

Localization of mucopolysaccharides, proteins and lipids in the buffalo tongue during prenatal life

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In literature histochemical study of carbohydrates, proteins and lipids in tongue has been reported in buffalo (Ramayya *et al.* 2000, Singh 2001), in sheep and goat (Roy *et al.* 1992, Singh *et al.* 1992) during postnatal life but no report is available during prenatal life. The mucopolysaccharides, poroteins and lipids play an important role in cellular differentiation so it is important to study their distribution pattern in the tissue during different stages of prenatal life to understand the process of histogenesis. With this purpose the present study was planned.

The study was conducted on tongues of 36 buffalo foetii of CVRL (curved crown rump length) 1.2 to 99.5 cm (age ranging from 34 to 298 days). The age of the foetii was estimated by using the formula given by Soliman (1975).

$Y = 28.66 + 4.496 \times (\text{CVR length} < 20 \text{ cm})$

$Y = 73.544 + 2.256 \times (\text{CVR length} \geq 20 \text{ cm})$

where, Y is the age in days and $\times$ is CVR length in centimeters.

Based on the CVR length the samples were divided into group 1, CVR length between 0–20 cm; group 2, CVR length above 20 cm and up to 40 cm and group 3, CVR length above 40 cm

The tissues were taken from different parts of the tongue and fixed in Neutral buffered formalin and Bouin's fixatives immediately after collection. The tissues were processed for paraffin blocks preparation by acetone benzene schedule (Luna 1968) and sections of 5–6 μm were cut. The sections were stained with periodic acid Schiff, alcian blue and bromphenol blue to demonstrate neutral and acid mucopolysaccharides and basic proteins. For demonstration of lipids, cryostat sections of fresh tissue having 10–12 μm thickness were cut at -20°C and stained with Sudan Black B (Chayen *et al.* 1969).

The histochemical observation of neutral

mucopolysaccharides, acid mucopolysaccharides, basic proteins and total lipids on the different layers and papillae of tongue have been summarized in Table 1.

Mucopolysaccharides

Neutral mucopolysaccharides (NMPS): The lamina epithelialis showed a moderate to strong reaction for NMPS in group 1 (Fig. a) and 2 whereas the reaction was weak to moderate in group 3. The interpapillary epithelium showed strong PAS reaction so it was correlated that these NMPS are to be utilized as a source of energy for mitotically dividing interpapillary epithelium during cellular differentiation and growth. At the same time the epithelium over the papillae showed a weak reaction for NMPS in group 1 as there was arrest of mitosis at the area for a particular period (Baratz and Farbman 1975) and the cells were not differentiating. Trott (1957) have reported that glycogen synthesizing enzymes are more in fetal than in adult epithelium.

Kulawik (2006) observed that the role of glycogen during the development of tongue was not only connected with the keratinization process, since the area of the lateral surfaces of the lingual body was covered with the nonkeratinized stratified squamous epithelium.

The propria submucosa, muscles and core of different papillae showed weak reaction for NMPS in group 1 which became weak to moderate in group 2 whereas in group 3 propria submucosa and core of papillae showed moderate reaction and muscles showed moderate to strong reaction (Figs c,d,f). Von Ebner's gland showed moderate reaction in group 2 and strong reaction in group 3. An intense PAS reaction has been reported in both serous and mucous acini of Von Ebner's gland in adult buffalo tongue (Lahkar 1985, Ramayya *et al.* 2000, Singh 2001). The basement membrane and connective tissue had moderate staining reaction.

Acid mucopolysaccharides (AMPS): The lamina epithelialis was weak to moderately positive for AMPS in group 1 and moderate to strongly positive in groups 2 and 3. The epithelium covering the papillae was weak to moderately

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Table 1. Histochemical distribution of neutral mucopolysaccharide, acid mucopolysaccharide, basic protein and total lipids in tongue during different stages of prenatal life

Structure	Group 1				Group 2				Group 3			
	NMPS	AMPS	Basic protein	Total lipid	NMPS	AMPS	Basic protein	Total lipid	NMPS	AMPS	Basic protein	Total lipid
Lamina epithelialis	++/+++	+ /++	+ /++	+ /++	++/+++	++/+++	++	++/+++	+ /++	++/+++	+++	++/+++
Papillae	#											
<i>Fungiform</i>												
Epithelium	+	+ /++	+ /++	+ /++	+ /++	+ /++	+ /++	++/+++	+ /++	+	++/+++	++/+++
Core	+	+ /++	+	+	+ /++	+ /++	+	+	++	++/+++	+ /++	+
<i>Circumvallate</i>												
Epithelium	+	+ /++	+ /++	+ /++	+ /++	+ /++	+ /++	++/+++	+ /++	+	++/+++	++/+++
Core	+	+ /++	+	+	+ /++	+ /++	+	+	++	++/+++	+ /++	+ /++
<i>Filiform</i>												
Epithelium	+	+ /++	+ /++	+ /++	+ /++	+ /++	+ /++	++/+++	+	+	++/+++	+
Core	+	+ /++	+	+	+ /++	+ /++	+	+	++	++/+++	+ /++	+ /++
<i>Conical</i>												
Epithelium	+	+ /++	+ /++	+ /++	+ /++	+ /++	+ /++	++/+++	+	+	++/+++	+
Core	+	+ /++	+	+	+ /++	+ /++	+	+	++	++/+++	+ /++	++/+++
<i>Lenticular</i>												
Epithelium	+	+ /++	+ /++	+ /++	+ /++	+ /++	+ /++	++/+++	+	+	++/+++	++/+++
Core	+	+ /++	+	+	+ /++	+ /++	+	+	++	++/+++	+ /++	+
Propria	+	+ /++	+	+	+ /++	+ /++	+ /++	+	++	++/+++	+ /++	+
submucosa												
Muscles	+	+ /++	+ /++	+	+ /++	+ /++	++	++/+++	++/+++	++/+++	+++	+++
Von Ebner's gland	*	*	*	*	++	++	++/+++	+ /++	+++	+++	++/+++	++

Dome shaped primitive papillae in group 1 and 2; *Absent; + weak; ++ moderate; +++ strong; NMPS, neutral mucopolysaccharides; AMPS, acid mucopolysaccharides.

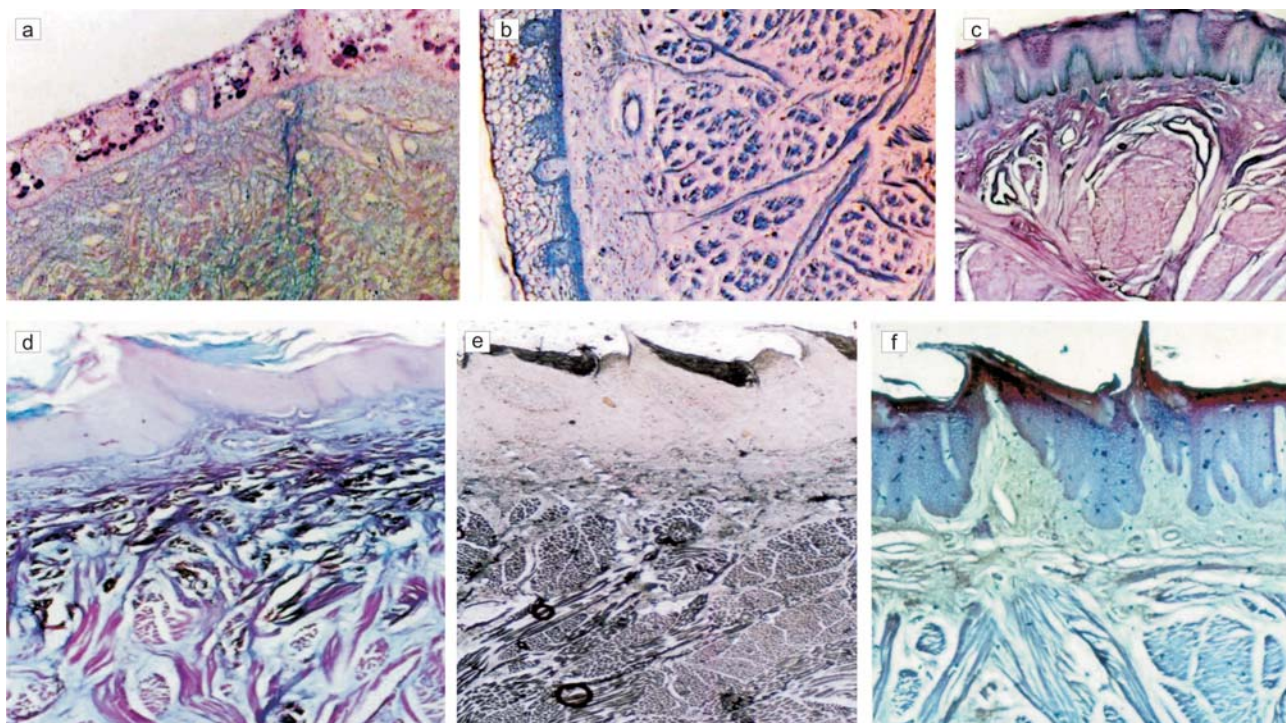
positive in group 1 and 2. All the layers of epithelium showed a weak reaction except for stratum corneum which was strongly positive. The core of the papillae was weak to moderately positive in group 1 and 2 but showed a moderate to strong reaction in group 3. Similarly propria submucosa and muscles were weak to moderately positive in group 1 and 2 but strongly positive in group 3 (Fig. d). The Von Ebner's gland were moderately positive in group 2 and strongly positive in group 3. A strong reaction was revealed by the cornified layer of the epithelium, earlier a moderate reaction was reported in the the cornified layer of the epithelium in adult buffalo tongue (Lahkar 1985, Ramayya *et al.* 2000 and Singh 2001).

Basic protein: In group 1 and 2 (Fig. b), epithelium and muscles showed weak to moderate reaction while the core of papillae and propria submucosa showed weak reaction for basic proteins. In group 3 lamina epithelialis and muscles showed strong reaction whereas the reaction in epithelium of papillae and Von Ebner's gland was moderate to strong. In the core of papillae and propria submucosa the reaction was weak to moderate. Singh (2001) reported in

buffalo tongue that the epithelium of tongue was strongly positive for basic proteins and secretory product of the acini. The content of basic protein was low to moderate in taste buds.

Lipids: In group 1, the epithelium was weak to moderately positive for Sudan Black B while the core of papillae, propria submucosa and muscles showed weak reaction. The epithelium showed moderate to strong reaction in group 2 and 3 while weak reaction was observed in the propria submucosa (Fig. e). The muscles showed moderate to strong reaction in group 2 and 3. Weak to moderate and moderate reaction was given by Von Ebner's gland in group 2 and 3 respectively. Singh (2001) reported moderate amount of lipid in the epithelium of the tongue of buffalo. The lipid content of the muscle was high as observed earlier in sheep and goat (Roy *et al.* 1992 and Singh *et al.* 1992).

The anterior aspects of filiform papillae contained the soft keratin producing cells which showed strong reaction and the epithelium covering the posterior aspect contains the hard keratin producing cells which have low affinity for Sudan Black B.



Figs a–f. **a.** 15.0 cm CVR length (96 days) foetus showing Alcian Blue reaction in epithelium (Ep) and propria submucosa (Ps). PAS-Alcian Blue×200; **b.** 21.4 cm CVR length (122 days) foetus showing Bromphenol Blue reaction. Bromphenol Blue×100; **c.** 39.0 cm CVR length (161.5 days) foetus showing Alcian Blue reaction in epithelium (Ep), propria submucosa (Ps) and bundles of muscle fibers (Mf). PAS-Alcian Blue×40; **d.** 85.5 cm CVR length (266 days) foetus showing Alcian Blue reaction in cornified layer of epithelium (Ep) and muscle fibers (Mf). Alcian Blue×40; **e.** 95.0 cm CVR length (288 days) foetus showing sudanophilic lipids in filiform papillae (Fi), propria submucosa (Ps) and muscle fibers (Mf). Sudan Black B×40; **f.** 95.0 cm CVR length (288 days) foetus showing Alcian Blue reaction in filiform papillae (Fi), propria submucosa (Ps) and muscle fibers (Mf). PAS-Alcian Blue×40.

SUMMARY

The present study was conducted on tongue of 36 buffalo foetii of CVRL 1.2 to 99.5 cm (age ranging from 34 to 298 days) divided into 3 groups. The tissues were processed and sections were stained with periodic acid Schiff, alcian blue and bromphenol blue to demonstrate neutral and acid mucopolysaccharides and basic proteins. For lipids, cryostat sections of fresh tissue were stained with Sudan Black B. The study revealed that neutral mucopolysaccharides contents was more in the differentiating epithelial cells in group 1. Acid mucopolysaccharides, basic proteins and lipids content in different structures of tongue were variable and has been discussed in relation to their significance with the period of prenatal life.

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