

Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.



**ELECTRONIC  
JOURNAL  
OF POLISH  
AGRICULTURAL  
UNIVERSITIES**

**2006  
Volume 9  
Issue 2  
Topic  
VETERINARY  
MEDICINE**

Copyright © Wydawnictwo Akademii Rolniczej we Wrocławiu, ISSN 1505-0297

KULAWIK M. 2006. DEVELOPMENT OF THE MUCOSA ON LATERAL SURFACES OF THE LINGUAL BODY IN THE PERIOD FROM DAY 15 OF PRENATAL LIFE TO 6TH MONTH OF POSTNATAL LIFE IN THE RABBIT **Electronic Journal of Polish Agricultural Universities**, Veterinary Medicine, Volume 9, Issue 2.

Available Online <http://www.ejpau.media.pl/volume9/issue2/art-07.html>

## **DEVELOPMENT OF THE MUCOSA ON LATERAL SURFACES OF THE LINGUAL BODY IN THE PERIOD FROM DAY 15 OF PRENATAL LIFE TO 6TH MONTH OF POSTNATAL LIFE IN THE RABBIT**

Mirosława Kulawik

*Department of Animal Anatomy, Agricultural University in Poznan, Poland*

### **ABSTRACT**

Studies were conducted on 77 tongues, collected from rabbits being at day 15, 18, 20, 22 and 26 of prenatal life and from rabbits at day 1, 15 and 30 and in the 6th month of postnatal life.

Tissues for analyses, collected from the lateral surfaces of the body of the tongue of rabbits, were studied under a light microscope (LM) and a scanning electron microscope (SEM). The thickness of the epithelium in successive periods of pre- and postnatal life was analyzed morphometrically.

As a result of the conducted studies it was shown that the epithelium covering the lateral surfaces of the tongue changes in the course of pre- and postnatal development from an epithelium consisting from 1-2 layers of cells into a nonkeratinized stratified squamous epithelium. The thickness of the epithelium increases in successive analyzed periods of life in rabbits. A rapid growth rate was found for the epithelium in the period from day 26 p.c. The lamina propria mucosae is observed in histological slides starting from day 22 p.c. In the same period the presence of elastic fibers was shown. Glycogen was found in the cytoplasm of the epithelial and mesenchymal cells from day 15 to 20 p.c.

**Key words:** rabbit, tongue, mucous membrane, development, morphometry.

### **INTRODUCTION**

On the basis of available literature it is known that numerous studies investigate the development of lingual papillae (gustatory and mechanical) in various animal species [4, 5, 6, 7, 8, 14] and in the human [16]. Researchers focus their attention also on the quantitative analysis of lingual papillae and taste buds [1, 10, 11, 13]. However, developmental changes in areas devoid of papillae on the tongue have not been scientifically documented.

The aim of this study was to follow the development of the mucosa on the lateral surfaces of the body of the tongue and to perform morphometric analysis of the thickness of the epithelium in the investigated area. The scope of the realized tasks included also the determination of the dynamics of changes occurring in successive periods of pre- and postnatal life in the rabbit.

## MATERIALS AND METHODS

Material for investigations consisted of tongues of 77 rabbits (*Oryctolagus cuniculus f. domestica*), which were collected at day 15, 18, 20, 22 and 26 of prenatal life (p.c.) and at day 1, 15 and 30, as well as the 6th month of postnatal life (p.p.). The age of fetuses was determined on the basis of the CRL value (Crown-Rump-Length) [3].

Tissues collected from the lateral surfaces of the lingual body were prepared for analyses conducted using a light microscope (LM) and a scanning electron microscope (SEM). Material for analyses under a light microscope was fixed in 10% neutralized formalin or Bouin's liquid and stained using the following methods: HE, Masson-Goldner, resorcin-fuchsin, orcein and PAS, along with control stainings using  $\alpha$ -amylase and dimedone.

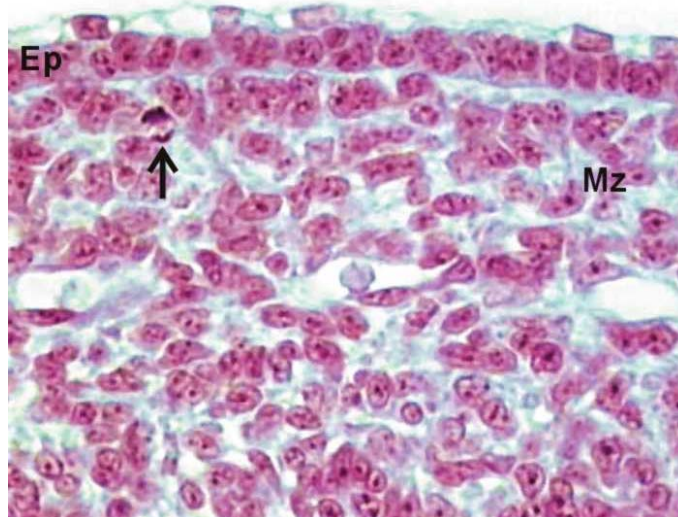
Morphometric studies were conducted on the basis of images of histological specimens, observed under a light microscope, mounted with a camera with a computer image analysis program MultiScan v. 6.08. Measurements were taken after system calibration using a micrometric scale (Zeiss Jena). The thickness of the epithelium covering the lateral surfaces of the lingual body in individual periods of the pre- and postnatal life of rabbits was analyzed morphometrically. On the basis of the obtained results the primary statistical characteristics were determined: mean ( $\bar{x}$ ), minimum value (Min), maximum value (Max) and standard deviation (SD). Using the F test statistical differences were determined for mean values of the measured trait in successive periods of pre- and postnatal life of rabbits.

Material for observations under a scanning electron microscope was fixed in the Karnovski solution. In order to study the connective tissue core, tissues originating from rabbits being at day 1 of postnatal life were placed for the period of two weeks in 10% NaOH. Tissues collected from rabbits being at day 15 and 30 and in the 6th month of postnatal life were placed in 10% NaOH for the period of 20 days. After maceration at room temperature tissue samples were washed in distilled water, and next immersed in 2% tannic acid solution for 12 h, again washed in distilled water, dehydrated in a series of alcohols with increasing concentrations and acetone, dried at critical point using carbon dioxide in an Emitech K 850 device and gold-sprayed. The preparations were studied under a scanning electron microscope Hitachi S-4200.

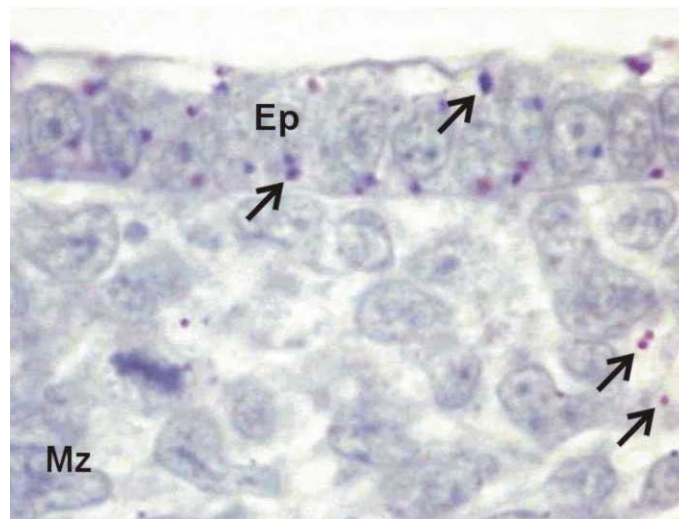
## RESULTS

In the period from day 15 to 20 of prenatal life in the rabbit, the lateral surfaces of the body of the tongue are covered with an epithelium consisting of 1-2 layers of cells. The epithelial cells from basal layer are high, cylindrical, while from the superficial layer cells are flattened. Under the epithelium the mesenchymal tissue is found (Fig. 1). Histochemical studies showed that in the cytoplasm of epithelial cells and the mesenchyme glycogen grains are arranged irregularly (Fig. 2).

**Fig. 1. Transverse section of lateral surface of the body of the tongue at day 15 of prenatal life of the rabbit. Ep – epithelium, Mz – mesenchyme, arrow – cell during mitosis. Light microscope, x 40, Masson-Goldner staining**

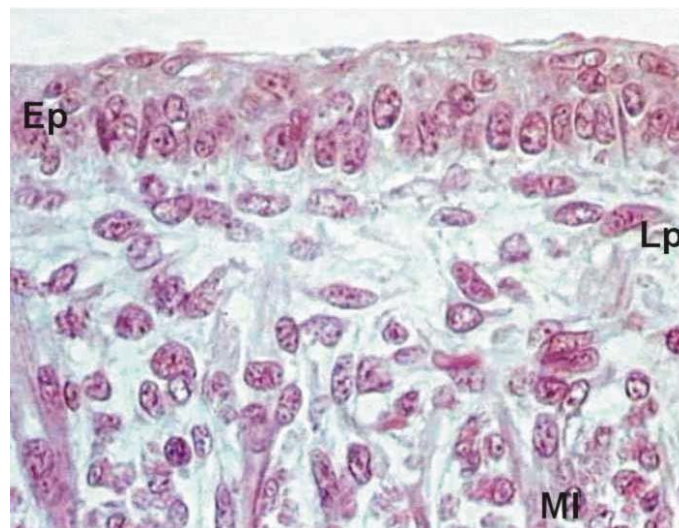


**Fig. 2.** Transverse section of lateral surface of the body of the tongue at day 15 of prenatal life of the rabbit. Ep – epithelium, Mz – mesenchyme, arrows – glycogen. Light microscope, x 100, PAS staining after dimedone blocking



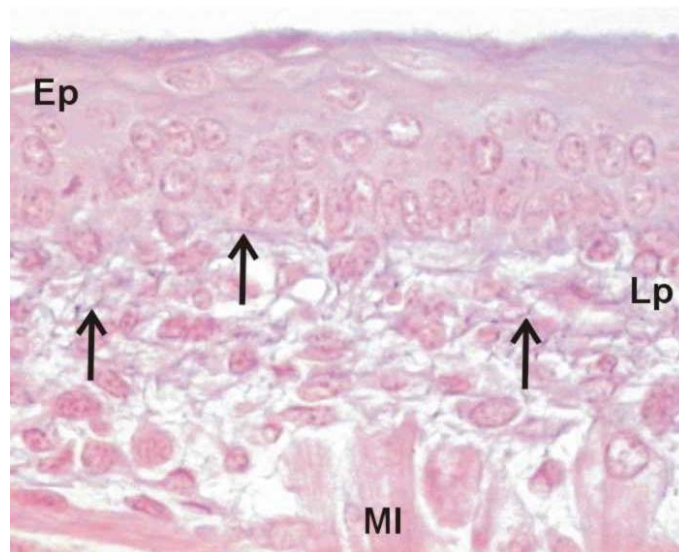
At day 22 of prenatal life the lateral surfaces of the lingual body in the rabbit are covered by an epithelium composed of 4-5 layers of cells. Cells of the basal layer are high, cylindrical and with a large oval nucleus. In this layer mitotic divisions were observed. In the intermediary layer cells are lower and their nuclei are round in outline. In the superficial layer cells and their nuclei are flattened. Under the basement membrane the lamina propria mucosae is found (Fig. 3). Very delicate elastic fibers were detected. The lamina propria mucosae forms in certain places slight protuberances, as a result of which the epithelium varies in thickness. In the investigated period no presence of glycogen was detected.

**Fig. 3.** Sagittal section of lateral surface of the body of the tongue at day 22 of prenatal life of the rabbit. Ep – epithelium, Lp – lamina propria mucosae, Ml – muscles of the tongue. Light microscope, x 40, Masson-Goldner staining



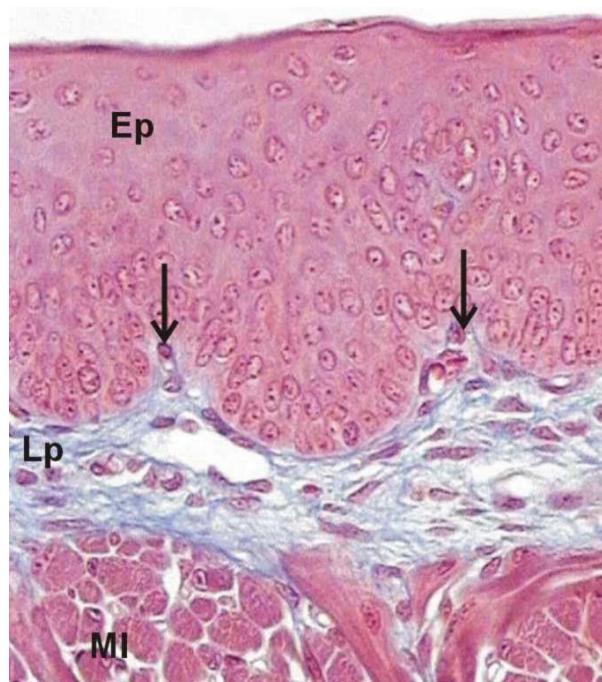
At day 26 of prenatal life, the epithelium covering the lateral surfaces of the lingual body is a nonkeratinized stratified squamous epithelium. Elastic fibers observed in the lamina propria mucosae are still delicate (Fig. 4).

**Fig. 4. Sagittal section of lateral surface of the body of the tongue at day 26 of prenatal life of the rabbit. Ep – epithelium, Lp – lamina propria mucosae, MI – muscles of the tongue, arrows – elastic fibers. Light microscope, x 40, resorcin-fuchsin staining**

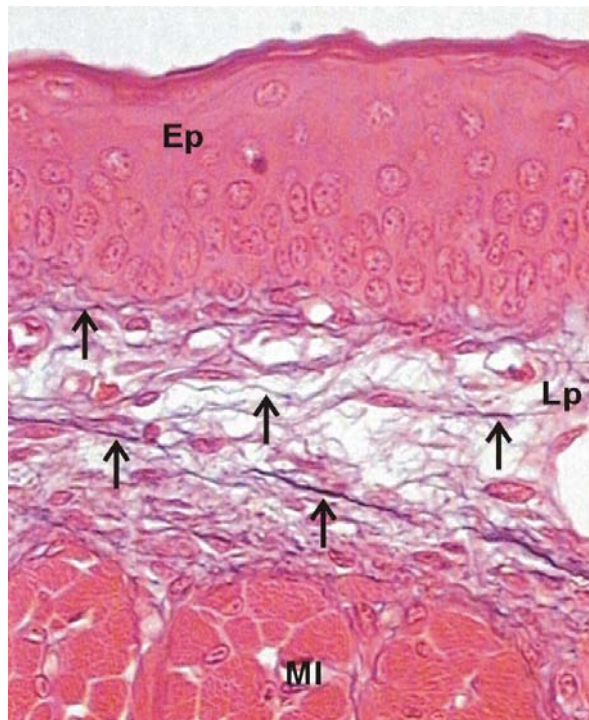


In the next investigated period, i.e. at day 1 of postnatal life, the lamina propria mucosae on the lateral surfaces of the lingual body forms distinct projections, extending towards the epithelium, modifying its thickness ([Fig. 5](#)). Elastic fibers are well-visible. They are arranged uniformly in the lamina propria mucosae and are arranged primarily perpendicularly to the longitudinal axis of the tongue ([Fig. 6](#)). Elastic fibers extend also to the area of prominences of the lamina propria mucosae.

**Fig. 5. Transverse section of lateral surface of the body of the tongue at day 1 of postnatal life of the rabbit. Ep – epithelium, Lp – lamina propria mucosae, which prominences are indicated by arrows, MI – muscles of the tongue. Light microscope, x 25, Masson-Goldner staining**



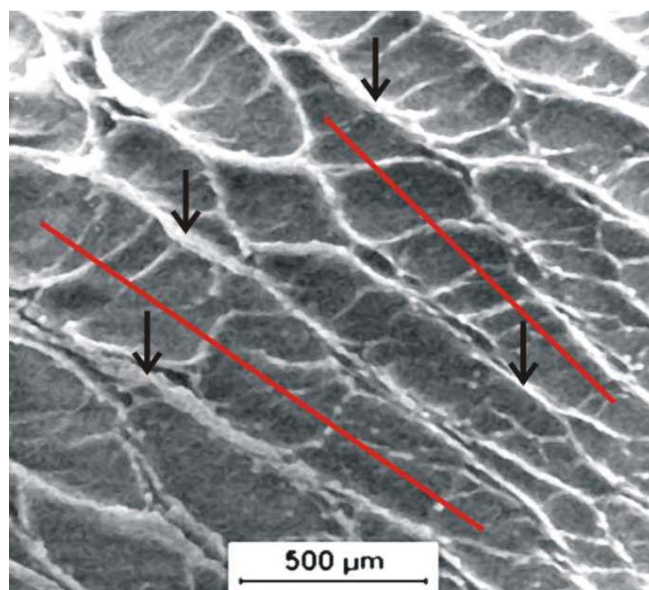
**Fig. 6.** Transverse section of lateral surface of the body of the tongue at day 1 of postnatal life of the rabbit. Ep – epithelium, Lp – lamina propria mucosae, MI – muscles of the tongue, arrows – elastic fibers. Light microscope, x 30, resorcin-fuchsin staining



At day 1 of postnatal life, after the removal of the epithelium with 10% NaOH, on the basis of observations under a scanning electron microscope (SEM) it was shown that prominences of the lamina propria mucosae on the lateral surfaces of the lingual body are numerous.

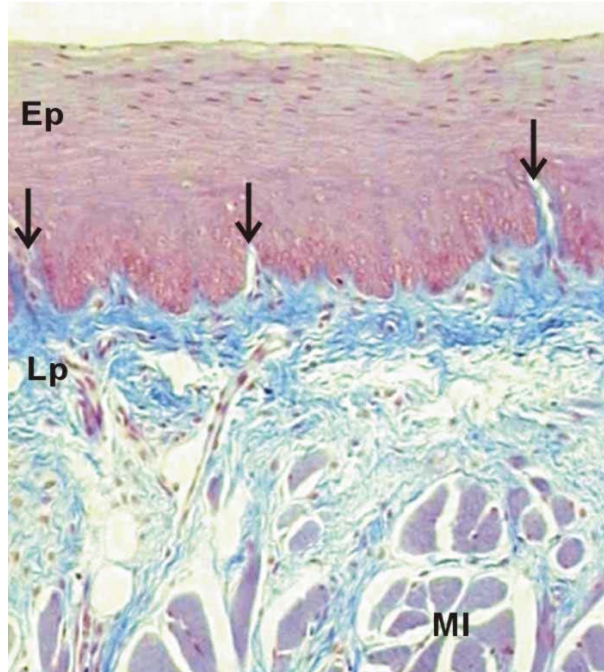
The nonkeratinized stratified squamous epithelium, covering the lateral surfaces of the tongue at day 15 p.c. in the rabbit exhibits varying thickness. It results from the presence of prominences of the lamina propria mucosae varying in height. Elastic fibers in the analyzed period are well-visible and arranged similarly as in the previous investigated period. Prominences of the lamina propria mucosae, observed under a scanning electron microscope (SEM) are numerous and surround groove-like hollows (Fig. 7).

**Fig. 7.** Connective tissue core of lateral surface of the body of the tongue at day 15 of postnatal life. Arrows indicate prominences of lamina propria mucosae, limited by groovy depressions marked with lines. Scanning electron microscope



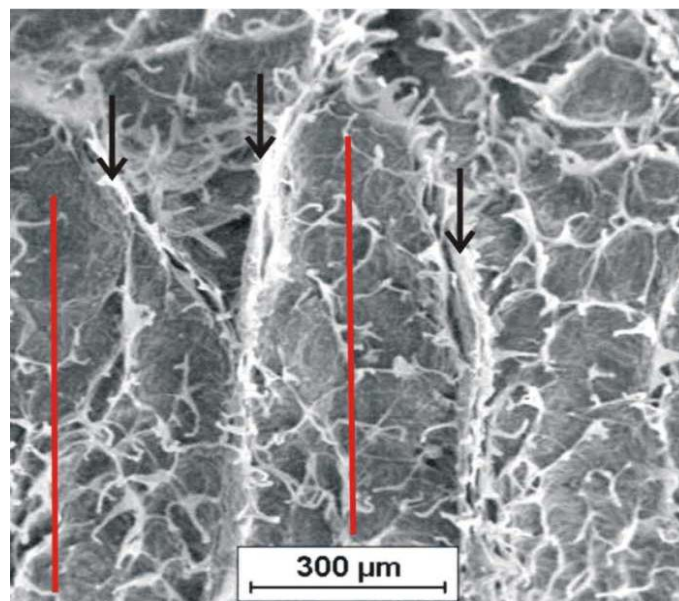
Studies conducted at day 30 of postnatal life showed prominences of the lamina propria mucosae modify the thickness of the epithelium on the lateral surfaces of the body of the tongue (Fig. 8). Elastic fibers are arranged primarily perpendicularly to the longitudinal axis of the tongue.

**Fig. 8. Transverse section of lateral surface of the body of the tongue at day 30 of postnatal life of the rabbit. Ep – epithelium, Lp – lamina propria mucosae, which prominences are indicated by arrows, MI – muscles of the tongue. Light microscope, x 10, Masson-Goldner staining**



Observations of connective tissue core specimens, after the previous removal of the epithelium with 10% NaOH, showed the presence of groove-like hollows, limited laterally by the prominences of the lamina propria mucosae. In the 6th month p.p. in the rabbit the nonkeratinized stratified squamous epithelium exhibits high diversification in thickness. The lamina propria mucosae forms numerous prominences. Elastic fibers are arranged similarly as in the previous periods of postnatal life, primarily perpendicularly to the longitudinal axis of the tongue. Examination of the connective tissue core under a scanning electron microscope (SEM) showed the presence of groove-like hollows, limited by prominences of the lamina propria mucosae (Fig. 9).

**Fig. 9. Connective tissue core of lateral surface of the body of the tongue at month 6 of postnatal life of the rabbit. Arrows indicate prominences of lamina propria mucosae, limited by groovy depressions marked with lines. Scanning electron microscope**



On the basis of morphometric examinations of the thickness of the epithelium covering the lateral surfaces of the body of the tongue in rabbits in the period from day 15 of prenatal life to the 6th month of postnatal life it was shown that the mean value of the investigated trait increased in successive periods of life (Tab. 1). The rapid growth rate of epithelium thickness was reported starting from day 26 of prenatal life.

**Table 1. Epithelium thickness of lateral surfaces on body of tongue in rabbits at individual stages of pre- and postnatal life**

Age	Number of animals	Number of measurements	Mean (µm)	Min (µm)	Max (µm)	SD
day 15 p.c.	3	100	10.77	9.29	11.98	0.70
day 18 p.c.	18	132	11.19	9.13	13.89	1.33
day 20 p.c.	7	105	11.27	9.09	14.40	1.37
day 22 p.c.	12	104	12.48	8.33	14.77	1.44
day 26 p.c.	20	113	38.42	11.32	98.56	23.12
day 1 p.p.	3	102	68.17	30.33	138.16	27.36
day 15 p.p.	3	102	95.30	35.22	208.13	47.00
day 30 p.p.	5	109	105.02	39.74	219.97	52.70
6 <sup>th</sup> month p.p.	6	110	131.08	40.85	235.53	48.20

Statistical analysis of epithelium thickness in the periods from day 15 to 22 of prenatal life did not show statistical differences ( $p > 0.05$ ). A statistically significant difference ( $p \leq 0.05$ ) was recorded between day 15 and 30 of postnatal life, whereas statistically highly significant differences in epithelium thickness ( $p \leq 0.01$ ) were shown for means of the other comparable periods of life of rabbits (tab. 2).

**Table 2. Statistical differences in epithelium thickness on lateral surfaces of body of tongue in rabbits at individual stages of pre- and postnatal life**

	day 15 p.c.	day 18 p.c.	day 20 p.c.	day 22 p.c.	day 26 p.c.	day 1 p.p.	day 15 p.p.	day 30 p.p.	6 <sup>th</sup> month p.p.
day 15 p.c.		0.42	0.51	1.71	27.66	57.41	84.53	94.25	120.32
day 18 p.c.	ns		0.09	1.29	27.23	56.99	84.11	93.83	119.90
day 20 p.c.	ns	ns		1.21	27.15	56.90	84.03	93.75	119.81
day 22 p.c.	ns	ns	ns		25.94	55.69	82.82	92.54	118.60
day 26 p.c.	**	**	**	**		29.75	56.88	66.60	92.66
day 1 p.p.	**	**	**	**	**		27.12	36.84	62.91
day 15 p.p.	**	**	**	**	**	**		9.72	35.79
day 30 p.p.	**	**	**	**	**	**	*		26.07
6 <sup>th</sup> month p.p.	**	**	**	**	**	**	**	**	

\*\*  $P \leq 0.01$  statistically highly significant difference

\*  $P \leq 0.05$  statistically significant difference

ns  $P > 0.05$  no statistically significant differences

Digits in white fields denote differences between mean values of analyzed trait

## DISCUSSION

The development of the mucous membrane on the lateral surfaces of the lingual body in the rabbit is a complex and log-term process, occurring in the pre- and postnatal life.

In the period from day 15 to 20 of prenatal life, the mucosa is not yet formed. The epithelium covering the lateral surfaces of the lingual body consists of 1-2 layers of cells, which are found on the well-visible basement membrane. The mesenchyme is found under the basement membrane. Histochemical analyses made it possible to identify glycogen in the cytoplasm of epithelial and mesenchymal cells. On the basis of available literature it is known that

glycogen is also found in epithelial cells of other organs in the digestive system, during their prenatal development. The presence of glycogen was shown in the esophagus in the mouse [2], in the stomach in the mouse [12] and in the small intestine in the sheep [15]. Mackenzie in his study [9] recorded the presence of glycogen in the epithelium of the oral cavity in the human up to the 5<sup>th</sup> month of prenatal life. That author reported that glycogen in the epithelium of the oral cavity serves the function of energy reserve for the future metabolic activity, connected e.g. with the keratinization process. On the basis of my investigations it may be concluded that the role of glycogen during the development of the tongue is not connected only with the keratinization process, since the area of the lateral surfaces of the lingual body analyzed in this study is covered with the nonkeratinized stratified squamous epithelium. The importance of glycogen in complex developmental processes has not been clarified to date.

Starting from day 22 of prenatal life in the rabbit in microscopic slides the presence of the lamina propria mucosae was reported, which undergoes structural diversification. In the lamina propria mucosae, starting from day 22 of prenatal life the presence of elastic fibers was shown. At day 22 and 26 of prenatal life elastic fibers are very delicate, while from day 1 of postnatal life they are distinct and arranged uniformly in the area of the lamina propria mucosae, mainly perpendicularly to the longitudinal axis of the tongue, similarly as it was shown in the mucosa on the dorsum of the tongue in the rabbit [7, 8]. Elastic fibers determine the motor ability of the tongue and changes in its shape, and ensure considerable functionality of this organ in the rabbit.

The lamina propria mucosae at day 22 of prenatal life forms small prominences, which in successive analyzed periods are larger. Between prominences of the lamina propria mucosae the epithelium is found, which exhibits varying thickness, as it was confirmed by the results of morphometric studies. Examinations conducted under a scanning electron microscope showed that prominences of the lamina propria mucosae from day 15 of postnatal life are limited by groove-like hollows. The epithelium covering the analyzed lateral surfaces of the lingual body changes during the pre- and postnatal development from an epithelium consisting of 1-2 layers of cells into the nonkeratinized stratified squamous epithelium.

On the basis of the measurements of epithelium thickness in the analyzed area the value describing this trait was shown to increase in individual periods of life in rabbits. The dynamics of changes in epithelium thickness on the lateral surfaces of the lingual body in successive periods of pre- and postnatal life is not uniform. Higher growth rate of the investigated trait was recorded starting from day 26 of prenatal life.

A lack of available literature, describing the development of the mucosa in the lateral surfaces of the lingual body in the rabbit, in other animal species and in the human makes it impossible to confront the results obtained in this study.

## CONCLUSIONS

1. The epithelium covering the lateral surfaces of the body of the tongue in rabbits in the period from day 15 of prenatal life to the 6th month of postnatal life changes from a 1-2-layer epithelium into a nonkeratinized stratified squamous epithelium.
2. In successive analyzed periods of pre- and postnatal life in the rabbit the thickness of the epithelium increases. A higher growth rate is observed for epithelium thickness starting from day 26 p.c.
3. From day 15 to 20 p.c. irregularly arranged glycogen grains are found in the cytoplasm of epithelial and mesenchymal cells.
4. The lamina propria mucosae is formed from day 22 p.c.
5. From day 22 p.c. elastic fibers are observed in the mucous membrane of the lateral surfaces of the body of the tongue.

## REFERENCES

1. Dasgupta K., Singh A., Ireland W.P., 1990: Taste bud density in circumvallate and fungiform papillae of the bovine tongue. *Histol Histopath.*, 5: 169-172.
2. Calvert R., Véza A., Ghania M., 1991: Development of esophageal epithelium in the fetal and neonatal mouse. *The Anatomical Record*, 230: 225-234.
3. Evans H.E., Sack W.O., 1973: Prenatal development of domestic and laboratory mammals: growth curves, external features and selected references. *Anat., Histol., Embryol.*, 2: 11-45.
4. Fujimoto S., Yamamoto K., Yoshizuka M., Yokoyama M., 1993: Pre- and postnatal development of rabbit foliate papillae with special reference to foliate gutter formation and taste bud and serous gland differentiation. *Microscopy Research and Technique*, 26: 120-132.
5. Iwasaki S., Yoshizawa H., Kawahara I., 1996: Study by scanning electron microscopy of the morphogenesis of three types of the lingual papilla in the mouse. *Acta Anatomica*, 157: 41-52.
6. Iwasaki S., Yoshizawa H., Kawahara I., 1997: Study by scanning electron microscopy of the morphogenesis of three types of lingual papilla in the rat. *The Anatomical Record*, 247: 528-541.
7. Kulawik M., 2005a: The development of the mucous membrane of the tongue with special emphasis on the development of fungiform papillae in the prenatal life of the rabbit. *EJPAU, Veterinary Medicine*, Volume 8, issue 4.



8. Kulawik M., 2005b: Morfologia błony sluzowej języka, ze szczególnym uwzględnieniem brodawek grzybowatych w okresie od 1. dnia do 6. miesiąca życia postnatalnego królika. [Morphology of the mucous membrane of the tongue, with special emphasis on fungiform papillae in the period from day 1 to month 6 of postnatal life in the rabbit] Acta Scientiarum Polonorum, Medicina Veterinaria, 4(2): 47-58 [in Polish].
9. Mackenzie I.C., 1984: In J. Meyer. C.A. Squier and S.J. Gerson (Eds.), The Structure and Function of Oral Mucosa, Pergamon, Oxford.
10. Miller I.J., Jr., 1989: Variation in human taste bud density as a function of age. Nutrition and the Chemical Senses., v 561 June 30.
11. Ojima K., 1998: Quantitative and distributive study of the fungiform papillae in the cat tongue in microvascular cast specimens. Annals of Anatomy, 180: 409-414.
12. Pipan N., Mam M., 1972: Autophagie und Glikogenverteilung im Magenepithel der Maus während der Differenzierung. Z. Mikrosk. Anat. Forsch., 85: 421-437.
13. Robinson P.P., Winkles P.A., 1990: Quantitative study of fungiform papillae and taste buds on the cat's tongue. The Anatomical Record., 225: 108-111.
14. Tichý F., 1991: The morphogenesis of circumvallate papillae and the differentiation of taste buds in the pig at 41 to 64 days of prenatal development. Acta Vet. Brno, 60: 99-110.
15. Trahair J., Robinson P., 1986: The development of the ovine small intestine. The Anatomical Record, 214: 294-303.
16. Witt M., Reutter K., 1997: Scanning electron microscopical studies of developing gustatory papillae in Humans. Chem. Senses., 22: 601-612.

*The study was carried out within the framework of a grant for a Ph.D. thesis supervision no. 5P06D01719 financed by the Committee for Scientific Research.*

---

Mirosława Kulawik  
Department of Animal Anatomy,  
Agricultural University in Poznan, Poland  
Wojska Polskiego 71 C, 60-625 Poznan, Poland  
phone: +48 061 8487629  
email: [kulawik@au.poznan.pl](mailto:kulawik@au.poznan.pl)

---

[Responses](#) to this article, comments are invited and should be submitted within three months of the publication of the article. If accepted for publication, they will be published in the chapter headed 'Discussions' and hyperlinked to the article.

---