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Comparative Histochemical Study of Mucin in Colonic Goblet Cells of Albino Rat, Goat & Dog

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Abstract:
Introduction: *Goblet cells secrete high molecular weight glycoprotein known as mucin which forms a protective mucus blanket throughout the G.I.T. The present work characterizes histochemically different types of mucin in colonic goblet cells & makes a comparative study in animal species of different food habit.*
Methods:- *After ethical clearance tissues were taken from the proximal part of colon of albino rat, goat and dog, processed and stained with Alcian blue pH-1 ,Alcian blue pH-2.5, Aldehyde Fuschin ,Periodic acid Schiff ,combination stains of Aldehyde Fuschin- Alcian blue pH- 2.5 and Periodic acid Schiff- Alcian blue pH 2.5.*
Result: - *Neutral mucin is present in small amount in colonic goblet cells of Albino rat and dog whereas traces in goat. Weakly sulphated acid mucin and sialomucin is present in substantial amount in Albino rat and moderate amount in goat and dog .Highly sulphated acid mucin is present in substantial amount in Albino rat and goat but moderate amount in dog. Acid mucin is predominantly found in colonic goblet cells of all these three animals.*
Discussion: *A comparative study indicated that there is similarity in the type of mucin secreted by colonic goblet cells of goat which is a herbivore, dog a carnivore and albino rat an omnivore. Both types of mucins i.e acid and neutral are present with predominance of acid mucin in their colonic goblet cells. Hence, food habit seems to have negligible impact on the type of mucins secreted by colonic goblet cells.* ***Conclusion :-****Food habits seem to have negligible impact on the type of mucin secreted by colonic goblet cells in animals.*
Keywords: *Goblet cells, Mucin, Ascending colon.*

I. Introduction

Goblet cell is a unicellular gland that secretes mucin. The formation of mucous in the basal part, its elaboration in the apical part and finally its expulsion by exocytosis has been described as different functional states of goblet cells (Moe, 1955,, Merzel & Lablond 1958, Freeman, 1966)[1,2,3].

Mucin,secreted by goblet cells forms a blanket of mucous on the mucosal surface of the intestine. This protective function is not only limited against the acidic gastric contents which reach the proximal part of duodenum , but also help in making the mucosa of intestine smooth and slippery for onward passage of food. This explains the gradual increase in the number of goblet cells from proximal to distal part of intestine, this naturally has led investigators in the direction of finding out the chemical nature of mucin.

Mucins have been referred to as mucopolysaccharide , glycosaminoglycanes (Jeanloz 1960)[4] and mucosubstances (Spicer et al 1960) [5]. More recently the term glycoconjugates has been suggested (Reid & clamp 1978) [6].

Histochemically mucins are classified into epithelial mucins and connective tissue mucins. The present study is restricted to epithelial mucins.

Epithelial mucins are further classified into: - Neutral mucins and acid mucins. Depending on sialic acid group or ester sulphate group acid mucins are classified into sialomaucins and Sulphomucins. (Reid J. Clamp JR 1978)[6].

Gastrointestinal tract is mainly concerned with digestion and absorption of food. It is expected that the food habit of animals might have some bearing on the type of secretion by goblet cells. As such, in the present work an effort is being made to histochemically characterize the different types of mucins in colonic goblet cells and make a comparative study in animals of different food habits.

II. Material & Methods

Materials: Animals of different food habits were chosen viz a herbivore, goat (Capra Indica), a carnivore dog (Canis Familiaris), and an omnivore albino rat (Rattus Norvegicus). After ethical clearance tissues were taken from the proximal part of ascending colon of these animals. Albino rat was anesthestised in laboratory under closed chloroform anesthesia after which it was dissected, the specimen from dog was obtained by anesthetising it with sodium barbitone and then dissecting it. Goat's specimen was taken from slaughter's house immediately after it was sacrificed.

Methods:-The obtained specimen was fixed in 2% calcium acetate in 10 % formalin. Then dehydrated by passing them through ascending grades of alcohol , cleaned in three changes of 1% celloidin in methyl benzoate, then passed through three changes in benzene then embedded in paraffin wax.All specimens were cut at a thickness of 6*micron* and were mounted on slides using egg albumin as adhesive. These slides were incubated for 24 hours at 37degree centigrade in incubator.

For histochemical study prepared slides were subjected to various staining techniques. Alcian blue pH 1 and aldehyde fuschin for highly sulphated acid mucin. Alcian blue pH 2.5 for weakly sulphated acid mucin and sialomucin. Simultaneous demonstration of highly and weakly sulphated acid mucin was done by using combination stains of alcian blue pH 2.5 – aldehyde fuschin and for simultaneous demonstration of acid and neutral mucins alcian blue pH 2.5 – periodic acid schiff was used. The results were tabulated and presence of acid and neutral mucins along with their amount of presence was compared in the above mentioned animals of different food habits.

III. Observations And Results

Histochemical study of the colonic goblet cells of animals of different food habits revealed the following results with alcian blue pH1 albino rat which is an omnivorous showed deep blue stain indicating the presence of substantial amount of highly sulphated acid mucin whereas, goat which is a herbivore and dog, a carnivore, goblet cells stained blue showing moderate amount of highly sulphated acid mucin..

Alcian blue pH 2.5 staining showed deep blue stained goblet cells in albino rat (Fig 1) and blue stained goblet cells in goat (Fig 2) and dog (Fig 3). This indicated the presence of weakly sulphated acid mucin and sialomucin in substantial amount in albino rat and in moderate amount in goat and dog .

Periodic acid Schiff stain in albino rat and dog gave the same picture in which goblet cells stained light magenta indicating the presence of small amount of neutral mucin. Goat's goblet cells showed very mild magenta stain indicating presence of traces of neutral mucin. With aldehyde fuschin albinorat and goat gave the same picture where their goblet cells stained deep purple indicating presence of substantial amount of highly sulphated acid mucin. Dog showed purple stained goblet cells indicating presence of moderate amount of highly sulphated acid mucin.

The combination stain by alcian blue pH 2.5- periodic acid schiff revealed moderate to deep blue and light magenta stained goblet cells in albino rat (Fig 4) indicating the presence of substantial amount of weakly sulphated acid mucin and small amount of neutral mucin. Whereas, in goat moderate blue and very mild magenta stains were taken by goblet cells (Fig 5) showing moderate amount of weakly sulphated acid mucin and traces of neutral mucin. In dog the goblet cells took moderate blue and light magenta stains (Fig 6) indicating the presence of moderate amount of weakly sulphated acid mucin and small amount of neutral mucin. For simultaneous demonstration of highly and weakly sulphated mucins alcian blue pH 2.5 – aldehyde fuschin was used which showed deep blue purple stained goblet cells in albino rat which indicated the presence of substantial amount of both weakly and highly sulphated acid mucin.Goat showed deep purple blue stained goblet cells (Fig 7) indicating presence of substantial amount of highly sulphated acid mucin and moderate amount of weakly sulphated acid mucin. Dogs goblet cells took bluish purple stain (Fig 8) showing presence of moderate amount of both weakly and highly sulphated acid mucin.

Thus in this study we found that both neutral and acid mucins are present in colonic goblet cells of the three animals with slight difference in the amount of mucins. Highly sulphated acid mucin is present in substantial amount in Albino rat and goat but in moderate amount in dog. Weakly sulphated acid mucin is present in substantial amount in albino rat but moderate amount in goat and dog. Sialomucin is present in moderate amount in dog and goat whereas albino rat has this in substantial amount. Neutral mucin is found in traces in goat but in small amount in albino art and dog. Comparative result has been tabulated in **Table -1**

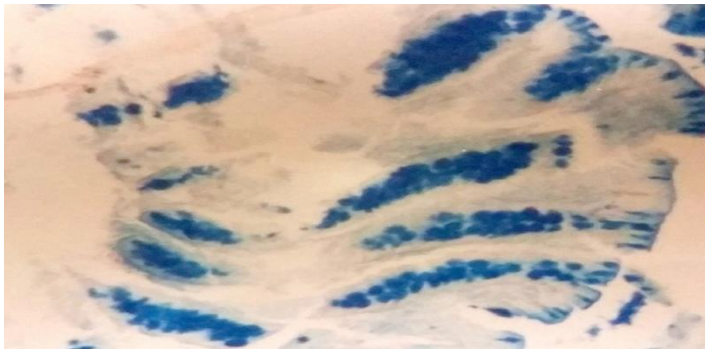

Fig.-1 Albino rat: - Ascending colon showing deep blue stained Goblet cells. (Alcian blue pH 2.5×80)

Fig-2 Goat: - Ascending colon showing moderately blue stained Goblet cells.(Alcian blue pH 2.5×80)

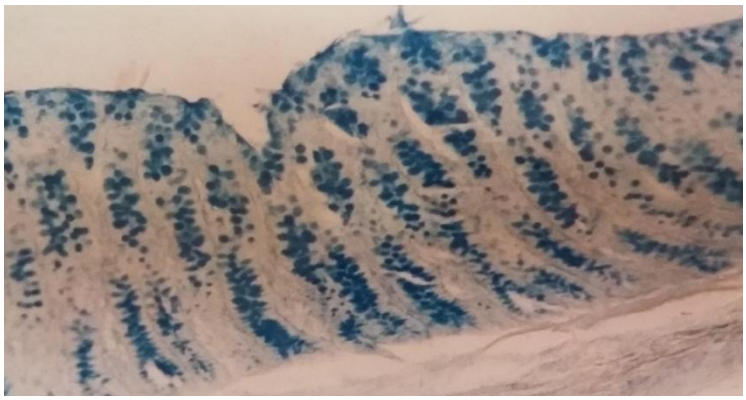

Fig-3 Dog: - Ascending colon showing moderately blue stained Goblet cells. (Alcian blue pH 2.5×80)

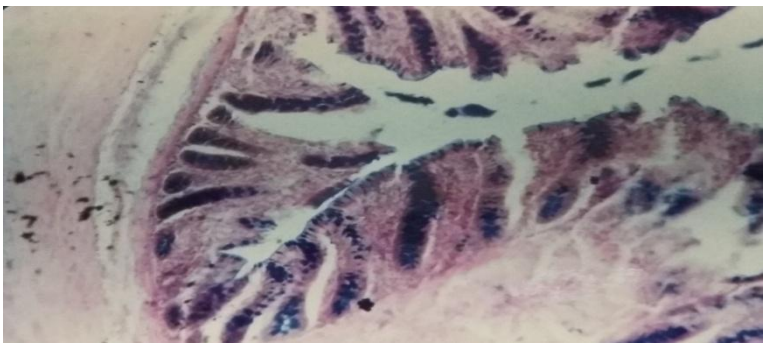

Fig.-4 Albino rat: - Ascending colon showing moderate to deep blue and light magenta stained Goblet cells. (Alcian blue pH 2.5-PAS×80)

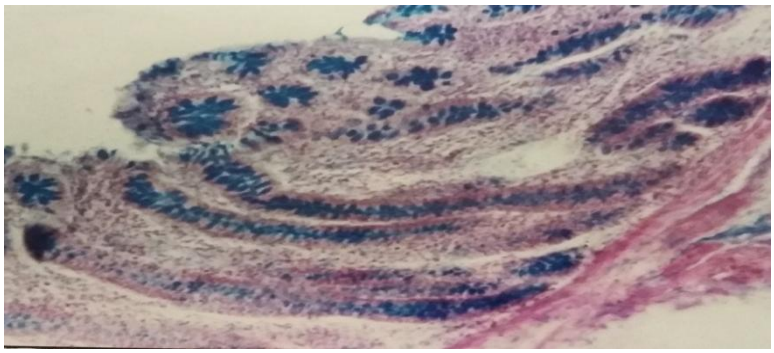

Fig-5 Goat: - Ascending colon showing moderate blue and traces of magenta stained Goblet cells.(Alcian blue pH 2.5-PAS×80)

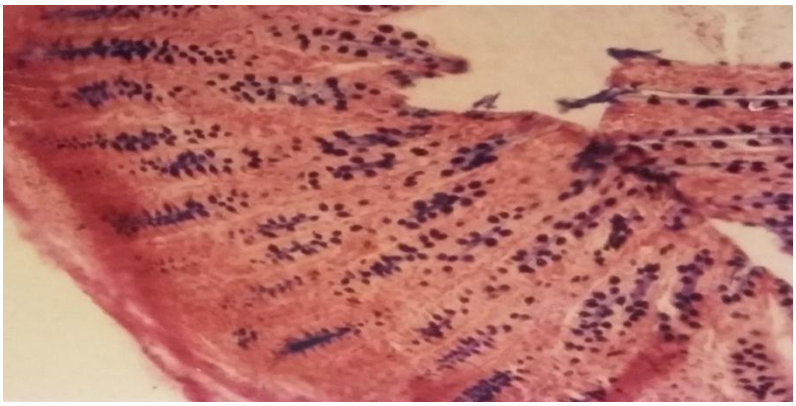

Fig-6 Dog- :- Ascending colon showing moderate blue and light magenta stained Goblet cells.(Alcian blue pH 2.5-PAS×80)

Fig-7 Goat: - Ascending colon showing deep purple blue stained Goblet cells (Aldehyde Fuschin- Alcian blue 2.5×80)

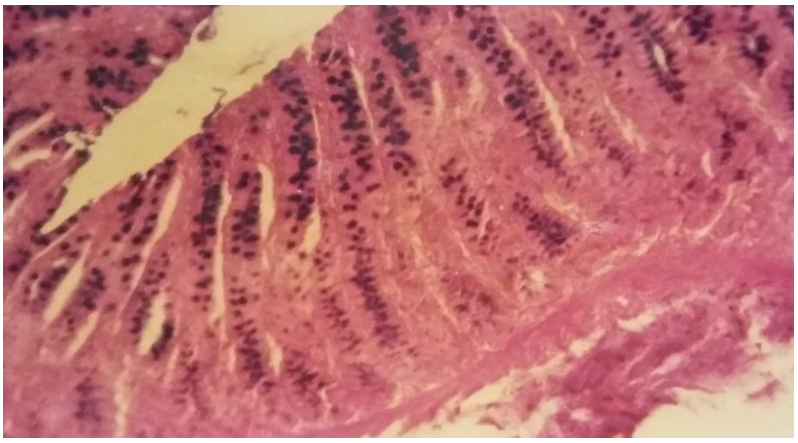

Fig-8 Dog: - Ascending colon showing bluish purple stained Goblet cells (Aldehyde Fuschin- Alcian blue2.5×80)

S.NO	Histochemical Stains	Albino Rat	Goat	Dog
1.	Alcian Blue PH-1	+++B	++B	++B
2.	Alcian Blue PH-2.5	+++B	++B	++B
3.	Aldehyde Fuschin	+++P	+++P	+++P
4.	PAS	+M	±M	+M
5.	Alcian Blue PH-2.5 -PAS	+++B +M	++B ±M	++B +M
6.	Alcian Blue PH-2.5- Aldehyde Fuschin	+++B +++P	++B +++P	++B ++P

Table-1:-Showing Comparative Histochemical Results in Animals of Different Food Habits
Keys to Symbols in Table:-

-ve =Negative staining.
± =Weak Staining.
+ = Slight Staining.
++ =Moderate Staining.
+++ =Strong Staining.
B =Blue.
M = Magenta
P =Purple.

IV. Discussion

In the present work an attempt has been made to identify the various types of mucins present in colonic goblet cells of animals of different food habits. A comparision among albino rat an omnivore, goat a herbivore and dog a carnivore has been demonstrated .Histochemical characterisation of mucins of colonic goblet cells, as undertaken, in this work is based on the staining procedures under different conditions of pH as suggested by different workers from time to time. (Gomori 1954, Mowry 1956)[7,8].

The precise occurrence of weakly sulphated , highly sulphated, sialomucin and neutral mucins has been studied in this work applying various histochemical methods as suggested by various workers. (Spicer 1967, Pearse 1968)[9,10].

The acidic sulphated mucins show affinity for cationic dyes like alcian blue at different pH level depending upon the degree of sulphation of mucins. The non- sulphated mucin found in secretory epithelium is sialic or sialomucin .Histochemically and chemically different from them is the neutral mucin.

All these mucins react variably to various histochemical tests which have been applied in this work. Identification of highly sulphated acid mucins is done using alcian blue pH 1 in which they stain deep purple.

Weakly sulphated acid mucins and sialomucins give blue stain , with alcian blue pH 2.5. Neutral mucins lack an acid radical and are periodic acid Schiff positive giving magenta stain. Simultaneous demonstration of highly and weakly sulphated acid mucins using aldehyde fuchsin – alcian blue pH 2.5 sequence gives bluish purple stain if both weakly and highly sulphated mucins are present .

If only highly sulphated mucin is present purple stain is given and only blue for presence of weakly sulphated acid mucin.

Simultaneous demonstration of neutral and acid mucins using alcian blue pH 2.5- Periodic acid Schiff gives bluish magenta when both acid and neutral mucins are present, only magenta when neutral mucin is found whereas only blue if acid mucin is present.

In the present work both acid and neutral mucins were observed in the colonic goblet cells of all the three animals with quantitative differences .Predominantly acid mucins were found which only same amount of neutral mucins.

Substantial amount of highly sulphated acid mucin was present in both albino rat and goat whereas dog had this in moderate amount. Moderate amount of weakly sulphated acid mucin and sialomucin were found in dog & goat whereas albino rat had the both in substantial amount.

Neutral mucin was found in traces in goat and in small amount in dog and albino rat.

No such comparative study of presence of type of mucin in colonic goblet cells has been found except one (Martin 1961) [11] who studied it in dog, rabbit , guineapig and rat but studied them using only periodic acid Schiff and mucicarmine stains . He found goblet cells to stain differently with periodic acid schiff in different animals. In rat they stained weakly and in dog they gave intense reaction.

The presence of weakly and highly sulphated acid mucins in intestinal goblet cells have been variably reported by different workers and to a certain extent confirm the present observations in the animals in which these mucins have been studied . However there is lack of comparative study.

Presence of sulphated acid mucin in the duodenal; goblet cells of ferret has been reported (Poddar & Jacob 1979)[12]. Gobelt cells of small intestine to contain both highly and weakly sylphated mucins have been studied (Siponen 1979).

Presence of sialomucins and neutral mucin in duodenum and colonic goblet cells of ferret was reported (Poddar & Jacob 1979)[12].

Colonic goblet cells in proximal colon to contain abundant sialomucin in the lower third of crypts in rat was also reported (Shamsuddin & Trump 1981)[13]. And this finding correlated with the finding of present study regarding colonic goblet cells of Albino rat.

Subbuswamy 1971[14] ,has noted that the goblet cells in small intestine produce predominantly neutral mucin while those of the colon produce predominantly acid mucin in human which is in keeping with observation recorded in the present work but in animals and further corroborated by another work which

reported sulphomucin in colonic goblet cells of rat (Shamsuddin & Trump 1981)[13]. Similar conclusion was reached in a study of colonic goblet cells associated with carcinoma in man (Dawson & Filipe 1982)[15].
However, there is no study which gave a comparative histochemical report of colonic goblet cells mucins and its correlation with the food habits of animals. Thus a comprative study in the present work gave a clear picture that food habits of animals have no impact on the type of mucins produced by colonic goblet cells.

V. Conclusion

Histochemical study of colonic goblet cells in animals of different food habits revealed a similar picture of presence of type of mucins with a slight quantitative difference. Both acid and neutral mucins with predominance of acid mucin are found in colonic goblet cells of albino rat , goat and dog who are omnivore, herbivore and carnivore respectively. Thus, food habits seem to have negligible impact on the type of mucin secreted by colonic goblet cells in animals.

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