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HISTOCHEMICAL STUDIES OF CARBOHYDRATES, ACID MUCOPOLY-SACCHARIDES AND PROTEINS IN THE TRACHEA OF BLACK BENGAL GOAT (C*apra hircus*)

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ABSTRACT

Histochemical studies of carbohydrates and proteins were conducted on the trachea of ten adult healthy Black Bengal goats (*Capra hircus*) of either sex. The goblet cells, tracheal glands and tracheal cartilages manifested intense positive reactions generally for carbohydrates, whereas connective tissue and muscle fibres were weak to moderately positive for these constituents. The epithelium, glands, cartilage matrix, perichondrium and muscle layers manifested strong reactions for acid mucopolysaccharides. Reaction for proteins was strong to intense in the epithelium, glands, perichondrium and muscle fibres, whereas other structures showed only weak or moderate reaction.

Keywords: Acid mucopolysaccharides, Black Bengal goat, carbohydrates, histochemistry, proteins, trachea

INTRODUCTION

Respiratory system performs vital functions in the body. Besides being responsible for conduction and exchange of gases, the respiratory organs are concerned with many other important functions like phonation, olfaction, body temperature regulation, excretion, acid-base balance, blood pressure maintenance, production and removal of many substances like histamine, prostaglandin E and prostaglandin F, etc., protection by means of muco-ciliary apparatus, alveolar macrophages, etc. Thus respiratory organs have a vital significance in the animal body on one hand and their disorders are fatal simultaneously on the other hand (Banks, 1993). Owing to the wide distribution and significant role of carbohydrate complexes and proteins in body tissues, the histochemical differentiation of these constituents in normal and pathological tissues has gained much importance in recent years (Culling *et al*., 1985). Since the trachea constitutes an important component of the bronchial tree in respiratory tract, the exploration of these constituents in its various structures could be useful in understanding the various aetiological factors responsible for disorders of these structures resulting into numerous diseases and malfunctions of respiratory tract (Pearse, 1968; Culling *et al*., 1985). Keeping the above facts in view, the present study was aimed to elucidate the intensities of carbohydrate complexes and proteins in various structures of the trachea of Black Bengal goat.

MATERIALS AND METHODS

The tracheas of 10 adult healthy Black Bengal goats of either sex of 1-2 year age, collected from the slaughter houses of Ranchi (Jharkhand), India were utilized for the present study. The specimens were fixed in 10% neutral buffered formalin. Small tissue pieces were collected from different representative

areas of tracheas. The tissues were processed by routine paraffin embedding technique. The paraffin sections were obtained at 6 µm thickness. Moreover, cryostat sections form fresh tissues were obtained at 15 µm. The sections were stained for carbohydrates by PAS stain without counter staining (Culling *et al.,* 1985), PAS stain with counterstaining (Humason, 1967); acid mucopolysaccharides by alcian blue method without counterstaining (Culling *et al.,* 1985), alcian blue method with counterstaining (Drury and Wallington, 1967) and proteins by mercuric bromophenol blue method (Humason, 1967) and Millon reaction (Culling *et al.,* 1985). Observations were done under compound microscope at 100, 400 and 1000 X magnifications.

RESULTS AND DISCUSSION

Histochemical studies of goat trachea revealed that goblet cells manifested intense reactions for carbohydrate complexes (Fig. 1). This suggested that their secretions contained high concentrations of PAS-positive materials. Plopper and Adams (1993) pointed out that the goblet cells of most species secreted primarily a sulphated glycoprotein as a major component of mucus. Banks (1993) proposed that mucous produced by goblet and mucous cells of submucosal glands of bronchial tree are glycoprotein in nature consisting of more than 50% carbohydrates. He, however, indicated that the nature of secretion could vary from one species to another. Many factors reportedly influence the secretory characteristics of these cells, and the glycoproteins present in mucins are rich in sulphated amino sugars and uronic acids termed as 'sulphomucins' or sialic acid called 'sialo-mucins'. Thus, the intense positivity shown by goblet cells for polysaccharides in present study indicated the presence of glycoproteins in mucus secreted by these cells. Further, goblet cells showed different phases of activity, some full with secretory materials, others pouring them onto the epithelial layer and some others already emptied. As per Banks (1993), many factors influence the secretory activities of uni-cellular glands including various anatomical and physiological conditions, metabolic status, environmental factors, apart from the conditions like stress, infection, surface irritation, etc. The present findings are in line with Yasear and Hassan (1992) who found these substances in goblet cells and tracheal glands of sheep and goats. Kahwa and Purton (1996) claimed that acidic and mixed muco-substances are produced by various epithelial cells of trachea in goats. In present study, epithelium of trachea excluding goblet cells exhibited only a weak reaction for carbohydrates in all the areas.

The tracheal glands showed intense positivity for carbohydrate complexes (Fig. 2). The glands seemed to be under different phases of activities. Similar observations were made by Mariassy *et al.* (1988) in sheep and Yasear and Hassan (1992) in sheep and goats. The tracheal cartilage exhibited intense positive reactions for carbohydrates and the perichodrium showed moderate to strong positivity in different portions of trachea (Fig. 1). The tracheal epithelium exhibited moderate to strong positive reactions for acid mucopolysaccharides (Fig. 3). The goblet cells showed weak reaction whereas lamina propria-submucosa exhibited strong reactions. The tracheal glands were generally strongly positive for acid mucopolysaccharides. The observation was in agreement with Banks (1993) and Plopper and Adams (1993). The reaction for these substances was strong in cartilage matrix and mild in chondrocytes whereas perichondrium showed strong to intense reaction (Table 1).

The epithelium manifested moderate to strong positive reaction for proteins (Fig. 4). A weak or negative reaction was observed in the goblet cells suggesting that mucins produced by these cells were either devoid of or possessed only meager quantities of proteins. The lamina propria-submucosa exhibited moderate reactions for proteins (Table 1). Different intensities varying from moderate to strong were noticed in the tracheal gland cells, suggesting various phases of their secretory activities attributable to various factors. Banks (1993) indicated that the glandular secretions of tracheo-bronchial glands of respiratory organs in domestic animals contained proteins in simple forms and conjugated proteins in form of glycoproteins. Further, an iron-binding protein, *lactoferrin* was present in their secretion which was responsible for immune protection against bacteria gaining access in the system.

Table 1: Histochemical reactions for carbohydrates, acid mucopolysaccharides and protein in the trachea of Black Bengal goats

Tracheal portion	Characters Studied	Epithelium	Cilia	Goblet cells	Basement membrane	Lamina propria submucosa	Tracheal glands	Cartilage matrix	Chondrocytes	Perichondrium	Blood vessel	Tracheal muscle	Tunica adventitia
Anterior portion	Carbohydrates	+	- to ±	++++	++	+	++++	++++	++++	++	++	++	+
	Acid mucopolysaccharides	++ to +++	+	+	++	+++	+++	+++	+	+++	+++	+++	++
	Protein	++ to +++	+	± to +	+++	++	++ to +++	±	++	++++	+++ to ++++	++++	++
Middle portion	Carbohydrates	+	±	++++	++	+	++++	++++	++++	+++	++	++	+
	Acid mucopolysaccharides	++ to +++	+	+	++	+++	+++	+++	+	+++ to ++++	+++	+++	++
	Protein	++ to +++	+	± to +	++ to +++	++	++	±	++	++++	+++ to ++++	++++	++
Posterior portion	Carbohydrates	+	±	++++	++	+	++++	++++	++++	++ to +++	++	++	+
	Acid mucopolysaccharides	++ to +++	+	+	++	+++	+++	+++	+	+++	+++	+++	++
	Protein	+++	+	± to +	+++	++	++ to +++	±	+ to ++	+++ to ++++	++++	++++	++
Extra-pulmonary bronchi	Carbohydrates	++	+	++++	+++	+ to ++	++++	++++	++++	+++	+++	++	++
	Acid mucopolysaccharides	++ to +++	+	+	++	+++	+++	+++	+	+++ to ++++	+++	+++	++
	Protein	+++	+	+	+++	++	++	±	+ to ++	++++	++++	++++	++

Note: ++++ = Intense reaction, +++ = Strong reaction, ++ = Moderate reaction, + = Mild (Weak) reaction, ± = Negative/weak reaction and - = Negative reaction

Although perichondrium generally manifested intense reaction for proteins, the matrix of tracheal cartilages showed negative to mild reaction and mild to moderate intensities in chondrocytes. The positivity of proteins in perichondrium was suggestive of its supportive and protective role. Blood vessels and muscle fibres of tracheal muscle generally exhibited intense positivity for proteins.

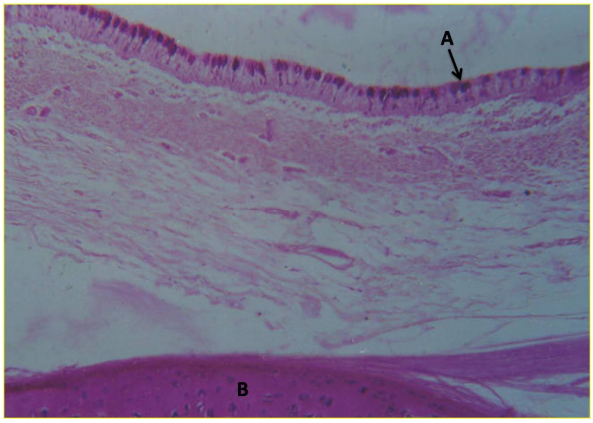

Fig. 1: Section of trachea of goat showing intense reactions for PAS in goblet cells (A) and cartilage (B). PAS x 100

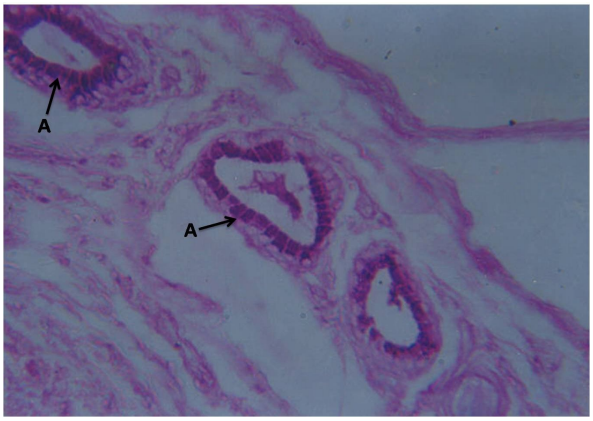

Fig. 2: Section of trachea of goat showing intense reactions for PAS in glands (A). PAS x 400

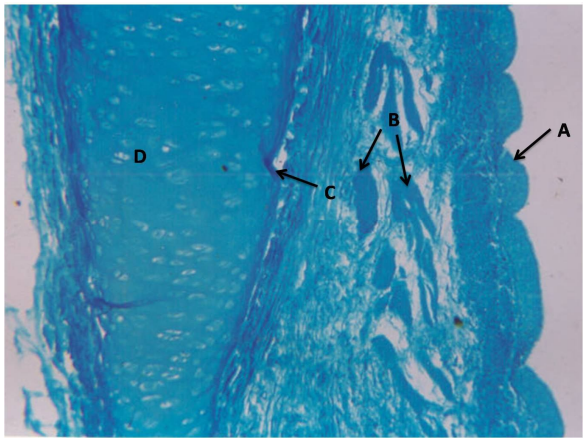

Fig. 3: Section of the trachea of goat showing different intensities of reactions for acid mucopolysaccharides in epithelium (A), muscle fibres (B), perichondrium (C) and cartilage (D).Alcian Blue Stainx100

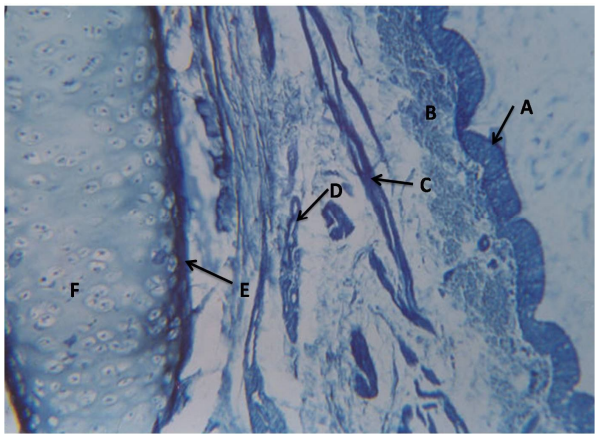

Fig. 4: Section of the trachea of goat showing different intensities of reactions for proteins in epithelium (A), connective tissue layers (B), muscle fibres (C), glands (D), perichondrium (E) and cartilage (F). Mercuric Bromophenol Blue Stainx100

REFERENCES

Banks, W.J. 1993. *Applied Veterinary Histology* (3rd edn.). Mosby Year Book, St. Louis, USA.

Culling, C.F.A., Allison, R.T. and Barr, W.T. 1985. *Cellular Pathology Technique* (4th edn.). Butterworth and Co. Ltd., London, UK.

Drury, R.A.B. and Wallington, E.A. 1967. *Carletons's Histological Technique* (4th edn.). Oxford University Press, New York, USA.

Humason, G.L. 1967. *Animal Tissue Techniques* (2nd edn.). W.H. Freeman and Co., San Francisco, USA.

Kahwa, C.K.B. and Purton, M. 1996. Histological and histochemical study of epithelial lining of the respiratory tract in adult goats. *Small Ruminant Research,* **20**: 181-186.

Mariassy, A.T., Plopper, C.G., George, S.T. and Wilson, D.W. 1988. Tracheobronchial epithelium of the sheep. IV. Lectin histochemical characterization of secretory epithelial cells. *Anatomical Record*, **222**: 49-59.

Pearse, A.G.E. 1968. *Histochemistry: Theoretical and Applied Volume I* (3rd edn.), Churchill Livingstone, Edindurgh, UK.

Plopper, C.G. and Adams, D.R. 1993. Respiratory system. pp. 136-152. **In:** *Textbook of Veterinary Histology* (4th edn.). (ed. H.D. Dellmann), Lea and Febiger, Philadelphia, USA.

Yasear, A.Y. and Hassan, N.H. 1992. Histomorphological study on the distribution of cells producing mucin in the trachea of sheep and goats. *Iraqi Journal of Veterinary Sciences,* **5**: 127-134.