A Study on the Prenatal and Postnatal Development of Rat Esophagus

SIÇAN ÖZOFAGUSUNUN PRENATAL VE POSTNATAL GELİŞİMİNİN İNCELENMESİ

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Summary

In this study, prenatal and postnatal development of rat esophagus was investigated using light microscope. For this purpose, esophaguses obtained from fetuses at 7, 10, 13, 15, 17 days of gestation and from newborn at 5, 10 days after birth were studied by histological methods.

At the 7th day of gestation esophagus had a simple columnar epithelium in the spreading mesenchyme. At this time, the other layers of esophagus were not seen. At the fetal 10th day, esophagus had a simple columnar epithelium. On the 13th day, mesenchymal cells surround esophagus and in this stage, esophagus epithelium was 4-6 cell thick. At the fetal 15th day, esophagus had stratified epithelium consisting of 2-3 row cells and was surrounded with circular myoblast layers. The esophageal epithelium of fetal rats of 17th day was stratified squamous of 4-5 cells thick. The longitudinal muscle layers were seen in this stage.

The esophageal epithelium was non-keratinized stratified squamous at postnatal 5th day. At the postnatal 10th day esophageal epithelium was keratinized stratified squamous type.

We are in the opinion that the findings obtained in this work enlighten other morphological investigations related to the development of rat esophagus, and contribute to literature.

Key Words: Esophagus, Prenatal, Postnatal, Development

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Özet

Bu çalışmada sıçan özofagusunun prenatal ve postnatal gelişimi ışık mikroskobu seviyesinde incelenmiştir. Bu amaçla, gebeliğin 7, 10, 13, 15, 17. günlerindeki fetuslardan ve doğum sonrası 5 ve 10. günlerdeki yenidoğanlardan alınan özofaguslar histolojik metodlarla incelenmiştir.

Gebeliğin 7. gününde yaygın mezenkim içindeki özofagus tek katlı prizmatik epitele sahiptir. Bu zamanda özofagusun diğer tabakaları yoktu. Fetal 10. günde özofagus tek katlı prizmatik epitele sahiptir. 13. günde mezenkimal hücreler özofagusu çevrelemiştir ve bu safhada özofagus epiteli 4-6 hücre kalınlığındadır. Fetal 15. günde özofagus 2-3 sıralı hücre içeren çok katlı epitele sahiptir ve sirküler myoblast tabakasıyla çevrelenmiştir. Fetal 17 günlük sıçanların özofagus epiteli 4-5 hücre kalınlığında çok katlı yassıdır. Longitudinal kas tabakası bu safhada görülmüştür.

5. postnatal günde özofagus epiteli çok katlı yassı keratinsizdir. Postnatal 10. günde özofagus epiteli çok katlı yassı keratinli tiptedir.

Sonuç olarak, bu çalışmada elde edilen bulguların sıçan özofagusunun gelişimi ile ilgili diğer morfolojik çalışmalara ışık tutacağı ve literatüre katkıda bulunacağı kanısını taşımaktayız.

Anahtar Kelimeler: Özofagus, Prenatal, Postnatal, Gelişim

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Esophagus is an organ that develops from the foregut part of the primitive intestinal canal. It is a muscular canal of the gastrointestinal system and its function is to carry food from the mouth to the stomach. It takes its structure from the stratified epithelium that develops from the endoderm, and the muscle and connective tissues coming out of the mesoderm (1-3).

After birth, it has been found that as a result of abnormal embryologic development, esophagus atresies does occur; however, the etiologic factors responsible for these pathologic conditions have not been fully explained (1, 4-7). Therefore, for a good understanding of the abnormal growth of the esophagus, it is necessary to have a knowledge of the normal embryologic developmental stages.

Despite the fact that a lot of research work was made in following esophagus development (4, 6, 8-17), little has been done in the stepwise variations that occur during prenatal and postnatal development. Hence, it is rightly thought that any study in this particulary direction will throw more hight on the development of the esophagus.

Material and Methods

In this study pregnant and newborn Wistar albino rats taken from Erciyes University Clinic and Experimental Research Laboratory were used. For one day and one night the male and female rats were kept in the same cage, after which the former was removed. This day of separation was counted as the zero (0 th) day of pregnancy. On the 7, 10,

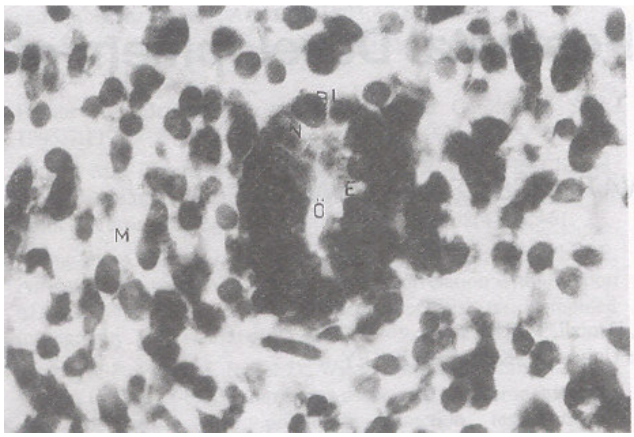

Figure 1. The general appearance of the 7 day old fetus esophagus. Found seated on the basal lamina (BL) are epithelial cells (E) containing large and oval nucleus (N) and surrounded by mesenchymal (M) cells. PAS, X 1250.

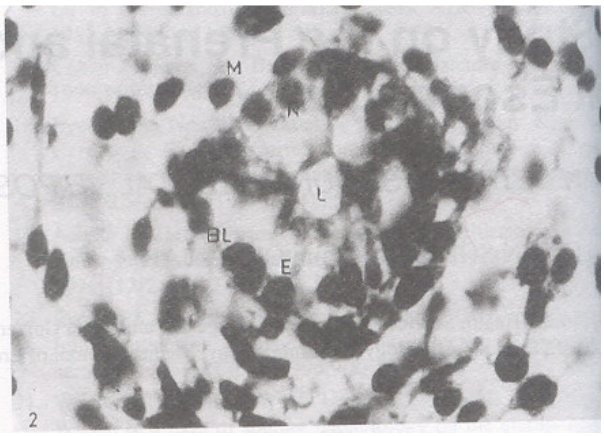

Figure 2. The general appearance of the 10 th fetal days esophagus. Epithelial (E) cells are of the prismatic type and the nuclei (N) close to the basal lamina (BL).L:lumen, M: Mesenchymal cells. PAS, X 1250.

13, 15 and 17 th days pregnancy, for each day the fetuses of five rats were removed. From each mother rat, between eight to twelve fetuses was found. For postnatal study, on the 5 th and 10 th days after birth, ten newborn rats were used for each day.

The whole body of the fetuses of the 7, 10 and 13 th days; the neck regions of the 15 and 17 th days old fetuses and that of the 5 day old rat; and the esophagus together with its surrounding tissues, of the 10 day old rats were fixed in 10% formaldehyde for 48 hours. After fixation, the tissues were taken through a dehydration process putting them in a series of alcohol solutions, in the order of increasing alcohol concentration. After clearing the tissues in xylol they were left for one night in paraffin at 60°C and then embedded in paraffin blocks.

Sections of 6 micron (μ) thick obtained by using Euromex Arnheim rotary microtom and using Hematoxylen+Eosin and Periodic Acid Schiff (PAS) reaction; and then the microphotographs obtained from an Olympus BH-2 light microscope were examined.

Results

7th Fetal Day

In the 7 day old fetus, the esophagus consists of only epithelial cells and the differentiations into the tunica (t.) submucosa and t. muscularis are not observable. The epithelial cells, seated on a prominent basal lamina show a single layered prismatic characteristics. While containing large oval shaped nuclei close to the basal lamina, the surroundings of the epithelial cells are filled with diffused mesenchym cells.

The average height of epithelial cells, during this period is 9.5 μ. However, the lumen of the esophagus only observed as a small fissure (Figure 1).

10th Fetal Day

The esophagus on the 10 th fetal day shows structural resemblance to that of the 7 th day. The epithelial cells are located on tiny, distinct basal membrane, maintaining the single layered prismatic character. However, cell distribution here is a bit sparse compared with that of the 7 th day. Epithelial cells contain oval or spherical shaped nuclei located near the lamina. The other layers developing from the mesenchym of the esophagus show no differentiation.

The average height of the esophagus is 13 μ, with measurement made from the dorso-ventrally located apical cells giving an average lumen diameter of 15 μ (Figure 2).

13th Fetal Day

The most outstanding feature of this period is the differentiation occuring in the wall of the esophagus. The esophagus consists of stratified epithelial cells and, t. submucosa and t. muscularis formed from mesenchymal cells are seen. T. submucosa is that large area found under the basal membrane which exhibits loose regular connective tissue characteristics. Under this layer forming the t. muscularis spindle shaped cells differentiating into myoblasts and with their long axes arranged in few layers in a circular from around the t. submucosa. At the outer most, however, are situated undifferentiated mesenchymal cells (Figure 3a).

The epithelium is made of 2-3 layers of cell. With the basal cells, containing oval nuclei, relatively prismatic in

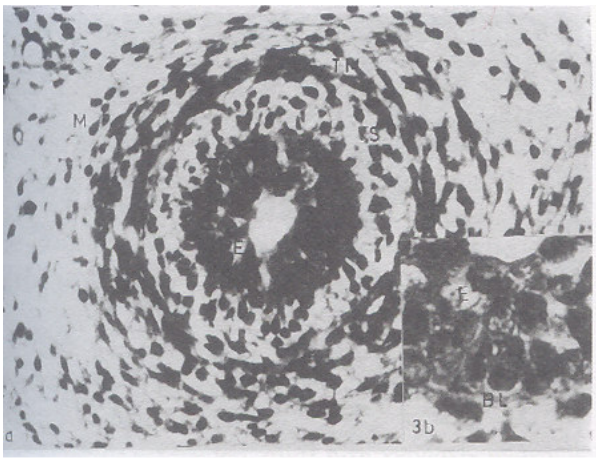

Figure 3. In the esophagus of the 13 th day fetuses (3a), t. submucosa (TS) and t. muscularis (TM) layers are prominent (3b). Epithelium (E) becomes stratified. BL: Basal lamina, M: Mesenchymal cells. (3a) Hematoxylen+ Eosin, X 500, (3b) Hematoxylen+ Eosin, X1250.

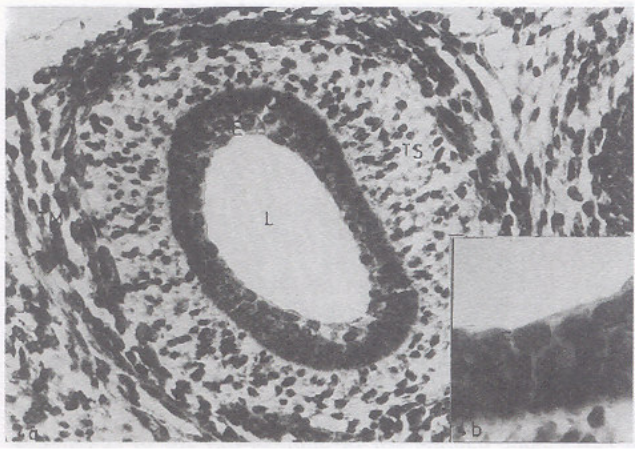

Figure 4. The general appearance of the esophagus (4a) and epithelium (4b) on the 15 th fetal days. Epithelium (E), lumen (L), t. submucosa (TS) and t. muscularis (TM). (4a) Hematoxylen+ Eosin, X 500, (4b) Hematoxylen+ Eosin, X 1250.

nature, the upper layers are spherical or oval and contain spherical nuclei (Figure 3b).

The epithelium of the esophagus at this stage is around 22 µ, while the width of the lumen is at an average of 19 µ.

15th Fetal Day

At this stage, the esophagus is made up of fully distinguishable, one from the other, of epithelium, t. submucosa and t. muscularis. It shows a change towards a stratified squamous epithelial type. T. submucosa is filled with cells of the connective tissue and in between them are located blood capillaries. T. muscularis is made of 6-8 layers of circularly oriented smooth muscle cells and shows continuity in a surrounding connective tissue (Figure 4a).

The epithelium is formed from 3-4 layers of cells. Cells at the basement are prismatic in shape and contain oval nucleus. In between them, with their deeply stained basophilic nuclei are found spindle-shaped cells. In the middle layer, arranged in 1-2 lines are located polygonal cells containing spherical nucleus. The cells surrounding the lumen, however, show a flattening trend with their oval shaped nuclei (Figure 4b).

During this period, the height of the epithelium is a bit reduced, compared with that of the 13 th day, and measures 12 µ. The average lumen width is 27 µ.

17th Fetal Day

It is observed that as the birth period approaches; the stratification of the esophagus becomes more prominent. In this period, one outstanding feature worth mentioning is the appearance of the regular structure of the lumen seen in the earlier stages, and the lamina propria becoming distinct. T. submucosa is of the loose, regularly arranged connective tissue type. T. muscularis turns into a thick double layer possesing in its interior circular and on the exterior longitudinally oriented muscle fibres. T. adventitia of trachea which connects it to the esophagus is of a loose connective tissue character (Figure 5a). The esophagus taking the form of stratified squamous epithelium consists of 4-5 layers. While the basal cells are prismatic, located in the middle are polygonal cells. However, at the top most are found with thin nucleus completely flattened cells (Figure 5b).

Measurement of the width of the esophagus lumen could not be made due to its acquiring of an irregular shape. With the height of the epithelium reaching an average 30 µ, it represents the widest form during the fetal period.

5th Day After Birth

The esophagus of the 5 th day after birth is irregular and stratified squamous epithelium in nature. T. submucosa is in loose connective tissue form. The t. muscularis consists of an interior made of circular and an exterior of longitudinally arranged smooth muscle cells; and at the outermost, the t. adventitia with its loose structure dose draw attention (Figure 6a). The epithelium is formed from 5-6 layers of cell. While the basal cells do not escape notice with their prismatic shapes, the cells above show a flattening trend towards the lumen and at the top most are found completely flattened cells (Figure 6b). At this stage, the epithelial height of the esophagus is 34 µ on the average.

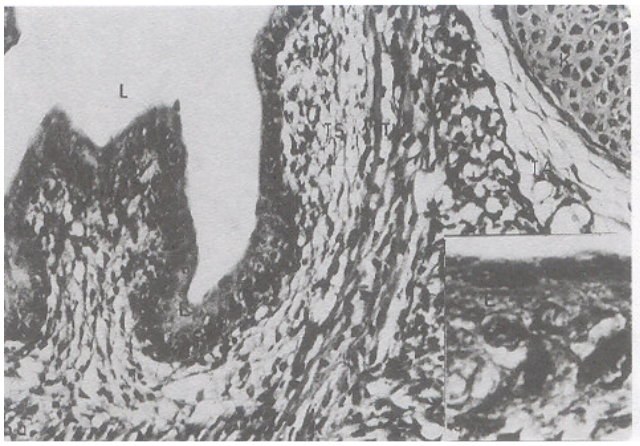

Figure 5. A wide lumen (L), the epithelium (E), t. submucosa (TS), t. muscularis (TM) and t. adventitia (TA) of esophagus are completely distinguishable. K: hyaline cartilage of the trachea (5a) PAS, X 250. (5b) Epithelium of esophagus. PAS, X 1250.

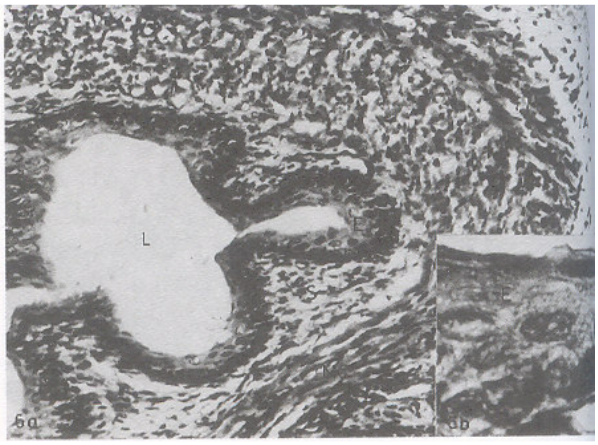

Figure 6. The general appearance (6a) of the esophagus and the epithelium (6b) on the 5 th day after birth. L: Lumen, E: epithelium, TS: t. submucosa, TMC and TML: The t. muscularis circular and longitudinal muscle layers, respectively, TA: t. adventitia. (6a) PAS, X 250, (6b) PAS, X 1250.

10th Day After Birth

The most striking feature of this period is the observation of keratinization. The t. submucosa, showing a loose connective tissue characteristic, contain several blood capillaries. The inside of the t. muscularis has circular and the outside made of longitudinally oriented muscle layer (Figure 7a). The epithelium of the esophagus which is stratified squamosal in nature has 5-6 layers. While the basal cells, containing oval nucleus, are prismatic in shape, the polygonal cells located in the middle posses spherical nucleus. During this stage, the epithelium of the esophagus shows keratinization and the cells situated under the keratohyaline layer contain many keratohyaline granules in their cytoplasm (Figure 7b).

In this period, the epithelial height is measured 26 μ on the average.

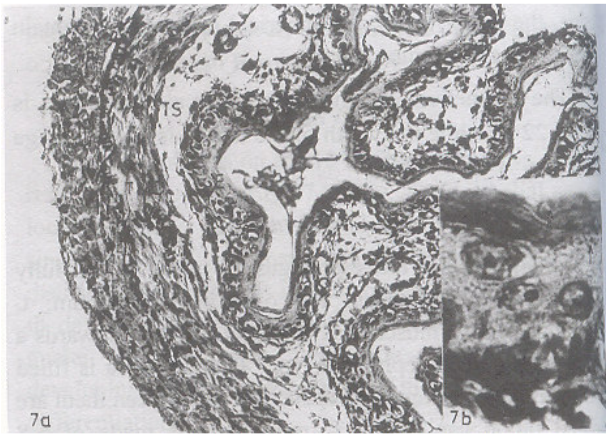

Figure 7. On the 10 th day after birth, the epithelium (E), t. submucosa (TS), the circular (TMC) and longitudinally (TML) oriented layers of the t. mucosa are distinguishable (7a) Hematoxylen+ Eosin, X 250. In the epithelium (7b), keratohyaline layer (arrow head) and under neath the cells with granule containing cytoplasm (arrow) are manifest. Hematoxylen+ Eosin, X 1250.

Discussion

Concerning the development of the esophagus, on the fetus of mice (13, 14, 15), chicks (11) and humans (4, 6, 8, 9, 11, 12) without any outside interference, different work was carried out in studying its normal development and different structural features that the epithelium acquires in the course of its growth was made known.

The study of Parakkal performed on mice made it clear that the epithelium of the esophagus in the beginning is made of single layered prismatic cells and afterwards turning into stratified squamous form. In this period of fast growth, it was shown that the surface cells are ciliated, afterwards these ciliated cells disappear and their place occupied by a permanent stratified squamous epithelia found in the adult. In another study carried out on mice it was found that, on the 15 th day of pregnancy, the epithelium has 2-3 layers and between the 15 th-18 th days, it consists of ciliated cells. In present study, on the 7 and 10 days old rat fetuses, we found that the esophagus composed of single layered prismatic cells; but at 13 days of gestation, we observed it is formed from 2-3 layers of cell. However, while making mention of the presence of ciliated cells within this period, we did not come across these ciliated epithelial cells in our work.

On studies conducted on human fetus, it was shown that while the esophagus epithelium during the 8 th week is single layered, prismatic and ciliated (4) and this same structure persisting in the 10 th week (8), fetuses of 40-45

and 49 mm in length were discovered to have 2-3 layers with cilia (9) and those between the 12 th and 16 th weeks were of the stratified prismatic epithelial forms (12). In our findings, those of the 7 th and 10 th days, possessing single layered prismatic epithelia, have esophagus structure showing resemblance to that in 8-10 week-old humans. However, nowhere in our work did we come across a stratified prismatic epithelial structure during this period.

In human fetus, it was discovered that the epithelium of the esophagus in the 4 th fetal month is stratified polygonal and then turns into a stratified squamous epithelia in the 5 th month (4, 8, 9, 11, 12). In chicks, however, the begining of flattening in apical cells is seen to occur on the 13 th fetal day while their complete flattening takes place between the 16 th and 18 th days (11). In our present study, it was found that, the stratified structure arising out of 2-3 layers of cell starts coming into view on the 13 th day, while the flat cells begin doing so on the 15 th day and continue until completion on the 17 th day. These changes seen in the epithelium runs parallel to the results of the above investigations.

In studies made on human esophagus culture (12), on the 12-16 th fetal weeks, due to their glycogen content, the epitelial cells and the basal membrane give a PAS (+) reaction and during this period, the epithelium was found to be stratified squamous in nature. We found similar results on the esophagus of the 17 day old fetus, in the course of our work. At this stage, it is of the stratified squamous epithelia type and a PAS (+) reaction appears.

In newborn mice, the esophagus epithelium is stratified squamous and up to the 8 th day shows no keratinization and during this period glycogen completely disappears. However, after 8 th day keratinization begins (13, 14). Initially, the keratohyalin granules are seen in the middle layer of the epithelium as small, dense granules but afterwards migrate upwards, and finally, with the disappearence of the organels form horny cells (13, 15). Showing similarity to the findings in our work, the epithelium on the 5 th day is 5-6 layered, of the stratified squamous type and non-keratinized region contain large numbers of keratohyalin granules.

The t. muscularis is formed by the myoblasts, which themselves result from the differentiation of the mesenchymal cells surrounding the epithelial tissue. In humans, it was realised that, firstly it is the circular muscle layer that appears, then in the 6 th week and in an 11 mm long embryo (9) begins to draw attention; the longitudinal muscle layer is not seen at this stage. In the 16 mm long embryo, the circular muscle layer is found to be well developed on the longitudinal muscle layer starts coming into view, but in the 24 mm embryo, both layers are seen fully developed (10). In our own work, even though there is no enough indication of differentiation into the t. muscularis in the esophagus of the 7 th and 10 th fetal day, by the 13 th day the t. muscularis starts coming into focus. Our findings are identical to those of Zhao and et. all (16, 17). During this period, as the myoblasts show a circular arragement to form the t. muscularis, the t. submucosa is also exposed. In the 15 day-old fetus while the circular layer is distinctly separated, the longitudinal muscle layer also begins its appearance. However, in the 17 day-old fetus both layers acquire their normal shapes, and by the 5 th and 10th days after birth, all the structures attain complete development.

In chicks, as a result of the proliferation of the esophagus epithelium, there is closure, during early embryonic stage, of the lumen (11), but in the human embryo, the lumen of the esophagus was found to be not completely closed (6, 10). In our investigations, on the esophagus of the rat, at none of the studied stages was it found to be totally closed, but rather the initially narrow lumen kept on widening in the course of its growth.

In conclusion during the course of its development, the rats esophagus show different epithelial forms and from the 13 th fetal day onwards starts gaining its structural properties up to the first days after birth when it attains full histological characteristics typical of the adult.

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