A Light Microscope Study of the Distribution of Muscle in the Frog Esophagus and Stomach

Masahide YOSHIDA

Laboratory of Biology, Kanagawa Prefectural College of Nursing and Medical Technology, 1-5-1 Nakao, Asahi-ku, Yokohama, 241-0815, Japan

Abstract

The present study reports light microscopical observations of the distribution of muscle in the esophagus and stomach of both the bull frog (Rana catesbeiana) and the African clawed frog (Xenopus laevis). The external muscle coat of the upper half of the esophagus in both species had several collagen coated bundles of striated muscle fibres around the circumference. These striated muscle bundles ran longitudinally from the pharynx to around the vicinity of the center of the esophagus. Beneath these striated muscle bundles was an inner circular layer of smooth muscle. In both species, the inner circular layer of smooth muscle was particularly thick in the region close to the pharynx. In the bull frog, the lower half of the esophagus lacked striated muscle. However, the circular smooth muscle layer, extending from the upper half of the esophagus, was also observed throughout the lower half of the esophagus. An outer longitudinal layer of smooth muscle developed towards the terminal portion of the esophagus such that in this region, both outer longitudinal and inner circular layers of smooth muscle were observed. Similarly in the African clawed frog, the inner circular layer of smooth muscle was continuous along the full length of the esophagus. Again, no striated muscle bundles were observed in the lower half of the esophagus. However, the outer longitudinal layer of smooth muscle was seen to develop in the middle region of the esophagus. Its muscle layer extended to the terminal portion of the esophagus. Thus, both outer longitudinal and inner circular layers of smooth muscle were observed throughout the lower half of the esophagus. In both frogs, the thickness of the outer longitudinal and inner circular layers of smooth muscle changed before and after the esophago-gastric junction. In both frogs, no muscularis mucosa was observed in the esophageal wall. However, in the lower half of the esophagus of the African clawed frog, small bundles of smooth muscle were observed here and there in the submucosa. A fully developed muscularis mucosa with both outer longitudinal and inner circular layers was observed in the upper stomach of both frogs.

Key words: rana catesbeiana, xenopus laevis, esophagus, muscle
Introduction

In the human esophagus, the proximal one-third of both longitudinal and circular layers of the muscularis propria consist wholly of striated muscle, whereas the remainder is smooth muscle (Christensen, 1975). The distribution of striated and smooth muscle in the vertebrate esophagus is reported to vary between species (Oppel, 1897). It is also stated that some marsupials and primates, for example, show the same distribution as in the human esophagus, while in amphibians, birds, and reptiles the esophagus is stated to consist wholly of smooth muscle. It is added that only the distal fifth or less of the esophageal muscularis propria is smooth muscle in the dog, guinea-pig, rabbit, rat, and the like. On the other hand, Oppel (1897) reports that the muscularis mucosa in all vertebrates studied consists of smooth muscle. Since this early work, there do not appear to have been any further reports of the morphology of amphibian esophageal muscle. Moreover, the muscle in the vicinity of the esophago-gastric junction of the frog has not been reported. This paper reports a light microscopic study of the distribution of muscle from the pharynx to the upper stomach in two species of frog.

Materials and Methods

Specimens of the bull frog *Rana catesbeiana* (3 females and 3 males, weighing 250–300 g) and of the African clawed frog *Xenopus laevis*, (3 females and 3 males, weighing 45–55 g) were used in this study. The upper alimentary canal from the pharynx (together with part of the buccal cavity) to the duodenum was quickly excised (Fig. 1). Adherent tissue around each preparation was carefully removed while the preparations were immersed in Ringer solution, using a pair of scissors and a pincette. The preparations were then immediately fixed with 10% neutral buffered formalin solution. From the preparation from *Rana*, the esophagus was excised together with part of the buccal cavity at the proximal end and approximately one-fifth of the stomach at the distal end. Similar trimming was made of the *Xenopus* preparations except that it included approximately one-third of the stomach. These excised preparations were then embedded in paraffin wax and sectioned at 8 µm thickness. After that, sections were usually stained with Mallory-Heidenhain’s azan stain. A few sections were stained with Pearse’s phosphotungstic acid haematoxylin (PTAH) stain in order to highlight the striations of striated muscle. Observations were made under a light microscope.

Results

Gross anatomy

Bull frog: The length of the esophagus ranged from 6–8 mm, although it was frequently difficult to determine the esophago-gastric junction due to muscular contraction of the preparation (Figs. 1-1, 1-2, 1-3). The esophagus opened into the stomach that expanded with a slight curve, with a thick wall at the fore part (esophageal end), but was somewhat thinner at the rear (duodenum end), with a constriction at the junction with the duodenum.

African clawed frog: The length of the esophagus was approximately 10 mm (Fig. 1-4). The
esophagus opened into the stomach that was egg-shaped or spherical, thin walled towards the duodenal end, with a constriction at the junction with the duodenum (Figs. 1-4, 1-5, 1-6).

Light microscope observations

In both the bull frog (Figs. 2 and 3) and the African clawed frog (Fig. 4), the composition of the external muscle of the esophagus showed variation between regions which were composed of
striated muscle and circular smooth muscle, only circular smooth muscle, or longitudinal and circular smooth muscle. The lumen of the esophagus was lined by a deeply folded mucosa (Figs. 2-1, 2-3). By the azan staining method, striated muscle and smooth muscle were stained red purple or red, and supporting tissues, that is, collagenous tissues, were stained blue (Figs. 2, 3).

A fuller description of the evidence for the presence of both striated muscle and smooth muscle will be described in III below. A detailed description of the distribution of both types of muscle in each species follows.

**Distribution of muscle in the wall of the esophagus and stomach of the bull frog**

a) The upper half of the esophagus: Outer bundles of striated muscle fibres and an inner thick circular layer of smooth muscle were observed around the entrance of the esophagus close to the pharynx (Figs. 2-1, 3-1). The striated muscle was observed intermittently down to the middle of the esophagus (Figs. 2-2, 2-3, 3-2). The thick circular layer of smooth muscle decreased in thickness down to the middle of the esophagus and then maintained this thickness as it extended into the lower esophagus (Figs. 2-2, 2-3). A muscularis mucosa was not observed in the upper half of the esophagus.

b) The lower half of the esophagus: Striated muscle was not observed on the outside of the muscularis externa (Fig. 2-4). The circular layer of smooth muscle, extending from the upper half of the esophagus, was observed throughout the lower half of the esophagus. An outer longitudinal layer of smooth muscle was, however, observed towards the terminal portion of the esophagus (Figs. 2-4, 3-3, 3-4). Thus, in the vicinity of the terminal portion of the esophagus, both outer longitudinal and inner circular layers of smooth muscle were observed. A muscularis mucosa was not observed (Fig. 2-4).

c) The esophago-gastric junction and its vicinity: On the esophageal side, there were outer longitudinal and inner circular layers of smooth muscle. The latter was a little thicker than in the middle of the esophagus (Fig. 2-4). On the gastric side, the thickness of the outer longitudinal layer of smooth muscle was similar to that in the esophageal end. By contrast, the inner circular layer of smooth muscle was thinner on the gastric side than at the esophageal end (Fig. 2-4). On the gastric side, gastric glands were observed, but a muscularis mucosa was not observed in the submucosa (Figs. 2-4, 3-3). From this region, the thickness of the wall of the alimentary tract increased gradually or rapidly as shown in Fig. 1.

d) Stomach: Both outer longitudinal and inner circular layers of smooth muscle became thicker as the diameter of the stomach increased (Fig. 2-5). Gastric glands also filled the submucosa (Fig. 2-5). Further, a muscularis mucosa was not observed (Figs. 2-5). A muscularis mucosa gradually appeared around the region at which the diameter of the stomach became approximately maximal (Figs. 2-6, 2-7, 3-5). The muscularis mucosa consisted of both longitudinal and circular smooth muscle layers (Figs. 2-8, 3-6).

**Distribution of muscle in the wall of the esophagus and stomach of the African clawed frog**

a) The upper half of the esophagus: The outer bundles of striated muscle and the inner thick circular layer of smooth muscle of the muscularis externa were observed around the entrance of the esophagus close to the pharynx. The striated muscle extended down to the
Fig. 2. Micrographs of representative regions observed in longitudinal sections of the esophagus and stomach of the bull frog (Azan staining): 1 and 2: the esophagus at the pharyngeal end; 3: the esophagus mid-way between the esophagus and stomach; 4: the esophago-gastric junction; 5: the fore part of the stomach (where the diameter of the stomach is increasing); 6 and 7: the stomach (at the point of maximum stomach diameter); 8: the muscularis mucosa in the fore-part of the stomach at higher magnification. Calibration Bar in 1–7 is 500 µm, in 8 is 100 µm. CM, circular layer of smooth muscle; CT, collagenous tissue; GG, gastric gland; LM, longitudinal layer of smooth muscle; M, mucosa; MM, muscularis mucosa; ST, striated muscle; SM, submucosa.
middle of the esophagus (Fig. 4-1), whereas the thick circular layer of smooth muscle again gradually decreased in thickness as it extended into the lower esophagus. No muscularis mucosa was observed in the upper half of the esophagus (Fig. 4-1).

b) The lower half of the esophagus: The lower half of the esophagus lacked the outer bundles of striated muscle seen in the upper half. The inner circular layer of smooth muscle continued from the upper half of the esophagus through the length of the lower esophagus. The outer longitudinal layer of smooth muscle gradually appeared in the mid-region of the esophagus (Fig. 4-2) and extended to the terminal portion of the esophagus. Thus both outer longitudinal and inner circular layers of smooth muscle were observed throughout the lower half of the esophagus (Figs. 4-2, 4-3). Moreover, additional small bundles of the smooth muscle were sporadically observed in the mucosa in the lower half of the esophagus (Figs. 4-2, 4-3, 4-4), but there was no clearly defined muscularis mucosa.

c) The esophago-gastric junction and its vicinity: The thickness of the circular layer of smooth muscle on the gastric side was distinctly greater than that on the esophageal side (Fig. 4-5). On the esophageal side, additional small bundles of smooth muscle were observed along
the mucosa (Fig. 4-5). On the gastric side, the muscularis mucosa was observed along the gastric glands in the submucosa (Fig. 4-5).

d) Stomach: There were outer longitudinal and inner circular layers of smooth muscle (Fig. 4-6). Moreover, the muscularis mucosa was observed along the gastric glands in the submucosa, with both longitudinal and circular smooth muscle layers (Fig. 4-6).
Evidence for the presence of striated muscle in the wall of the esophagus

In the bull frog, around the outside of the muscularis externa there are a number of muscle bundles which are invested in collagenous sheaths (Figs. 2-1, 2-3, 3-1, 3-2). The muscle fibres within these muscle bundles appear to be cut longitudinally, transversely or obliquely (Figs. 2-1, 2-3, 3-1, 3-2) when sectioned either longitudinally or transversely. Consequently, it would appear that these muscle bundles are oriented in irregular directions. In transverse sections of the upper half of the esophagus, a number of such muscle bundles are observed around the circumference of the esophagus. Thus it would appear that these muscle bundles containing striated muscle fibres run longitudinally from the pharynx to the middle of the esophagus. In the African clawed frog, similar sheathed muscle bundles were observed with the same distribution and orientation (Fig. 4-1).

In both frogs, PTAH staining confirmed that the connective tissue ensheathed muscle bundles were composed of striated muscle fibres. PTAH staining did not show any striations in the smooth muscle layers of the muscularis externa, but clearly demonstrated distinct striations in the cells within the ensheathed muscle bundles (Fig. 5).
Discussion

In the frog esophageal wall, bundles of striated muscle which are invested in a collagenous sheath are found on the surface of the muscularis externa of the upper half of the esophagus (Figs. 2–5). The results presented above confirm that the tissue is striated muscle. The layers of muscle which form the bulk of the muscularis externa were not invested in a collagenous sheath and showed no evidence of striations. This muscle is clearly smooth muscle.

The muscle of the esophageal wall is usually dispersed as an inner circular layer and an outer longitudinal layer. Christensen (1975) describes that, in human esophagus, the proximal one-third of both longitudinal and circular layers of the muscularis propria consists wholly of striated muscles, whereas the remaining muscle is smooth muscle. In addition, Fujita et al. (1984) describes that the middle one-third of the human esophagus consists of a mixture of both striated and smooth muscle. The distribution of these two types of muscle in the esophagus differs among various vertebrate lineages (Oppel, 1897). Some marsupials and primates, for example, show arrangements common to that of the human, while the esophagus consists of wholly smooth muscles in amphibians, birds, and reptiles (Oppel, 1897). Only the distal fifth or less is smooth muscle in the dog, guinea-pig, rat, and the like (Christensen, 1975).

The longitudinal and circular smooth muscles observed in the present study correspond to the smooth muscle described by Oppel (1897). However, the striated muscle bundles invested in their collagenous sheaths which extend from the pharyngeal end to the middle of the esophagus, have been reported for the first time in the present study. It has also been described that while the muscularis mucosa observed in the vertebrate esophageal wall is also smooth muscle, it is found as a weak muscle layer only in the lower esophageal wall of the frog (Oppel, 1897). In the present study, a muscularis mucosa was not observed in the esophageal wall of either species of frog (Figs. 2–4). However, scattered small bundles of smooth muscle were observed here and there along the mucosa of the lower half of the esophagus of the African clawed frog (Figs. 4-2, 4-3, 4-4). While these may not represent a fully defined muscularis mucosa, further detailed morphological study is needed of the distribution of smooth muscle in this region. In both the bull frog and the African clawed frog, the muscularis mucosa along the gastric glands in the submucosa of the stomach, seemingly consisting of both longitudinal and circular smooth muscle layers, gradually became a significant feature with distance from the esophagus (Figs. 2, 3, 4). In both frog esophagus, gastric glands were not observed.

The thick circular layer of smooth muscle, present in the vicinity of the pharynx, extended toward the lower part of the esophagus with a gradual decrease of its thickness (Fig. 2-2). The thickness of smooth muscle layers in the esophagus of the bull frog and the African clawed frog varied before and after the esophago-gastric junction. In mammals, an upper sphincter and a lower sphincter are present in the esophagus (Ingelfinger, 1958; Christensen, 1975). The abovementioned thick circular layer of smooth muscle in the vicinity of the pharynx may play a role in the tonic closure of the upper sphincter. The change in the thickness of the muscle layers before and after the esophago-gastric junction may be associated with the tonic closure of the lower sphincter. It is described that the muscularis propria of the human esophago-gastric junction does not form a thick anatomical sphincter, but that there is a physiological sphincter.
mechanism (Burkitt et al., 1993). Moreover, it is possible that in the frog the outer striated muscle bundles might be related to the action of swallowing. Therefore, further studies are needed on the possible functions of both these putative sphincter muscles and the striated muscle bundles in the frog esophagus.

In conclusion, on the basis of the current study it is not possible to conclude that the striated muscle bundles that have been described have a structural or functional role in the frog esophagus. Neither is it possible to conclude that a muscularis mucosa is absent in frog esophagus. But this study provides a much clearer understanding of the distribution of muscle along the length of the esophagus and also in the stomach of these two species of frog.

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(Received September 7, 2001: Accepted October 23, 2001)