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DISTRIBUTION OF PHOSPHATASES, OXIDOREDUCTASES AND ESTERASES IN SUBLINGUAL SALIVARY GLAND OF BUFFALO DURING NEONATAL DEVELOPMENT

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ABSTRACT

The present study was aimed to elucidate the distribution of various enzymes in sublingual salivary gland of neonatal buffalo (n = 10), ranging from 1 to 3 months age, and correlate these enzyme systems with the secretory activity of gland. At 1 month age, a uniform moderate to strong positive diffused granular alkaline phosphatase activity was observed in serous acini and ductal epithelium. A moderate positive glucose-6-phosphatase and succinic dehydrogenase activity was seen close to the basement membrane. At 2 months age, glutamic dehydrogenase showed moderate activity in basal portions of serous acinar cells, while succinic dehydrogenase activity was strong, granular and evenly distributed in basement membrane of serous cells. Weak activity of monoamine oxidase and moderate non-specific esterase activity was seen. At 3 months age, alkaline phosphatase showed strong positive perinuclear activity in acinar cells, while glucose-6-phosphatase showed a moderate to strong activity in acinar cells and in ductal epithelium. Moderate to strong positive non-specific esterase activity was localized in nerve fibres of intralobular and interlobular connective tissues. Glucose-6-phosphate dehydrogenase and nicotinamide adenine dinucleotide diaphorase showed strong positive granular activity in basement membrane of serous acinar cells and ducts. The high basal activities of oxidoreductases were because of basal mitochondria and were associated with high energy production to meet the energy requirement for active and rapid transport of ions and water.

Key words: Buffalo, histoenzymic, neonates, sublingual salivary gland

INTRODUCTION

The sublingual salivary gland contributes to the secretion of saliva which plays vital role in maintaining ruminants healthy by facilitating mastication and deglutition, helping in restoration of normal ruminal pH and microbial protein synthesis to be used as dietary proteins. Saliva contains water, various enzymes, mucopolysaccharides and lubricating glycoproteins. It has an important role in providing lubrication for eating and vocalization, aids digestion and supplies saliva for pH buffering (Moghaddam *et al.,* 2009). In general, the major salivary glands in herbivores are better developed than those of carnivores. The salivary glands may be classified on the basis of their

secretions as serous, mucous or seromucous (mixed) glands. The distribution of these types varies from species to species (Konig and Liebich, 2004). Saliva is secreted into oral cavity via a series of ducts in ductal system. Dysfunction of salivary secretion (hyposalivation) causes xerostomia (dry mouth) and sequentially leads to severe dental caries as well as oral mucosal disorders (Featherstone, 2000). The salivary glands also secrete IgA, potassium and sodium (Aspinall and Reilly, 2004).

Enzyme histochemistry serves as a link between biochemistry and morphology. It can be used to locate many enzymes including phosphatases, dehydrogenases and esterases. Alkaline phosphatase is associated with ionic exchange across the membrane. It is found abundantly in endothelium of smaller blood vessels and also occurs in the cells specialized for endocytosis and pinocytosis. Glucose-6-phosphatase is related to carbohydrate metabolism. Succinic dehydrogenase is found in all aerobic cells and is essential part of Kreb's cycle. Diaphorases activities are indicative of mitochondrial activity and cytoplasmic electron transport. These enzymes serve as biochemical markers for tissue damage. Therefore, it is imperative to assess the accurate localization of these enzymes in sublingual salivary gland of buffalo during neonatal life and thereby correlate with the physiological functions of tissues. Various prenatal as well as postnatal studies have been done on this gland in gerbil (Ichikawa *et al.,* 1989), Arabian camel (Asgah *et al.,* 1990) and goat (Narasimhan *et al.,* 1999), however, there is no detailed information about the histoenzymic studies of buffalo sublingual salivary gland during neonatal development, therefore, the present work was aimed to observe the distribution of various enzymes in sublingual gland of neonatal buffalo.

MATERIALS AND METHODS

The fresh unfixed tissues from the sublingual salivary gland of buffalo neonates (n = 10), ranging from 1 to 3 months of age, were collected in 2014-15 winter and stored in liquid nitrogen. These tissues were subjected to cryostat sectioning at -20°C with Leica CM 1950 cryostat microtome. The sections of 10-12 µm thickness were obtained on clean glass slides and were incubated with different substrates at 37°C to study the distribution pattern of different enzymes. For alkaline phosphatase (AKPase) estimation, naphthol AS-MX phosphate disodium salt in combination with Fast Blue RR was used as substrate. After incubation for 30 min, the AKPase was estimated as per simultaneous coupling azo dye method of Barka and Anderson (1963). Glucose-6-phosphatase (G-6-Pase) was estimated by using glucose-6-phosphate and lead nitrate as substrate by lead nitrate method of Barka and Anderson (1963). Various oxidoreductase viz., succinic dehydrogenase (SDH), lactic dehydrogenase (LDH), glutamic dehydrogenase (GDH), glucose-6-phosphate dehydrogenase (G-6-PD), monoamine oxidase (MAO), nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase) and nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase) were estimated by standard method of bound enzyme by nitro BT method (Pearse, 1972). Non-specific esterase (NSE) was estimated by naphthol acetate method of Barka and Anderson (1963).

RESULTS AND DISCUSSION

At 1 month age, a uniform moderate to strong positive diffused granular alkaline phosphatase activity was observed in serous acini and ductal epithelium, however mucous cells showed doubtful to weak perinuclear activity. Asgah *et al.* (1990) observed that the sublingual salivary glands of

Arabian camel (*Camelus dromedarius*) had considerable activities of alkaline phosphatase, succinic dehydrogenase and non-specific esterase. Myoepithelial cells were also strongly positive. A moderate positive glucose-6-phosphatase activity was seen close to the basement membrane. This enzyme was firmly bound with mitochondrial framework (Chayen *et al*., 1969).

At 2 months age, the periductal nerve plexus showed strong alkaline phosphatase activities. Succinic dehydrogenase activity was strong, granular and evenly distributed in basement membrane of serous cells and interlobular duct (Fig. 1). Similar findings were observed in case of prenatal pig (Zhou *et al.*, 2010). Glucose-6-phosphate dehydrogenase activity was moderate to strong in serous acinar cells whereas mucous acinar cells showed weak activity. These results are in agreement with Ichikawa *et al*. (1989) in gerbil. Monoamine oxidase showed weak positive granular activity in acinar cells and epithelium of ducts (Fig. 2) and play a major role in the regulation of cellular metabolism (Chayen *et al*., 1969). Weak to moderate activity of non-specific esterase was seen in close vicinity of the striated ducts but serous cells showed moderate activity (Fig. 3). However non-specific esterase positive hypoluminal axons were observed in relation to striated duct and myoepithelial cells.

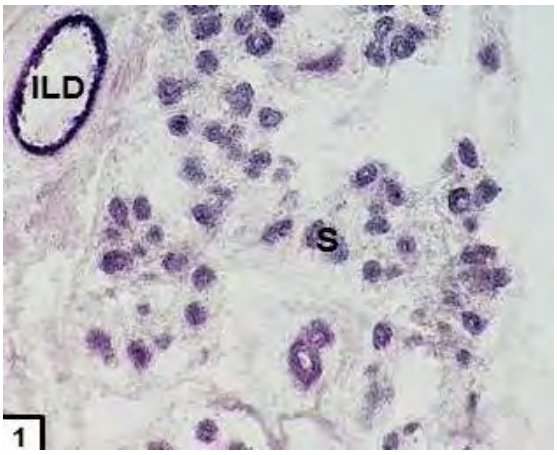


Fig. 1: Photomicrograph of sublingual salivary gland of neonatal buffalo showing strong positive succinic dehydrogenase activity in the serous acinar cells (S) and in the epithelium of interlobular duct (ILD) (Nitro BT method x 100)

At 3 months of age, alkaline phosphatase showed strong positive perinuclear activity in serous acinar cells while the ducts showed moderate activity (Fig. 4). These results are in agreement with Pospieszny *et al*. (2010) in case of pig. Glucose-6-phosphatase showed a moderate to strong activity in the acinar cells and in ductal epithelium, especially towards basal side. Similar results were reported in prenatal parotid salivary gland of buffalo (Singh and Singh, 2014). A higher activity of these phosphatases reflected higher secretory activity in sublingual salivary gland.

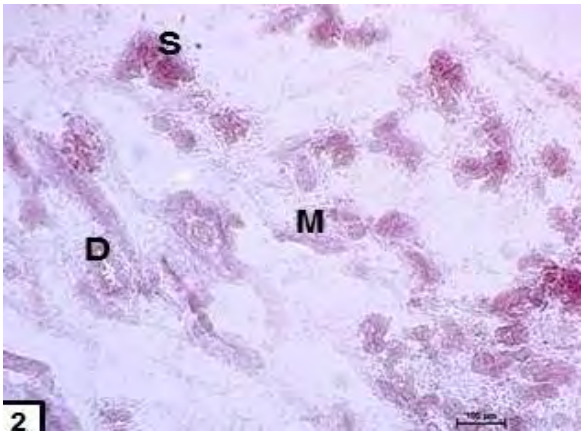


Fig. 2: Photomicrograph of sublingual salivary gland of neonatal buffalo showing weak positive granular monoamine oxidase activity in serous (S), mucous (M) and ductular (D) cells (Nitro BT method x 100)

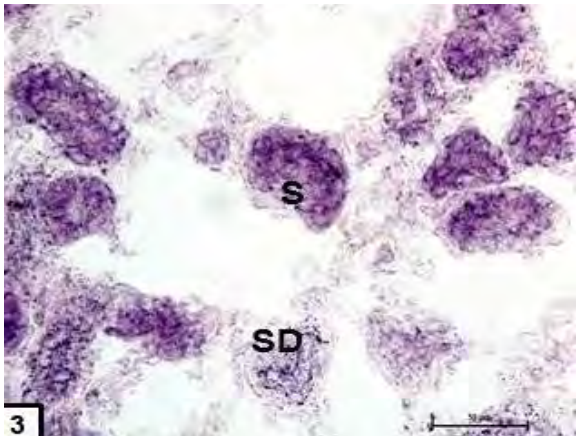


Fig. 3: Photomicrograph of sublingual salivary gland of neonatal buffalo showing weak to moderate activity of non-specific esterase in striated ducts (SD) but moderate activity in serous cells (S) (Naphthol acetate method x 400).

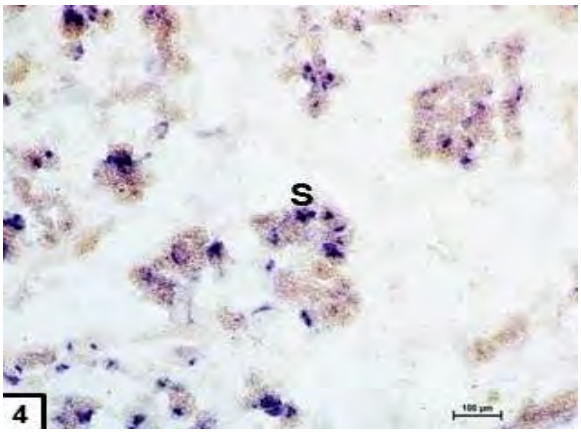


Fig. 4: Photomicrograph of sublingual salivary gland of neonatal buffalo showing strong positive alkaline phosphatase activity in the serous acinar cells (S) (Simultaneous coupling azo dye method x 100)

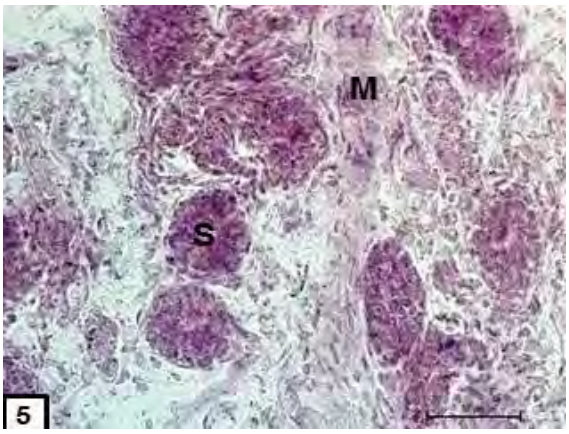


Fig. 5: Photomicrograph of sublingual salivary gland of neonatal buffalo showing strong positive glutamic dehydrogenase activity in serous acinar cells (S) and weak activity in mucous cells (Nitro BT method x 400)

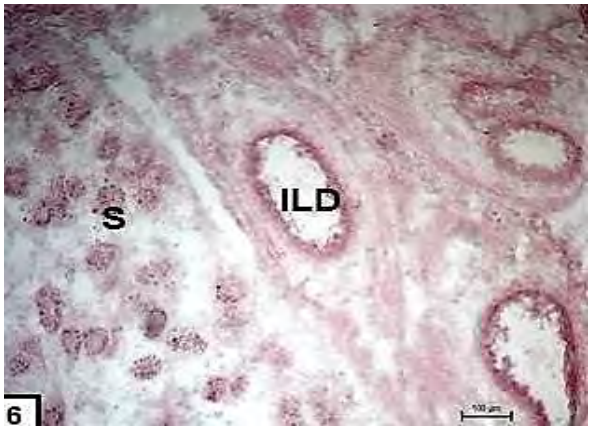
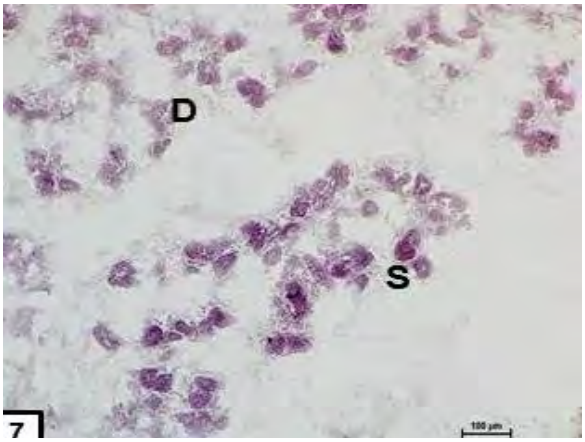


Fig. 6: Photomicrograph of sublingual salivary gland of neonatal buffalo showing fine, granular and more basal lactic dehydrogenase activity in serous acinar cells (S) as well as in interlobular ducts (ILD) (Nitro BT method x 100)



Narasimhan *et al*. (1999) reported that the lingual glands of goat showed mild alkaline phosphatase activity and moderate succinic dehydrogenase activity. Glutamic dehydrogenase showed strong activity in the basal portions of serous acinar cells while mucous acinar cells were devoid of it (Fig. 5). The lactic dehydrogenase activity was fine, granular and more basal in serous acinar cells as well as in interlobular ducts at 3 months of age (Fig. 6). Moderate non-specific esterase activity was localized in nerve fibres of intralobular and interlobular connective tissues. Glucose-6-phosphate dehydrogenase showed strong positive granular activity in the basement membrane of serous acinar cells and ducts (Fig. 7), however its activity was observed in the lumen of acinar cells and along the intercellular canaliculi in pig (Pospieszny *et al.,* 2010). A strong positive activity of nicotinamide adenine dinucleotide-diaphorase was observed in serous acinar cells and in ductal epithelium (Fig. 8) which was indicative of mitochondrial activity as well as including in cytoplasmic electron transport system (Chayen *et al.,* 1969). Similar

Fig. 7: Photomicrograph of sublingual salivary gland of neonatal buffalo showing strong positive glucose-6-phosphate dehydrogenase activity in basement membrane of serous acinar cells (S) and ducts (D) (Nitro BT method x 100)

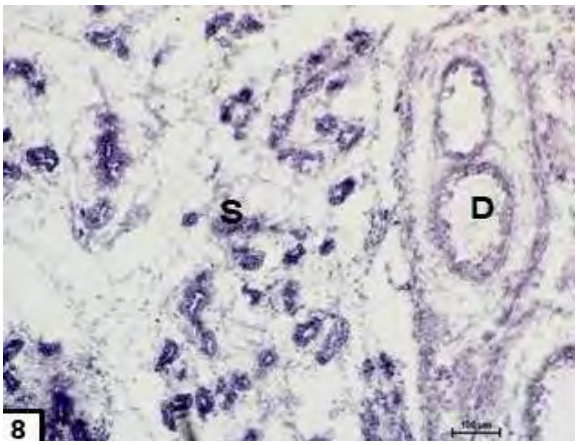


Fig. 8: Photomicrograph of sublingual salivary gland of neonatal buffalo showing strong nicotinamide adenine dinucleotide-diaphorase activity in the ductular epithelium (D) and in the serous cells (S) (Nitro BT method x 100)

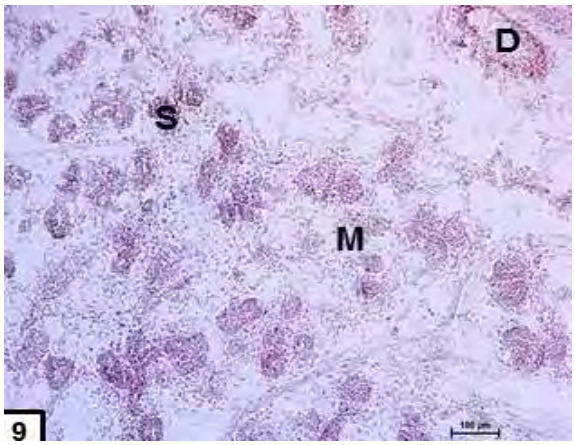


Fig. 9: Photomicrograph of sublingual salivary gland of neonatal buffalo showing moderate activity of nicotinamide adenine dinucleotide phosphate diaphorase in serous (S), mucous (M) and ductular (D) cells (Nitro BT method x 100)

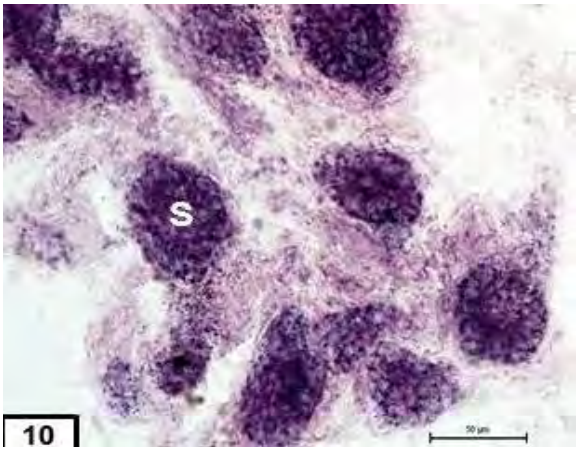


Fig. 10: Photomicrograph of sublingual salivary gland of neonatal buffalo showing strong positive succinic dehydrogenase activity in serous acini (S) (Nitro BT method x 400)

findings have been observed in submandibular salivary gland of prenatal buffalo by Singh and Singh (2014). Nicotinamide adenine dinucleotide phosphate diaphorase showed moderate activity in the acinar cells and ducts, especially towards basal side (Fig. 9). The basement membrane of serous acinar cells and striated ducts showed strong activity for succinic dehydrogenase (Fig. 10). The distribution of all these enzymes in sublingual gland of neonatal buffalo has been summarized in Table 1. Oxidoreductases were strongly demonstrated in serous acini and in ductal cells with higher activity in striated and interlobular ducts. The presence of oxido-reductases indicated increased physiological activity of different components of sublingual salivary gland. Similar findings have been noticed in the sublingual salivary gland of pig (Pospieszny *et al*., 2010). The strong activity of these enzymes in buffalo sublingual gland indicated that this species needs more energy and copious secretion for digestion of concentrate and roughage when compared to other ruminants. The overall distribution pattern of oxidoreductases in sublingual salivary gland of neonatal buffalo neonates reflected higher physiological activity in acinar cells. These enzymes probably formed a mutual complementary system regulating salivary buffer capacity (Parkkila *et al*., 1990). The striated and large excretory ducts exhibited strong activity for all these group of enzymes indicating metabolic activity of cells. The enzyme activity in duct epithelium increased with advancement of age.

Conclusion: The present study revealed that the sublingual salivary gland of buffalo neonates at 1 month age were moderate to strongly positive for phosphatases, oxidoreductases and esterases; however, strong activity was observed at 2 months age, which progressively increased with the

Table 1: Histoenzymic distribution of phosphatases, oxidoreductases and esterases in sublingual salivary gland of neonatal buffalo

Enzymes	Sublingual salivary gland								
	Group I			Group II			Group III		
	Serous cells	Mucous cells	Ducts	Serous cells	Mucous cells	Ducts	Serous cells	Mucous cells	Ducts
Phosphatases									
Alkaline phosphatise	++/+++	+	++/+++	+++	+	+++	+++	+	++
Glucose-6-phosphatase	++	0/+	++	+/++	0/+	++	++/+++	+	++/+++
Oxidoreductases									
Succinic dehydrogenase	++	+	++	+++	+	+++	+++	+	+++
Lactic dehydrogenase	++	0/+	++	++	+	++	++/+++	+	++/+++
Glutamic dehydrogenase	+/++	0/+	++	++	+	++	+++	+	
Glucose-6-phosphate dehydrogenase	++	+	++	++/+++	+	+/++	+++	+	+++
Monoamine oxidase	+/++	+	+/++	+	+	+	+/++	+	++
NADP diaphorase	+/++	0/+	++	+/++	0/+	++	++	+	++
NAD diaphorase	++	+	++	++	+	++	+++	+	+++
Esterases									
Non-specific esterase	+/++	0/+	++	++	0/+	+/++	++/+++	+	++

Group I = 1 month; Group II = 2 months; Group III =3 months; 0 = negative; + = weak; ++ = moderate; +++ = strong

advancement in age. Strong to intense activity was observed at 3 months age, which reflected higher secretory activity of sublingual salivary gland. The serous acinar cells as well as ductal epithelium showed positive activity to these group of enzymes. The high basal activities of oxidoreductases were because of basal mitochondria and were associated with high energy production to meet the energy requirement for active and rapid transport of ions and water.

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