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Histochemical studies on the rectum of pig (*Sus scrofa domesticus*)

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Abstract
Ten rectums from recently slaughtered adult pigs were used in the current experiment. The tunica muscularis and lamina muscularis responded positively to the McManus PAS stain for glycogen, while the lining epithelium and tunica submucosa did not. Following treatment with saliva, all layers of the rectum for McManus demonstrated PAS-negative reactions for glycogen and mucopolysaccharide. The lining epithelium stained with PAS-Alcian blue pH-2.5 responded strongly positively to the sulphated mucosubstance, however, it reacted only moderately and negatively to PAS-Alcian blue pH-1.0 and pH-0.4, respectively. The keratinized stratified squamous epithelium showed a positive reaction for pre-keratin and keratin but simple columnar epithelium showed a negative reaction for pre-keratin and keratin in Dane's stain.

Keywords: Histochemical, pig, rectum, tunica, and PAS stain

Introduction
The rectum is the last segment of the alimentary canal, and between the pelvic brim and the anus, it almost runs straight. It has thicker and more dilatable walls than the rest of the intestines. The mesorectum, which is the continuation of the mesenteric folds, keeps it attached to the dorsal wall of the pelvic cavity (Raghavan, 1964) [9].
Feces are temporarily stored within the rectum. Through the descending colon, feces are transported to the rectum by the regular muscular contractions known as peristalsis. The rectal walls stretch as the contents fill it from within, stimulating stretch receptors from the nervous system located there causing the need to pass waste product, a process known as defecation. (Barrett, 2019) [2].
Body temperature as measured from the rectum. In order to increase the amount of drug that reaches the systemic circulation with the least amount of modification, several medications are also taken via the rectal route. The per-rectal route is said to be a more efficient way to provide drugs (Lowry, 2016) [5].
Structural similarity between the human and pig rectum was determined using histological and morphological techniques. Based on this finding, we may conclude that pigs can be used as bio-models in medical studies to rebuild rectal pathology and the creation of a fresh approach to illness prevention using proctological disease diagnosis and therapy (Plakhotnyi *et al.*, 2021) [8]. Interest in conducting the current study and clarifying the histology of the rectum of the pig was prompted by a shortage of previous research on the rectum of pigs.
The current study subject was the mature rectum of a pig (*S. s. domesticus*). The research samples were subjected to histochemical analyses at the CVAS, Bikaner, Department of Anatomy.

Materials and Methods
The rectum of a mature pig (*S. s. domesticus*) was selected as the subject of the current study. In the Department of Anatomy at CVAS, Bikaner, performed histochemical analyses on the research samples. An examination of the rectum was presented using the 10 samples. The rectum samples were collected from recently killed adult pigs from the Bikaner slaughterhouse that were explicit of any digestive system pathologies.

Representative rectum samples will be taken from identical sites, fixed for 48 or 18 hours in Bouin's fluid or 10% formalin, respectively, rinsed the next day in flowing tape water, dehydrated with progressively higher alcohol concentrations (i.e., 50%, 70%, 90%, and finally absolute alcohol I, II, and III), clear in chloroform, and finally impregnated with paraffin. Blocks of paraffin were prepared, numbered, and stored in the freezer at 4°C (Luna-1968). Using a semi-automatic microtome, sections of around five-micron thickness were created and mounted on the albumenized slides, dried and stained for general histochemical findings.

Table 1: Following staining procedures were used in this study

S. N.	Name of stain	Purpose	References
1	McManus's (PAS) method	Used for glycogen demonstration	Singh and Sulochana, 1997.
2	McManus's (PAS) method with saliva	Used for glycogen demonstration	Singh and Sulochana, 1997.
3	PAS-Alcian blue pH-0.4 method	Used for mucosubstances	Luna, 1968.
4	PAS-Alcian blue pH-1.0 method	Used for mucosubstances	Luna, 1968.
5	PAS-Alcian blue pH-2.5 method	Used for mucosubstances	Luna, 1968.
6	Dane's stain	Used for pre-keratin, keratin and mucin demonstration	Luna, 1968.

Results and Discussion

McManus's Glycogen (PAS) method

The lining epithelium displayed a negative response to glycogen (Fig. 1). The findings of Morales (1980) [7], who looked at the histochemistry of bovine intestinal epithelium, were in contrary to those of the current investigation. He observed the complex carbohydrates on the intestinal epithelium's apical surface. Zaher *et al*. (2012) [12] in *Coturnix coturnix* discuss that the carbohydrates were localized in the rectal glands, goblet cells, and the surface mucosal epithelium with the PAS method in the form of magenta colouration.

In Mc Manus PAS-stain, the lamina propria exhibited a mild positive reactivity for glycogen. In McManus PAS-stain, the lamina muscularis displayed a positive response for glycogen (Fig. 1). This study findings agreed with those of Morales (1980) [7] for cattle and Kadam *et al.* (2009) [4] for sheep, cattle, and goats.

Tunica muscularis displayed a positive reaction to carbs in McManus PAS-stain, and tunica serosa displayed a slightly positive response to glycogen (Fig. 2). In accordance with the findings of Kadam *et al.* (2009) [4] in cattle, sheep, and goats.

In McManus PAS-stain, the tunica submucosa showed a negative response for glycogen (Fig. 1). The results of the current investigation were in opposition to those of Kadam *et al.* (2009) [4] who found that the intensity of the colour reaction to PAS-positive material in the wall of the caecum, colon, and rectum ranged from mild to intense in sheep, goats, and cattle.

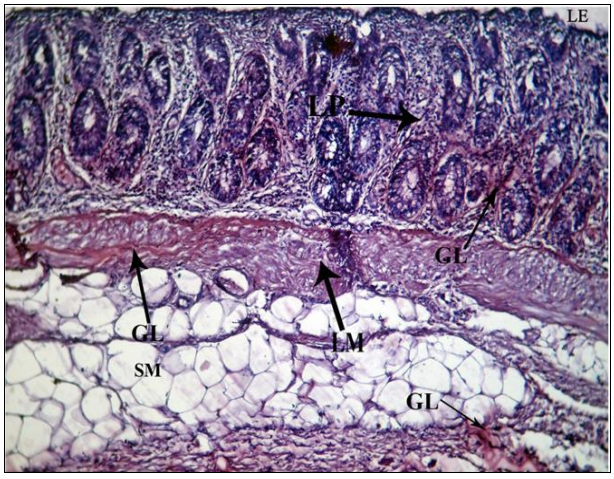

Fig 1: Photomicrograph of the rectum of pig showing PAS-positive reaction. LP-Lamina propria, LM:-Lamina muscularis, GL-Glycogen and SM:-Sub-mucosa. (Mc Manus method of PAS for glycogen, 100 X)

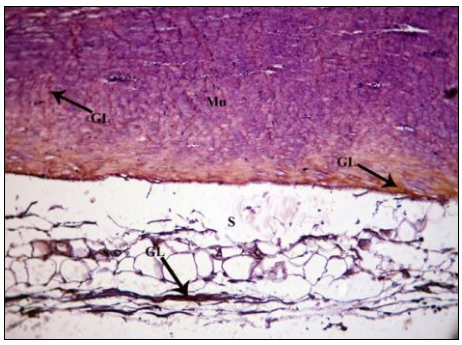

Fig 2: Photomicrograph of pig rectum showing PAS-a positive reaction. Mu-Muscularis, S-Serosa and GL-Glycogen. (Mc Manus method of PAS for glycogen, 100 X)

McManus's (PAS) method with Saliva used for Glycogen demonstration

Lamina propria, lamina muscularis, and the epithelium lining the tunica mucosa each revealed a PAS-negative response for glycogen and mucopolysaccharide for Mc Manus after treatment with saliva (Fig. 3). The results of this study agreed with those of Morales (1980) [7] for cattle as well as Kadam *et al.* (2009) [4] for cattle, sheep, and goats.

After treatment with saliva, tunica serosa, muscularis, and submucosa layers demonstrated PAS-negative reactions for glycogen and mucopolysaccharide for Mc Manus (Figs. 3 and 4). The conclusions of the current study were in harmony with those of the sheep, goats, and cattle reports by Kadam *et al.* (2009) [4].

The PAS response in all three regions anterior, central, and caudal parts of the rectum were identical.

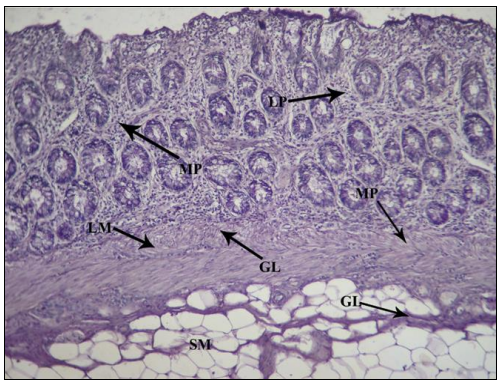

Fig 3: Photomicrograph of pig rectum showing PAS-a negative reaction. LP:-Lamina propria, LM:-L. muscularis, SM:-Sub-mucosa, GL-Glycogen and MP-Mucopolysaccharide. (PAS with saliva stain use for mucosubstance demonestratio, 100 X)

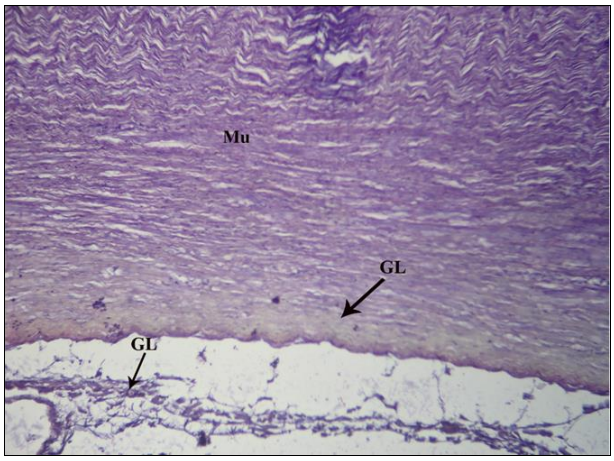

Fig 4: Photomicrograph of pig rectum showing PAS-negative reaction. Mu-Muscularis, S-Serosa and GL-Glycogen. (PAS with saliva stain used for mucosubstance demonstration, 100 X)

The PAS-Alcian blue reaction used for mucopolysaccharide materials

The lining epithelium responded strongly to the sulphated mucosubstance in the pH-2.5 stain with PAS-Alcian blue (Fig. 5) as well as moderately with pH-1.0 stain of PAS-Alcian blue (Fig. 6). This finding coincided with studies conducted on humans by Filipe (1969) [3], and guinea pigs and rabbits by Sheahan and Jervis (1976) [10], but it demonstrated a negative response to the PAS-Alcian blue pH-0.4 stain (Fig. 7).

In the PAS-Alcian blue stain with pH-2.5 and pH-1.0, the lamina propria responded somewhat positively to neutral mucosubstance and mostly positively to sulphated mucosubstance (Figs. 5 and 6). The results were almost identical to those obtained in guinea pigs and rabbits by Sheahan and Jervis (1976) [10]. When stained with PAS-Alcian blue pH-0.4, the lamina propria responded moderately positively to acidic sulphated mucosubstance and positively to neutral mucosubstance (Fig. 7). The result recognized with those of Zaher *et al.* (2012) [12] who studied *Coturnix coturnix*.

The rectum goblet cells displayed an intense blue colour and an intensely positive reaction to sulphated mucosubstances in PAS-Alcian blue both pH-2.5 stain and pH-1.0 stain (Figs. 5 & 6), whereas goblet cells demonstrated a strong positive reaction with acidic sulphated mucosubstances in PAS Alcian blue stain with pH-0.4 (Fig. 7). These results were comparable to those of Filipe (1969) [3] for humans. The results were somewhat consistent with those of Sheahan and Jervis (1976) [10], who found that in the distal colon of guinea pigs, dense goblet cells containing both neutral and sulfomucin were only found at the terminal part of the crypts whereas the lower part revealed an increase in the number of sulfated vacuolated cells, with some regions having sulfated vacuolated cells lining the entire crypt. The acid mucosubstances were more prevalent than the neutral mucins in the rabbit colon. Goblet cells showing almost entirely acid mucins were combined with those expressing only neutral mucins. Goblet cells exclusively displayed neutral and sulfomucins in the colon of the cat, with sulfomucins often present in higher concentrations. Goblet cells occasionally displayed neutral mucins. When stained with PAS-Alcian blue pH 0.4, goblet cells from the rectum displayed a strong positive response to acidic sulphated mucosubstances (Fig. 7). The findings were mostly consistent with those of AL-Samawy *et al.* (2019) [1] for camels.

The PAS-Alcian blue pH-2.5 stain demonstrated that the lamina muscularis reacted positively to neutral mucosubstance and moderately positively with sulphated mucosubstance (Fig. 5), and moderately positively with acidic sulphated mucosubstance in the PAS-Alcian blue stain at pH-0.4 (Fig. 7). The findings did not agree with those of Sheahan and Jervis (1976) [10] in both the guinea pig and rabbit, or with those of Zaher *et al.* (2012) [12] in Coturnix coturnix, but they did not agree with those of the PAS-Alcian blue stain at pH-1.0 (Fig. 6).

Tunica submucosa responded slightly positively to sulphated mucosubstance when PAS-Alcian blue stain was used at pH-2.5 (Fig. 5), but only moderately positively to sulphated mucosubstance when PAS-Alcian blue stain at pH-1.0 (Fig. 6), then positively to acidic sulphated mucosubstance when PAS-Alcian blue stain pH-0.4 used (Fig. 7). The results were similar with those of Filipe (1969) [3] who studied humans.

The tunica muscularis layer exhibited a moderate positive reaction for sulphated and neutral mucosubstance in the PAS-Alcian blue satin at pH-2.5 stain (Figs. 5 & 8), and a positive reaction to sulphated mucosubstance in PAS-Alcian blue stain at pH-1.0 (Fig. 9), however slightly positive reaction for acidic sulphated mucosubstance and moderate positive reaction with neutral mucosubstance in PAS-Alcian blue stain at pH-0.4 (Fig. 10). The results were partial congruence with the Zaher *et al.* (2012) [12] in *Coturnix coturnix*.

In the PAS-Alcian blue stain pH-2.5 tunica serosa displayed a strong positive reaction for sulphated mucosubstance as well as a positive reaction for neutral mucosubstance (Fig. 8), and a strong positive reaction with sulphated mucosubstance in the PAS-Alcian blue stain at pH-1.0 (Fig. 11), While the PAS Alcian blue stain at pH-0.4 produced a slightly positive reaction with acidic sulphated mucosubstance and a moderately positive reaction with neutral mucosubstance (Fig. 10). The results were somewhat consistent with those obtained in the all guinea pig, rabbit, and cat by Sheahan and Jervis in (1976) [10].

Table 2: The many layers of the rectum pig histochemical retorts

Particulars	McManus's (PAS) method used for Glycogen	McManus's (PAS) Method with Saliva used for Glycogen demonstration	PAS-Alcian Blue stain reaction with pH-1.0	PAS-Alcian blue stain reaction with pH-2.5	PAS-Alcian blue stain reaction with pH-0.4
Epithelium	-	-	++	++++	-
Lamina Propria	+	-	+	+	++
Lamina muscularis	+++	-	-	++	++
Tunica submucosa	-	-	++	+	++
Tunica muscularis	+++	-	+++	++	+
Tunica Serosa	+	-	++++	++++	+

Note: + +++ = intense or strong, +++=positive, ++ = moderate, + = weak or slightly or mild and – = negative.

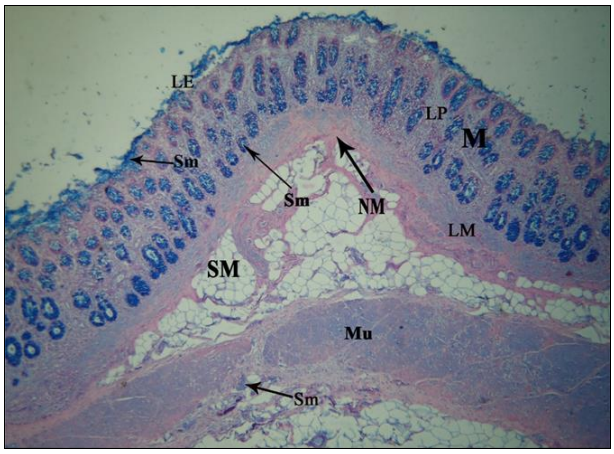

Fig 5: Photomicrograph of pig rectum presenting M:-Mucosa, SM:-Sub-mucosa, Mu-Muscularis, NM:-Neutral mucosubstance and Sm:-Sulphated mucosubstance. (PAS-Alcian blue stain used for mucosubstance at pH-2.5, 40X)

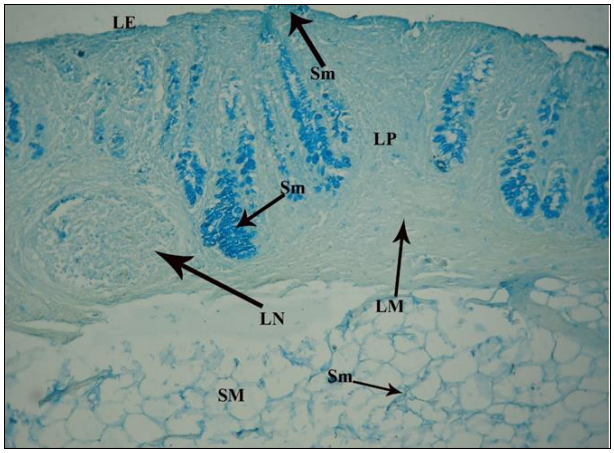

Fig 6: Photomicrograph of the rectum of pig PAS-positive reaction. LE:-Lining epithelium, LP:-Lamina propria, LM-L. muscularis, LN-Lymphatic nodule, SM:-Sub-mucosa and Sm-Sulphated mucosubstance. (PAS-Alcian blue stain used for mucosubstance at pH-1.0, 100X)

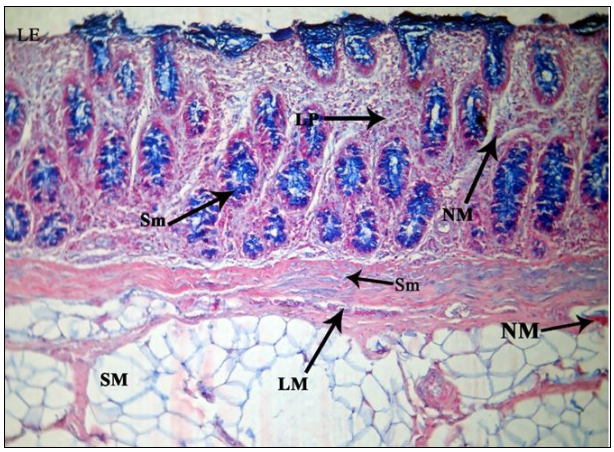

Fig 7: Photomicrograph of pig rectum display PAS-a positive reaction LP-Lamina propria, NM-Neutral mucosubstances, LM-Lamina muscularis, SM-Submucosa and Sm-Sulphated mucosubstance. (pH-0.4, 100X, PAS-Alcian blue stain for mucosubstance)

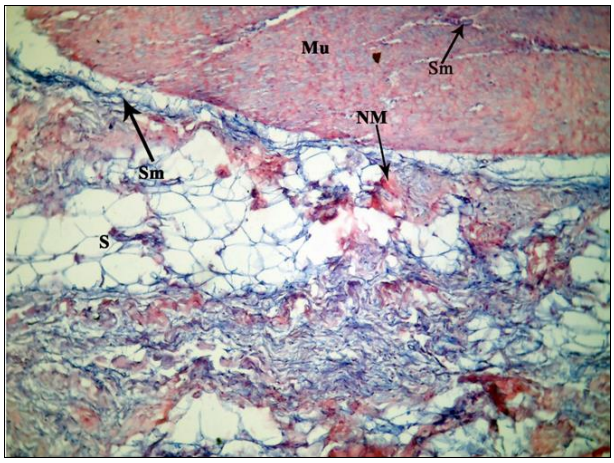

Fig 8: Photomicrograph of pig rectum presenting Mu-Muscularis, S-Serosa, NM-Neutral mucosubstance and Sm:-Sulphated mucosubstances. (PAS-Alcian blue reaction for mucosubstance at pH-2.5, 100X)

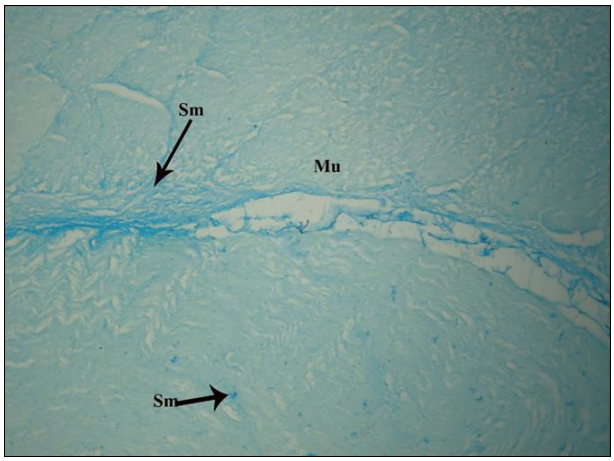

Fig 9: Photomicrograph of the rectum of pig PAS-positive reaction. Mu-Muscularis and Sm:-Sulphated mucosubstance. (PAS-Alcian blue stain for mucosubstance demons. at pH-1.0, 100X)

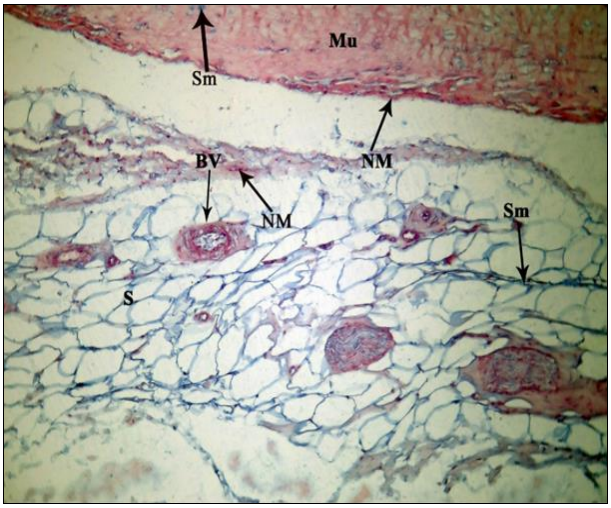

Fig 10: Photomicrograph of pig rectum viewing PAS-a positive reaction. Mu Muscularis, NM-Neutral mucosubstance: Sm:-Sulphated mucosubstance, S-Serosa and BV-Blood vessels. (PAS Alcian blue reaction for mucosubstance at pH-0.4, 100 X)

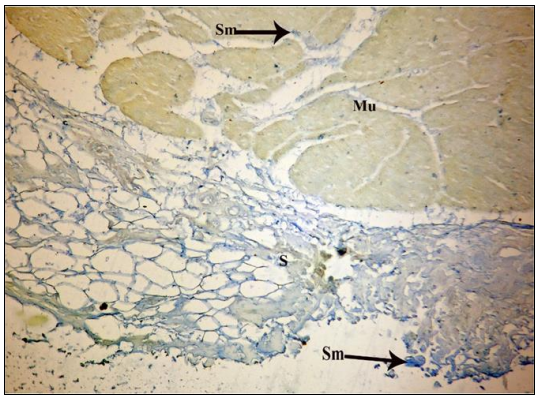

Fig 11: Photomicrograph of pif rectum displaying PAS-a positive reaction, Mu-Muscularis, S-Serosa and Sm-Sulphated mucosubstance. m(PAS Alcian blue reaction for mucosubstance at pH 1.0, 100X)

Dane's method for pre-keratin, keratin and mucin

Dane's method was used for the demonstration of pre-keratin, keratin and mucin. The rectal gland showed a strong positive reaction for acid mucopolysaccharide (Fig. 12 and 14).

The keratinized stratified squamous epithelium showed a positive reaction for pre-keratin and keratin but simple columnar epithelium showed a negative reaction for pre-keratin and keratin (Fig. 12 and 14).

For pre-keratin and keratin, other than the tunica mucosa, demonstrated all negative reactivity (Figs. 12 and 13).

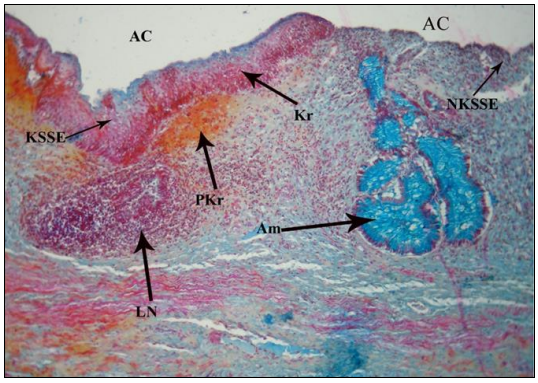

Fig 12: Photomicrograph of AC-Anal canal of pig presentation a positive reaction for Am-Acid mucopolysaccharide, Kr-Keratin, PKr-Pre-keratin, NKSSE-Nonkeratinized stratified squamous epithelium; KSSE-Keratinized stratified squamous epithelium, LN-Lymphatic nodule. (Dane's stain for Pre-keratin, keratin and mucin, 100 X)

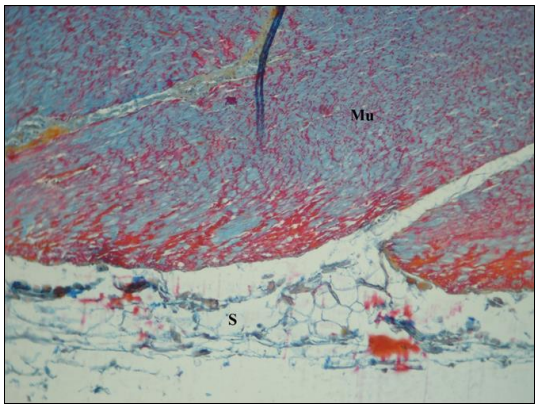

Fig 13: Photomicrograph of pig rectum display a negative reaction for PKr-Pre-keratin, Kr-Keratin, and Am-Acid mucopolysaccharide in Mu-Muscularis and S-Serosa. (Dane's stain for Pre-keratin, keratin and mucin, 100 X)

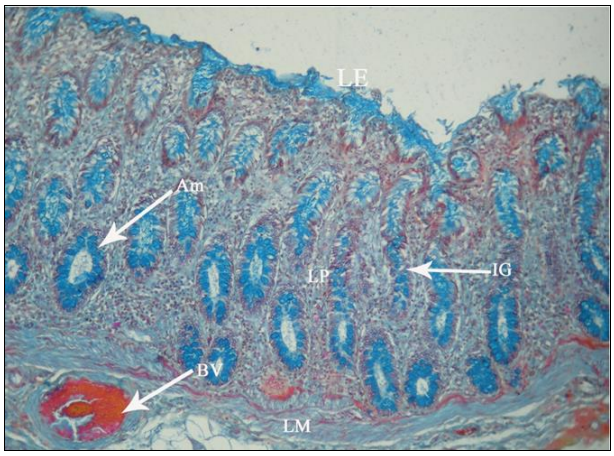

Fig 14: Photomicrograph of pig rectum shows LE-Lining epithelium, LP-Lamina propria, LM-Lamina muscularis, BV-Blood vessel, IG-Intestine gland and Am-Acid mucopolysaccharide. (Dane's stain for pre-keratin, keratin and mucin, 100 X)

Conclusion

When McManus with saliva, every layer of the rectum displayed a PAS-negative response to glycogen and mucopolysaccharide.

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