

Comparative Histological Studies of the Esophagus Wall of *Oryctolagus cuniculus* Rabbit Adult, Young and Lactating Using Light Microscope

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Abstract

Background: As the different parts of the alimentary canal, the esophagus is an important part of it, because it facilitates the passage of food material to the stomach. The purpose of this study was to compare the structure of the esophageal wall of rabbit before weaning (lactating) and after weaning (young and adult) to determine the structural changes that accompany the changes of the feeding pattern from lactating to adult.

Result: we collected fifteen rabbit. Five animals were randomly choised from each stage (young after weaning, young before weaning and adult). Esophagus were removed after anesthesia, processed and examined on light electron microscope. Histologically, the esophageal wall of *Oryctolagus cuniculus* composed of the four layers as any other mammals. mucosa with stratified non keratinized squamous epithelium was observed and it give strong reaction with (PAS) in adult rabbit while in lactating ones it give negative reaction and moderate reaction in young rabbits. Muscularis mucosa layer was absent in lactating rabbit.

Conclusion: From these studies, we observed some differences than other mammals may be related to the physiological pattern and behavior of feeding.

Keywords: Esophagus; Rabbit; Lactating; Young-adult; Light microscope; Histochemical studies

Introduction

Many previous authors describe the digestive organs of other mammals such as Egyptian bats in which showed some differences between them [1,2].

In vertebrate animals the secretion from the gastrointestinal wall contains mucoid substances [3-9]. The histological structure of the esophagus rabbit and guinea pig incomplete. Therefore, the present study was concentrated on rabbit and rat with different stages. The esophagus wall has four layers from inside to outside (mucosa, submucosa, muscularis and serosa). The mucosa is composed of three layers, the stratified squamous epithelium, a lamina propria and a lamina muscularis. The keratinization is different in different mammals in pig and in ruminants.

In African Giant Rats (AGRs), the epithelial lining of the esophagus was non keratinized stratified squamous cells. The mucous glands were absent. Give with Alcian Blue (AB), Periodic Acid Schiff (PAS) and Alcian Blue-Periodic Acid Schiff (ABPAS) negative [10].

The present work aims to illustrate the differences in the histological structure of the esophagus wall of rabbit through lactating and after weaning by the use of light microscope.

Materials and Methods

Animals

Fifteen animals of *Oryctolagus cuniculus* collected alive from Abu-Rawash, Giza Governorate. Five animals were lactating and depend only on suckling milk from their mothers average weighs were 257 g. five animals were after weaning but before maturity feed on different kinds of food and had free access to water. Their weighs were about 1.0 kg. Five adult animals also were procured also feed on different kinds of food and had free access to water. Their weighs average was 1.99 kg.

Histological examination

Animals were anesthetized and esophagus of adult, Young, and lactating animals were removed. The esophagus were fixed in 10% neutral buffered formalin for 24 h. Dehydrated in ascending grades of ethyl alcohol, cleared by xylene and embedded in paraffin. Sections of 5 μm thickness were mounted and stained with Haematoxylin and Eosin method [11].

Histochemical examination

Periodic Acid Schiff procedure (PAS). And azan stains used [12].

Statistical analysis

Statistical presentation and analysis of the present study was conducted, using below.

Mean value $\left(\bar{x}\right)$: the sum of all observations divided by the number of observation:

$$\left(\bar{x}\right) = \frac{\sum x}{n}$$

Where $\left(\bar{x}\right)$ = mean value,

$\sum x$ = The sum of individual observations,

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n=number of observations.

Standard deviation [SD]: It measures the degree of scatter of

individual varieties around their mean:
$$SD = \sqrt{\frac{\sum |x - \bar{x}|^2}{n - 1}}$$

Where $\sum(x-x)$ is the sum of squares of the difference between each observation (X) and the mean value of all observations(X)

Standard student “t test”: test of significance of the difference between two means:

The calculated “t” was compared with tabulated one at different levels of significance at the degree of freedom (DF):

$$DF = (D + n_2) - 2$$

Where:

X=The mean value of group L,

X 2=The mean value of group II.

SD1=The standard deviation of group I. SD2=The standard deviation of group II. n1=the number of observations of group L,

n2=The number of observations of group II.

Analysis of variance [ANOVA] tests: According to the computer program SPSS for Windows. ANOVA test was used for comparison among different times in the same group in quantitative data.

Results

Morphometric study

The esophageal length of rabbit was in lactating 3.62 ± 0.97 cm, in Young 6.97 ± 0.46 cm and in adult 8.19 ± 0.26 cm (Table 1).

Microscopic study

Histologically, the esophageal wall is composed of mucosa, sub mucosa, muscularis and serosa. The mucosa characterized with a non-keratinized squamous.

Lactating rabbit

The esophageal wall composed of serosa of loose connective tissue, the muscularis of three layers (outer longitudinal muscle layer-middle circular muscle layer-inner longitudinal), sub mucosa of loose connective tissue (Lamina propria) and the mucosa of non-keratinized squamous epithelium. All the above layers were poorly developed by using hematoxylin and eosin stain (Figure 1). By using Periodic acid Schiff reagent for mucus secretion, we observed that the muscular layer give strong purple color reaction, weak reaction with inner layer of mucosa and very weak reaction with lamina propria (Figure 2). With azan stain the lamina propria react with aniline blue give weak reaction but the muscular layer react with azocamine and give moderate reaction (Figure 3).

| Esophagus length rabbit | Lactating | Young | Adult |
|-------------------------|---------------------|-----------------|-----------------|
| Range | 2-5.2 | 6.2-7.8 | 7.8-8.6 |
| Mean \pm SD | 3.62 ± 0.97 | 6.97 ± 0.46 | 8.19 ± 0.26 |
| F. test | 183.286 | | |
| p. value | 0.001 | | |
| Lactating and Young | Lactating and Adult | Young and Adult | |
| 0.001 | 0.001 | 0.001 | |

Table 1: Histogram showing the esophageal length (cm) and body weight (kg) of rabbit.



Figure 1: Transverse section of lactating *O. cuniculus* esophagus showing muscularis layer, submucosa, and mucosa contain non keratinized stratified squamous epithelium H&E.



Figure 2: Transverse section of lactating *O. cuniculus* esophagus showing strong reaction with muscularis layer, weak reaction with inner layer of mucosa (lumen) and very weak reaction with basal lamina PAS.



Figure 3: Transverse section of lactating *O. cuniculus* esophagus showing muscularis layer, submucosa, and mucosa contain lamina propria and non-keratinized stratified squamous epithelium Azan.

Young rabbit

The esophog wall composed of serosa of loose connective tissue, the muscularis three layers (outer longitudinal muscle layer and the inner longitudinal) more thickness than lactating rabbit, submucosa of loose connective tissue (Lamina propria) containing blood vessels and nerve ending, thin muscularis mucosa and mucosa of non-keratinized squamous epithelium more thickness than lactating rabbit. The entire above developed layer give moderately. All the above layers were developed by using hematoxylin and eosin stain (Figure 4). By using Periodic acid Schiff reagent for mucus secretion, we observed that the muscular layer give negative reaction, very weak reaction with inner layer of mucosa and very weak also reaction with lamina propria (Figure 5). With azan stain the lamina propria react with aniline blue give negative reaction but the muscular layer react with azocamine and give moderate reaction (Figure 6).

Adult rabbit

The esophageal wall composed of serosa of loose connective tissue, the muscularis three layers (outer longitudinal muscle layer and the inner longitudinal) more thickness than young rabbit, submucosa of loose connective tissue (Lamina propria), thin muscularis mucosa and mucosa of non-keratinized squauamous epithelium more thickness than young rabbit. All the above layers were well developed by using hematoxylin and eosin stain (Figure 7). By using Periodic acid Schiff reagent for mucus secretion, we observed that the muscular layer give

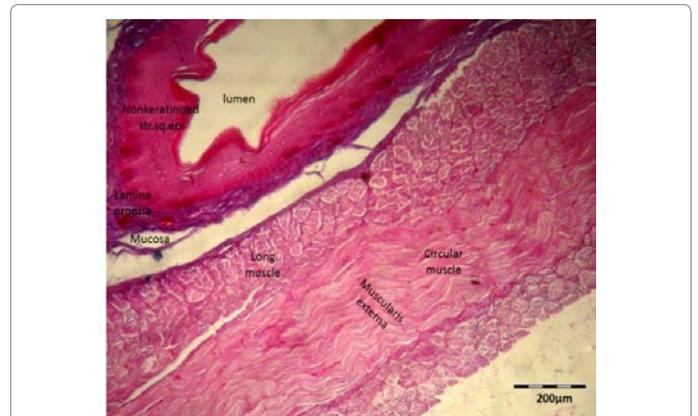


Figure 6: Transverse section of lactating *O. cuniculus* esophagus showing muscularis layer, submucosa, and mucosa contain lamina propria and non-keratinized stratified squamous epithelium, Azan.

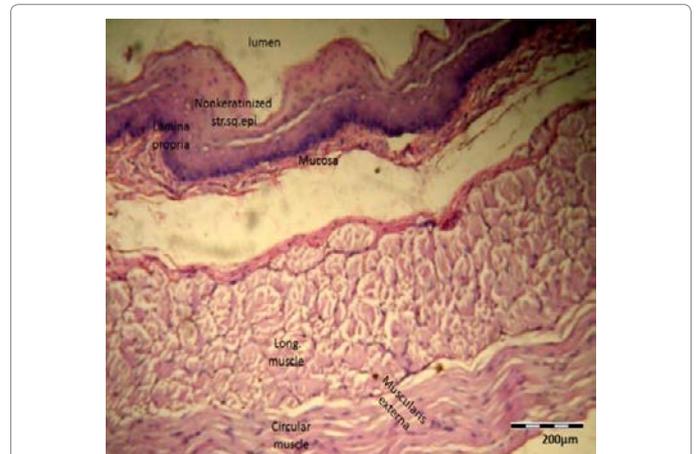


Figure 7: Transverse section of adult *O. cuniculus* esophagus showing muscularis layer, submucosa, and mucosa contain muscularis mucosa and non-keratinized stratified squamous epithelium H&E.

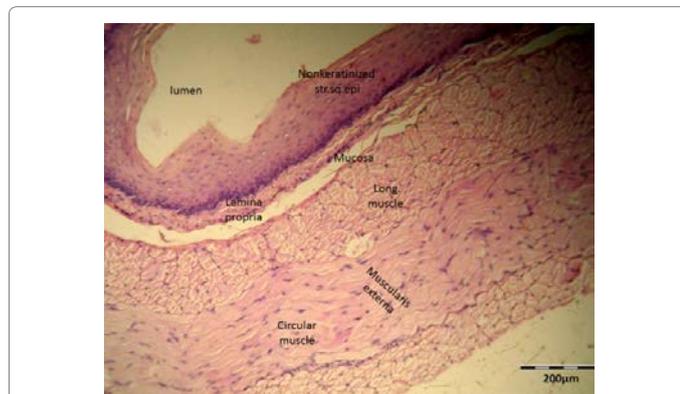


Figure 4: Transverse section of young *O. cuniculus* esophagus showing muscularis layer, submucosa, and mucosa contain thin layer of muscularis mucosa and non-keratinized stratified squamous epithelium H&E.



Figure 5: Transverse section of young *O. cuniculus* esophagus showing moderate reaction with muscularis layer, inner layer of mucosa (lining lumen) and basal lamina PAS.

poor reaction, strong reaction with inner layer of mucosa and moderate reaction with lamina propria (Figure 8). With azan stain the lamina propria react with aniline blue give negative reaction but the muscular layer react with azocamine and give moderate reaction (Figures 9 and 10).

Discussion

The esophagus is the first part of the alimentary canal and was divided into the cervical, thoracic and abdominal portions. As observed for rabbit [13]. However, Rudolf and Strombery [14] noticed that the esophagus of the rat deviated slightly to the left around the cervical region.

The inner wall of the esophagus in the present animals show non keratinized stratified squamous epithelium as the same observed of the African Giant Rat [8] (*Cricetomys gambianus*-Waterhouse, 1840) [10,15].

The esophagus is important organ [16] for passage of food from the mouth to the stomach. The present animals (Rabbit – herbivorous needs this mucous to increase the viscosity of the esophagus which is important allowing the passage of large food. The mucous barrier is also an important factor in the protection of the esophagus from damage. In the lactating rabbit the mucus which secreted from the mucosal layer



Figure 8: Transverse section of adult *O. cuniculus* esophagus showing weak reaction with muscularis layer and strong reaction with inner layer of mucosa (lining lumen) and basal lamina PAS.

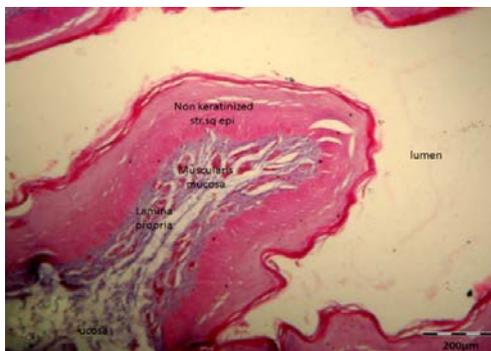


Figure 9: Transverse section of lactating *O. cuniculus* esophagus showing muscularis layer, submucosa, and mucosa contain lamina propria and non-keratinized stratified squamous epithelium.

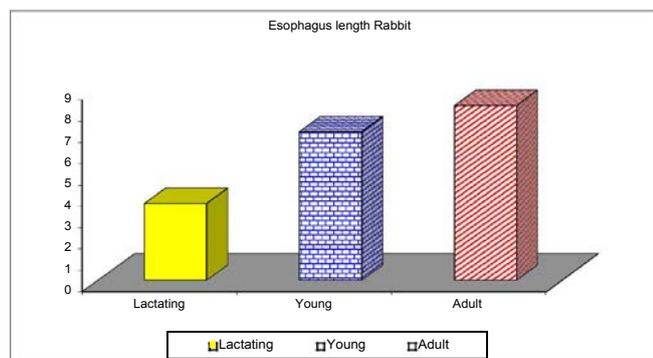


Figure 10: The non-keratinized squamous to Esophageal wall of 3 (Lactating, young, adult) rabbits.

or from the salivary gland give strong reaction may be due to feeding habit. This observation agrees with that of Ali et al. [17] but not with those ones of Elliott et al. [18-20].

Conclusion

From the present study, it was observed that the esophagus of rabbit had no esophageal glands and lamina muscularis and because of the absence of these glands, histochemical reactions showed AB and PAS, and AB-PAS negative results. There are structural changes in the rabbit esophagus before, after weaning and after maturity. These changes may be related to the type of food at different stages and feeding habit.

Ethics Approval

Faculty of Science Tanta University.

Ethics Committee

Zoology Department - Tanta University.

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