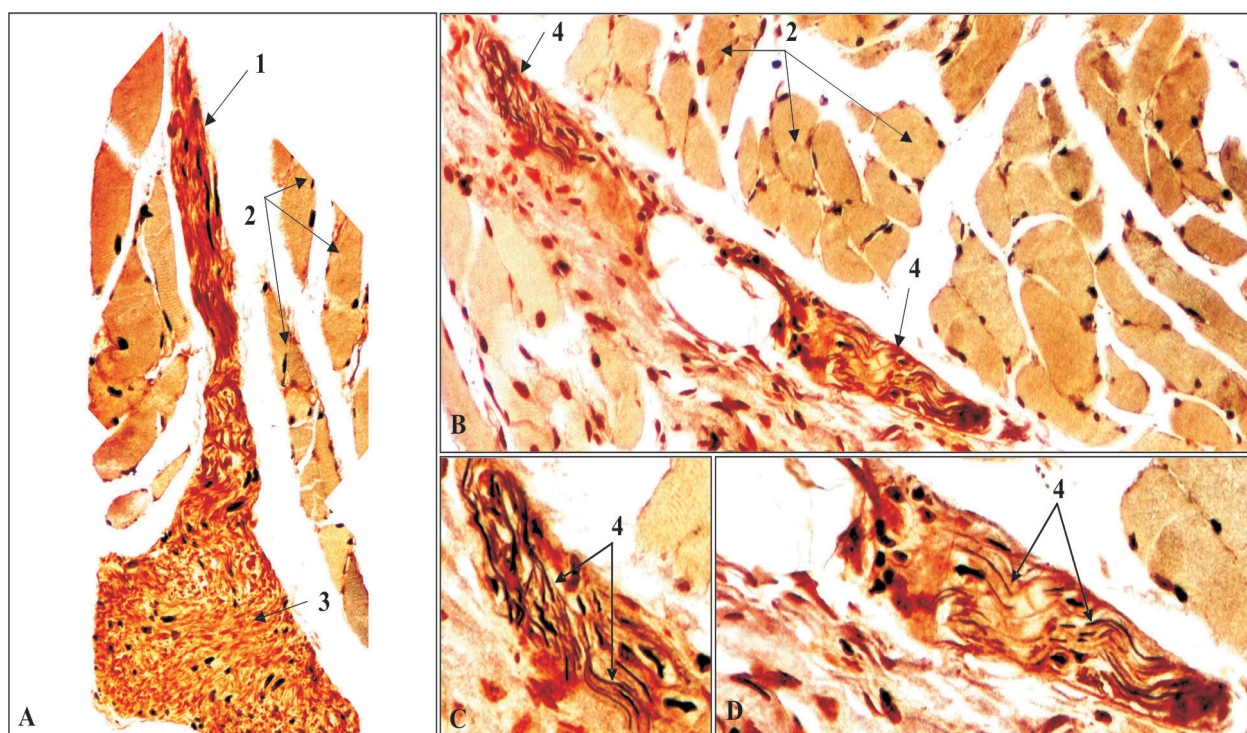


Figure 3 – A. Nervous branches longitudinally crossed (1) in the interstitium between skeletal muscular fascicles (2) and in continuity rapports with a smooth muscular pencil (3); **B, C, D.** The exam with ob. $\times 20$ reveals numerous neurofibrils (4) with sinusoidal and parallel trajet, in the structure of nervous branches (Silver impregnation on block Cajal; oc. $\times 7$, ob. $\times 20$, and $\times 140$)

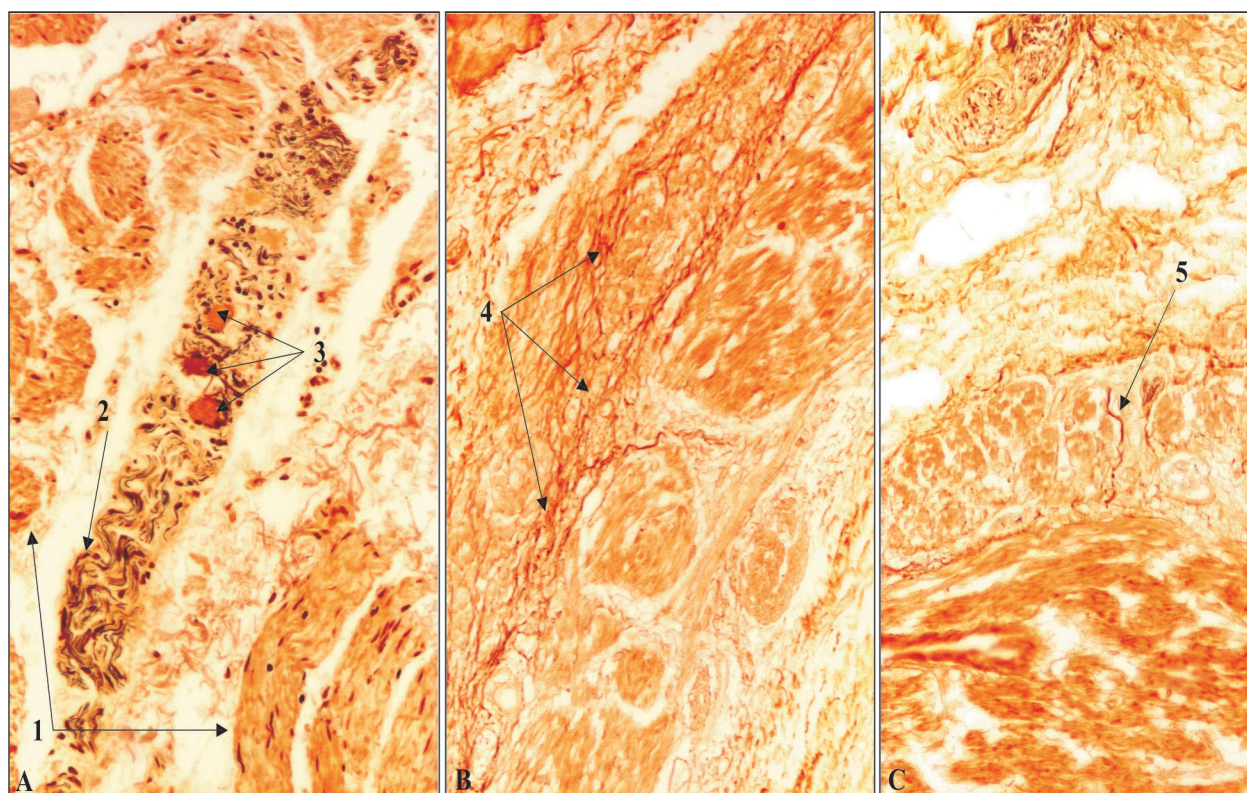


Figure 4 – A. In lamina propria muscularis (1) it is easy to observe the presence of a nervous structure longitudinally sectioned (2) which has continuity rapports with a neuroganglion, which contains multipolar neurons (3); **B.** At the limit between the circular and longitudinal layers of the muscular tunic it is a nervous plexus (4), which contains muscular fascicles; **C.** In the longitudinal layer there are interstitial cells Cajal (5) (Silver impregnation on block Cajal; oc. $\times 7$, ob. $\times 4$ – B, $\times 10$ – A, and $\times 20$ – C; $\times 28$ – B, $\times 70$ – A, and $\times 140$ – C)

The nervous branches appear in the intramuscular spaces, either on longitudinal sections, or on transversal ones (Figure 3).

The microtendons are crossed by fascicles of neurofibrils. The sarcolemma of the skeletal muscular fibers is tangent at the neurofibrils visualized through the method Cajal of silver impregnation on the bloc.

In the lamina propria mienterica we highlighted two neuronal structures: a nervous plexus and a neuroganglion annexed to a nervous structure (Figure 4 A).

We have also identified neurofibrils with helicoidal trajectory around the fascicles of the muscular cells. Numerous neurofibrils, distributed around the circular muscular fascicles, realize a perimuscular luxuriant net (Figure 4 B).

Interstitial cells of type Cajal were visualized in the longitudinal stratum of the muscular tunica (Figure 4 C).

☐ Discussions

Two major functions are recognized for the muscular tunic: on the one hand, the transport of the alimentary bolus, and on the other, the prevention of the oesopharingian or gastro-oesophageal retrograde flux.

The muscular tunic gives the oesophagus the particularities of its integration within the importing system of the matter.

Numerous questions have been asked about it, but the answer contains many controversies:

- why is the presence of two types of muscular tissue in the oesophageal wall necessary with human beings: skeletal-voluntary ?;
- what is and how is the propulsion in the case of the oesophagus realized ?;
- is there an organization of the oesophagus muscular structure in "motility units" ?;
- which is the mechanism through which the alimentary bolus crosses the cavity of the oesophagus ?;
- which of the neuronal structures located in the strata of the muscular tunic have motor function and which have a sensitive function?;
- by what mechanisms are the superior and inferior orifices of the oesophagus closed or opened ?;
- why in the mienteric plexus of the oesophagus are there neurofibers with myelin support and in the mienteric plexus of the intestine there are only neurofibers without myelin support ?;
- if we admit that the propagation of the contraction wave along the oesophagus is the result of the sequential exercitation of some dynamic successive units, do there exist any structures with functions of automatism in the wall of the oesophagus ?

The microanatomical analysis of the relations and reciprocal interactions of the structural elements of the oesophageal wall highlighted numerous controversies on:

- the stereodistribution of the types of the muscular fascicles (skeletal and smooth);
- the neuromotor systems implied in the biocinematics of the oesophagus and, last but not least,

- the biomechanics of the propulsion of the alimentary bolus.

It is known that with dogs and rabbits the musculature of the oesophagus is of the striate type. Although with humans it is accepted that the oesophagus contains two types of muscles in the muscular tunica (skeletal and smooth), there still are controversies on their stereo-distribution and their geometric location.

Their reference areas vary, according to the author: some authors divide the oesophagus into three sectors nominated as superior third, medium third and inferior third, and others into four sectors called fourths.

The variability of the stereo-distribution of the muscular fascicles with human beings can explain the existent discordance between the classical and contemporary descriptions. Some authors have noticed the presence of the skeletal muscular fascicles only in the superior third and of the smooth ones in the medium and inferior thirds [1, 4].

Other authors have inversed the above mentioned relations and described the presence of the skeletal muscle in the two superior thirds and of the smooth muscle in the inferior third [5–7].

Numerous authors remarked the absence of a rigor in delimiting the distribution limits of the two types of muscular fascicles. They remarked the presence of the striate muscle only in the first fourth of the superior half and of the smooth muscular fascicles only in the inferior half of the oesophagus. The authors notice that in the second fourth of the superior half there exists a gradual replacement of the skeletal muscle with the smooth one [1, 8, 9].

Later this conception was adopted by numerous contemporary authors [8, 10–12].

In describing the neuromotor systems there are controversies, too, among anatomists on the one hand and physiologists, on the other. An important contribution in the nuance and variation of the innervations of the oesophageal musculature was brought by considering the sympathetic system as the principal source of neurofibrils for the construction of the oesophageal lumen, and the vague nerve as the main opposite nerve [1].

From the topographical point of view, a topographical detachment of the oesophageal innervations has been noticed: the tracheal part is innervated by the recurrent nerve and the pharyngeal nerves, and the infrabronchial part, innervated by the vague nerves [1].

A new neuronal system has recently been nominated as "neoronal enteric system" and it contains elements of the intrinsic innervation: the mienteric plexus, the sub mucous plexus and the visceral ganglions.

Remarkable progress has been made in the neurotransmitters area, so as at the moment it is considered that there exists an enteric neuronal excitatory system by which the contraction of the muscular fascicles is realized with two neurotransmitters – acetylcholine and substance P – and an inhibitory enteric neuronal system of the intestinal

tube musculature by the intervention of two neurotransmitters— vasoactive intestinal peptid (VIP) and the nitric oxide (NO). From the bio-cinematic point of view, two contractile units can be taken into consideration at the level of the oesophagus: the unity of propulsion of the content of the oesophageal cavity and the sfincterian unities for closing and opening of the pharyngo-oesophageal and oesogastric orifices.

Remarkable progress has been made by considering the registration values of the intraoesophageal pressures and of their determination during the deglutition phases: 100 cm water, in the oral phase; 40 cm water in the pharyngo-oesophageal junction and 40–160 cm water in the oesophageal phase [13, 14].

By the study of the stereo-distribution of the miocinematic structures we highlighted two important phenomena: the participation of the muscular strata in the morpho-differentiation of the oesophagus and their implementation in the realization of the variable morphology of the oesophagus in its functional dynamics and ontogenesis. We have considered that the muscular tunica has a heterogeneous stereo-topography, both from the micro-structural and micro-stereo-topographical points of view.

The internal stratum of the muscular tunica, wrongly called stratum circulare, actually contains fascicles of muscular cells (smooth) that inscribe either on ellipsoidal routes or on circular routes with reduced obliquity. The external stratum of the muscular tunic, called stratum longitudinale, contains in the two inferior thirds a combination of striate and smooth muscular fascicles. The multipolar neurons from lamina propria muscularis have a compact distribution. The interstitial Cajal type cells are involved in the automatism of the esophageal wall [15].

From the analysis of our observations, it results that the mechanical progression of the alimentary bolus is assured by the contraction of the muscular structures upstream the alimentary bolus and the relaxation of those downstream.

In conclusion, there exists a temporal dissociation of the contractions and relaxations of the motor units: the upstream contraction has two effects and these are the impediment of the reflux of the alimentary bolus and its progression in the oesophageal cavity.

It is very difficult to realize an argumentation if the visceral ganglionar neurons have sensitive neurons, of association or motor, although it is claimed that they would have a sensitive function, the motricity being assured by the neurofilaments from the vague nerve. The dissociation of the innervations was continued, and it was affirmed that the sub mucous plexus and the mienteric one ensure the intrinsec innervations of the structures of the oesophageal wall.

It is important to remark the contribution of the pharmacodynamics research on the differentiation of the smooth musculature of the skeletal musculature, and it was demonstrated that atropine blocks the peristaltic answer at deglutition in that part of the oesophagus where the smooth musculature is predominant and curare has the same effect in that part with skeletal musculature.

Conclusions

The co-existence in the architecture of the oesophagus of two microcinematic systems structurally divergent, but functionally convergent represents microanatomical arguments for the nomination of a new type of muscles – the oesophageal muscle – anatomo-functionally different from the skeletal striate muscle, from the smooth visceral muscle, from the cardiac muscle or from the embryonic type muscle of the excitocductor system.

The extra cellular matrix of the muscular tunic of the oesophagus contributes to the organization of the endo- and peri-misium, as well as to the three structures involved in the biomechanics of the oesophageal muscle: the connective-vascular septa, the microtendons that have in their structure receptors of the peristaltic reflex and, finally, the lamina propria mienterica strewn with numerous neuronal structures.

The co-existence of the stereo-distribution of the monostructured fascicles (skeletal muscular fibers or smooth muscular cells) with bistructured fascicles (skeletal-smooth) gives the oesophagus a heterogeneous architecture.

The progression of the alimentary bolus is possible through the tempo-spatial succession of the constrictions and dilatations of the oesophageal lumen, by the activities of the “oesophageal muscle” which acts like a “multiunitary neat muscle”.

Lamina propria mienterica, which realizes the border between the circular and longitudinal strata, contains two neuronal structures of its own, but anatomically inserted the neural mienteric plexus which is equivalent to the Auerbach plexus and the neuroganglion of the mienteric plexus, equivalent to the visceral neuroganglion Cajal–Retzius.

The peristaltic wave is a “tempo-spatial image” of the succession of contractions of the upstream muscular structures and of relaxations of the downstream structures generated in a reflex way, through the transit of the oesophagus by the alimentary bolus.

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