

## Comparative Histochemical Study on Lingual Glands of Some Mammals

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**Abstract:** In tongue the, lingual glands secrete saliva containing mucin which forms a protective layer on mucosal surfaces of tongue. Being viscoelastic, mucin is responsible for maintaining lubrication and hydration of the surface. The glands adapt to the necessities of each species. Keeping this in mind we conducted a comparative histochemical study on sublingual glands of five species of mammals for characteristic of composition of salivary secretion. Tissues from all five mammals were stained by alcian blue ph 1.0 before & after active methylation and by aldehyde fuchsin for Highly sulphated acid mucopolysaccharides. For Weakly sulphated acid mucopolysaccharides they were stained by Alcian blue ph 2.5 before and after mild methylation. For Simultaneous demonstration of sulphated and Neutral mucopolysaccharides PAS procedure was applied with alcian blue at ph 1.0. To look for metachromatic properties Toluidine blue at ph 2.0 and 4.4 was used. For simultaneous demonstration of highly and weakly sulphated acid mucopolysaccharides aldehyde fuchsin - alcian blue ph-2.5 sequence was used while for Glycogen and neutral mucopolysaccharides PAS procedure and. PAS phenyl hydrazine were used. On examination at different magnification only specimen from rabbit was found to have the serous glands as purely serous, where as in the rest of animals the serous alveoli were lined by seromucous cells with variable presence of different types of mucopolysaccharides. The presence of moderate amount of weakly sulphated mucopolysaccharides and neutral mucopolysaccharides was found in dog. In albino rat, rabbit and guinea pig highly sulphated mucopolysaccharides were predominant in mucus alveoli followed by weakly sulphated mucopolysaccharide; but in goat and dog, main mucopolysaccharides were weakly sulphated mucopolysaccharides. Highly sulphated forms were absent in goat and insignificantly present in dog. Except goat, in all animals the same cells in same mucus alveoli, showed the presence of both sulphated and neutral forms.

Tongue, Lingual gland, Mucus alveoli, Sulphated Mucopolysaccharide,

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### I. Introduction

Tongue, located in the oral cavity, develops from two different sources and these two embryological different parts form body and root of tongue. The body forms anterior two third of the tongue and is freely mobile with the help of its skeletal muscles. Root of the tongue forms the posterior third of it and is attached to the floor of oral cavity. On the dorsal surface of the tongue the terminal sulcus and foramen caecum mark the junction of root and the body of tongue plays role in speaking, chewing and swallowing food which needs lubricated surface all over inside oral cavity for its free movement. This requirement of lubrication is fulfilled by the salivary gland's secretion.

The posterior part of tongue contains two sets of minor salivary glands Cheng (2009)<sup>1</sup>. Piludu(2006)<sup>2</sup> stated that the posterior deep lingual glands, known as von Ebner's glands, are a group of tubuloacinar serous glands located beneath the circumvallate and foliate papillae of the tongue, and their ducts open into and through the base of the papillae ..

The posterior superficial lingual glands, sometimes called Weber's glands, are located on the lateral margin at the level of the foliate papillae and in the root of the tongue behind the circumvallate papillae, and the ducts of the posterior superficial lingual glands open into the crypts of the lingual tonsils. Crabill (1993)<sup>3</sup>

A sticky substance mucus enables food and other substances bind tightly and slide freely through the gastrointestinal tract and prevents epithelium damage (Guyton and Hall 2007)<sup>4</sup>. The main components of mucus, is mucin which forms a protective layer on most mucosal surfaces in the body. Saliva containing high amounts of mucin tends to be viscoelastic, an important characteristic which causes retention of saliva on oral mucosal surfaces to maintain lubrication and hydration of the surfaces (Proctor, 2016)<sup>5</sup>. As with other organs salivary glands are also adapted to the necessities of each species. In this context, we conducted a comparative

histochemical study on the sublingual gland in five different species of vertebrate animals to study characteristic of their glandular structure and secretion

## II. Material And Methods

Study was conducted in department of anatomy in Rajendra Institute of Medical Sciences, Ranchi for the thesis work of PG. Samples of lingual glands were obtained from tongues of the five vertebrate animals –

1. albino rat (*Rattus norvegicus*; Order rodentia),
2. guinea pig (*Cavia-cobaya*; Order rodentia),
3. rabbit (*Lepus ruficavidapus*; Order lagomorpha),
4. goat (*Capra indica*; Order artiodactyls) and
5. Dog (*Canis familiaris*; Order carnivore).

Sample from all five animals were taken from, tongue, posterior pharyngeal part and vallete papillary region. After fixation tissue processing and staining was done for the histochemical study. Histochemical characterization of lingual gland's mucin as under taken in this work was based on staining procedure under different conditions of pH and enzymatic digestion, as suggested by different workers from time to time Gomory (1950)<sup>6</sup>, Mowry, R. w. (1956)<sup>7</sup>, Spicer (1967)<sup>8</sup>, Bancroft and Stevens (1982)<sup>9</sup> and pearse (1984). In the present work an attempt was made to identify the various types of mucin as characterized by different histochemical reactions. For different reactive substances in the mucin following series of activities were used –

A. Highly sulphated acid mucopolysacchrides. Alcian blue ph- 1.0 (lev and spicer, 1964).

1. Alcian blue ph- 1.0 (lev and spicer, 1964).
2. Alcian blue ph -1.0 after active methylation (Spicer et al, 1967).
3. Aldehyde fuchsine (Spicer and Meyer, 1960).

B. Weakly sulphated acid mucopolysacchrides.-

1. Alcian blue ph- 2.5 (Spicer et al, 1967).
- 2) Alcian blue ph- 2.5 after mild methylation (Spicer et al, 1967).

C. Simultaneous demonstration of sulphated and Neutral mucopolysacchrides with :- 1)

1. Alcian blue ph 1.0 pas procedure D. For metachromatic properties at different ph

1. Toludine blue (ph 2.0).

2. Toludine blue (ph 4.4).

E. For simultaneous demonstration of highly and weakly sulphated a.m.p.s.

1. Aldehyde fuchsine - alcian blue ph-2.5 sequence (Spicer and Meyer, 1960)

F. For Glycogen and neutral mucopolysacchrides:-

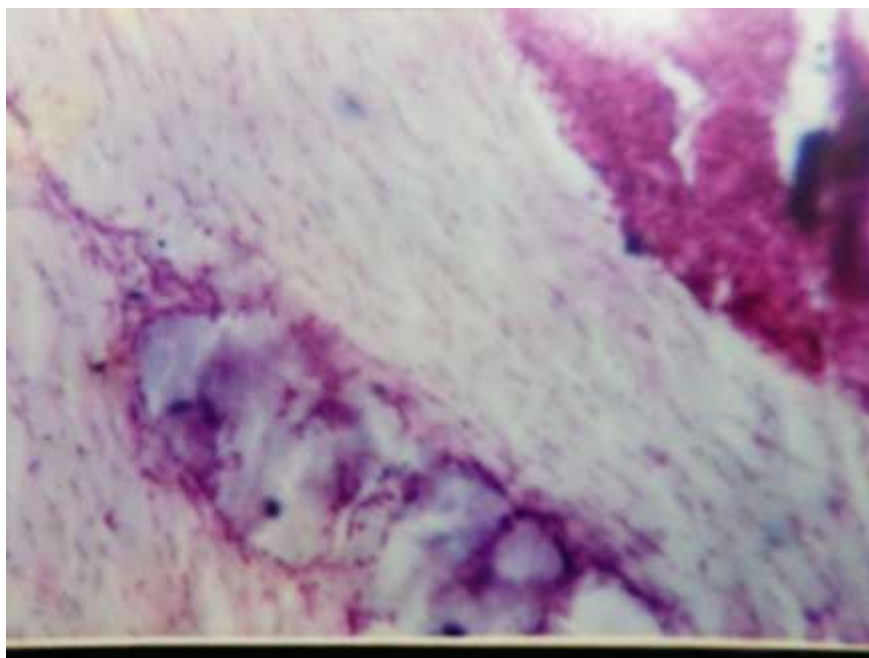
1. Pas procedure (Spicer et al, 1963)
2. Pas phenyl hydrazine (Spicer digestion Spicer et al 1967).

## III. Result

Serous alveoli of lingual gland in Albino rat stained light purple with toludine blue at pH 4.4. It stained light to moderate blue with alcian blue at pH- 2.5; this alcianophilia was diminished after mild methylation. Again it stained blue with alcian blue at p<sup>H</sup> -1.0, thus alcianophilia was abolished by active methylation. With PAS it stained faint red and with Alcian blue pH-1. With PAS some alveoli stained blue while others showed both blue and red reaction in the same alveoli thus indicating the presence of both sulphated and neutral mucopolysacchrides.

All the serous alveoli of lingual gland in Rabbit remained nonreactive to all the stains Serous gland in Guinea pig- with toludine blue it gave moderate blue reaction at pH – 4.4 and blue purple at pH -2.0. With alcian blue at pH 2.5, it stains light blue, but this alcianophilia is diminished after mild methylation. With rest of the staining procedures, it remains almost nonreactive. This staining reaction indicative of the presence of weakly sulphated A.M.P.

Serous alveoli of lingual gland in Guinea pig gave moderate blue reaction with toludine blue at pH 4.4 and blue purple at pH -2.0, while with alcian blue at pH 2.5, it stained light blue, but this alcianophilia was diminished after mild methylation. With rest of the staining procedures including PAS, it remained almost nonreactive (Fig. 1). This staining reaction indicated the presence of weakly sulphated A.M.P.



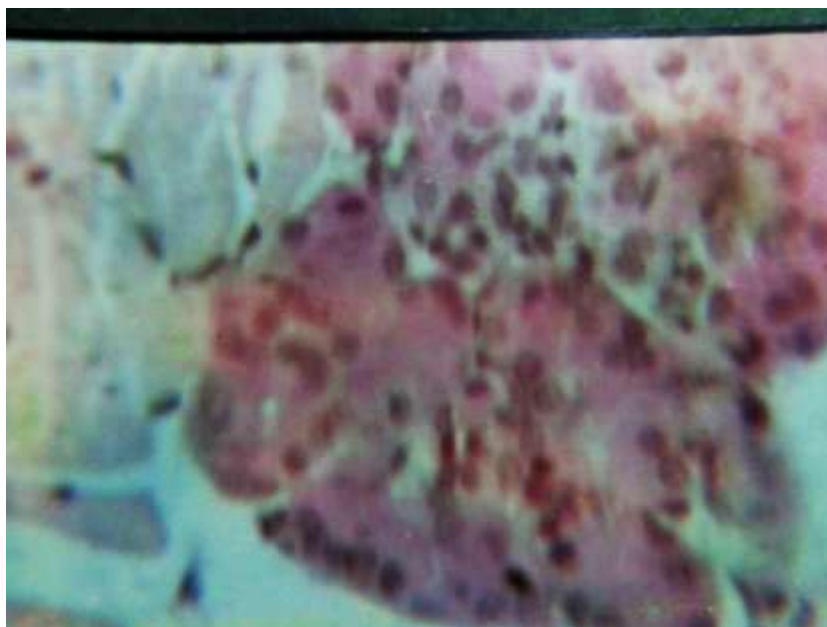
**Fig. 1** Serous and mucus acini in posterior part of tongue of Guinea pig near vallete papilla. Both alveoli are unstained (PAS 125 X)

Serous cells of lingual gland in Goat stained faint blue with alcian blue at  $p^H$ - 4.4 ,again faint blue with alcian blue at  $p^H$ - 2.5 labile to mild methylation and a faint bluish red reaction with alcian blue (Fig. 2), PAS and light blue reaction with aldehyde fuchsine AB 2.5 , indicating the presence of very small amount of weakly sulphated and neutral mucopolysacchrides .



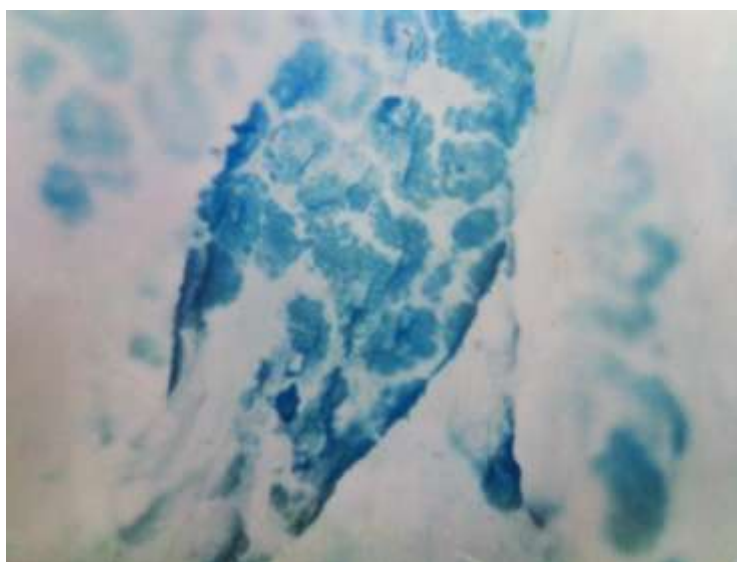
**Fig. 2** Serous alveoli in Vallete papillary region of Goat showing light bluish red staining of cytoplasm (Alcian blue Ph 1.0 PAS 125 X)

Serous gland of lingual gland in in Dog stained light blue with toluidine blue pH 4.4, again light blue with alcian blue pH 2.5, labile to mild methylation and again light blue with aldehyde fuchsine AB PH 2.5, and light red with AB pH 1 PAS Procedure (Fig.3) indicating the presence of small amount of weakly sulphated and natural polysaccharides.



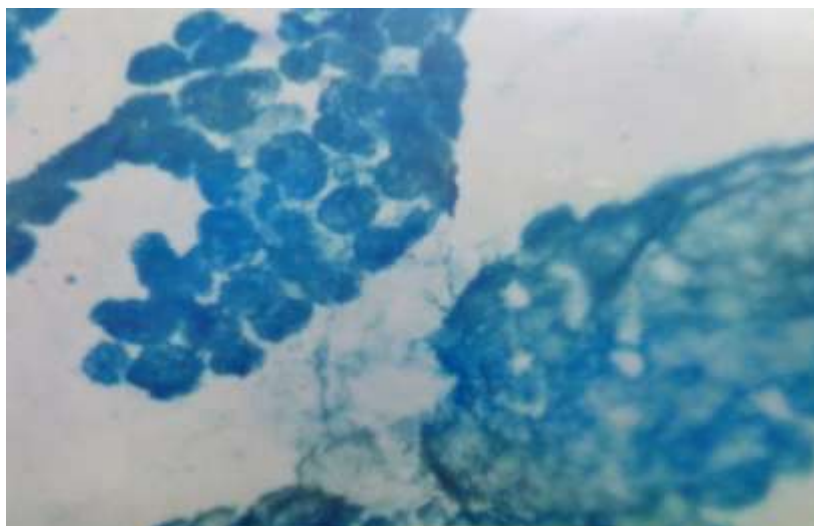
**Fig. 3** Serous alveoli in vallete papillary region in the tongue of Dog showing light red staining of cytoplasm (PAS-500 X)

Mucous alveoli of lingual gland in Albino rat stained deep blue purple with T.B. pH 4.4, and light purple at pH-2.5, with AB pH 2.5 & pH -1.0, they can stain light blue. This affinity to alcian blue is almost nonreactive to mild methylation, but liable to active methylation. With AF it gave light to moderate purple reaction and with AF-AB pH 2.5 reaction some alveoli stained moderate purple, others moderate blue (Fig.4), while in some the mucous cells give moderate blue-purple reaction with PAS, the staining is faint red but with alcian pH-1-PAS, some alveoli stain blue while others stain blue-red.



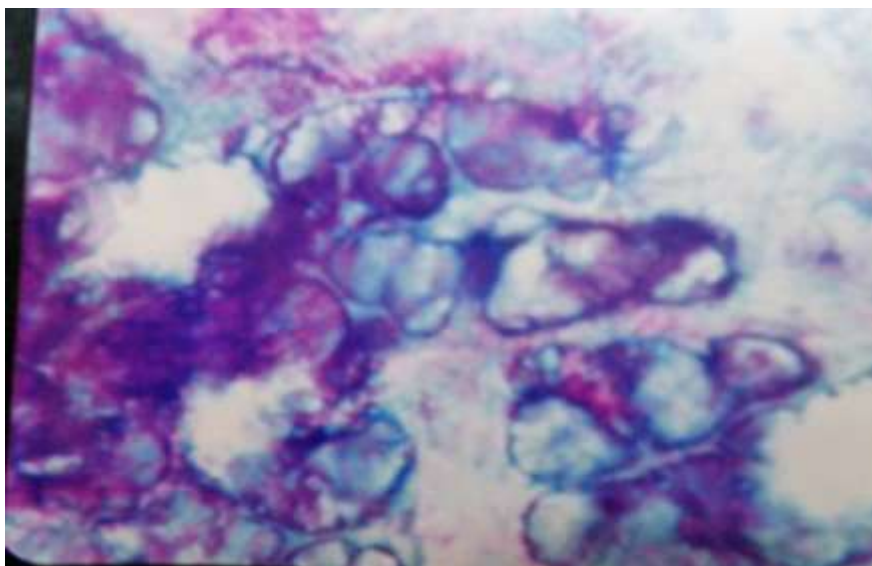
**Fig. 4** Mucous gland in pharyngeal part of tongue of Albino rat showing light to moderate blue staining of cytoplasm (Alcian blue pH2.5- 125X)

The mucous cells of lingual gland in rabbit stained light purple, with T.B. pH 4.4 but deep purple at pH-2; with AB 2.5 & pH 1.0, it gave light to moderate blue reaction (Fig.5). This Alcianophilia was abolished by active methylation but was refractory to mild methylation, with AF, they stain deep purple and with AF-AB pH 2.5, Most of the alveoli stained moderate blue while few gave blue purple reaction. PAS gave faint red reaction and with AB-PH -1 PAS, some alveoli stain light blue, while others give light blue-red reaction. These staining reactions indicate the presence of a substantial amount of highly sulphated A.M.P. and a small amount of weakly sulphated M.P.



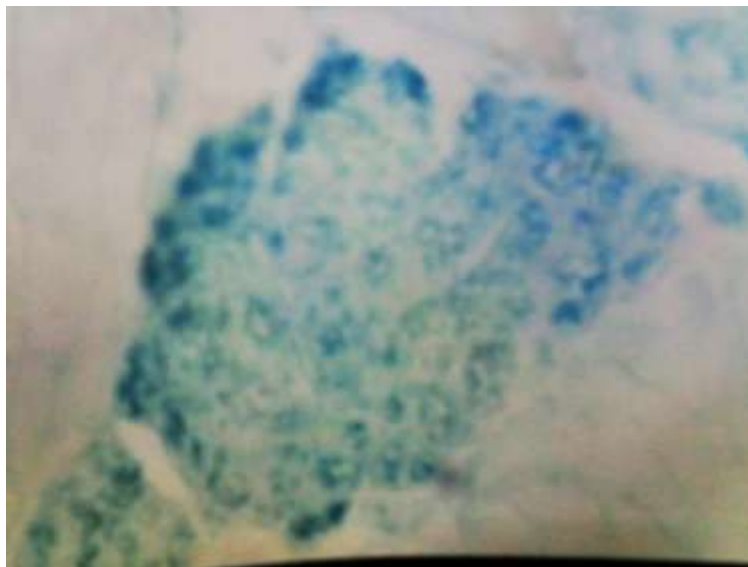
**Fig. 5-** Mucous gland in posterior part of tongue of Rabbit showing light to moderate blue staining of mucus alveoli ( Alcian blue pH 1, 125 X)

The mucous cells of lingual gland in Guinea pig stained deep purple with TB at P<sup>H</sup> 4.4 and 2, faint blue with AB at ph 2.5 and light blue with AB ph 1.0 ; the former was resistant to mild methylation .With AF and AF –AB PH 2.5 it gave deep purple reaction (Fig. 6) .These mucous alveoli were nonreactive to PAS but with AB PH 1.0 PAS most of the alveoli showed light blue staining on the luminal surface , while others gave a faint ,blue red reaction. All these procedure indicate the presence of amount of highly sulfated A.M.P.and only traces of weakly sulphated A.M.P.



**Fig. 6-** Mucus gland in posterior part of tongue of Guinea pig showing light blue staining in some alveoli and moderate purple staining in others ,some had stained blue purple (Aldehyde Fuchsine- Alcian blue Ph 2.5 - 125 X)

In Goat the mucous alveoli of lingual gland stained light blue with Alcian blue at pH2.5 (Fig.7), this alcianophilia was abolished by mild methylation. With rest of the stain procedure it remained nonreactive indicating the presence of only small amount of weakly sulphated A.M.



**Fig. 7** Mucus gland in pharyngeal part of tongue of Goat showing light blue staining of cytoplasm (Alcian blue pH2.5 125 X)

The mucous cells of lingual gland in Dog stained moderate blue, With TB at pH -4.4, and moderate purple with aldehyde fuchsin. With AB at pH -2.5 they stain moderate blue & this alcianophilia is slightly diminished after mild methylation. It is nonreactive to AB at pH -1.0 -PAS they stain light red, this reaction is almost abolished after

Phenyl hydrazine treatment, but undergoes on change after diastase digestion. With AB at pH -1.0 -PAS, some alveoli stain light blue, others light red and some give blue-red reaction. These staining reactions indicate the presence of moderate amount of weakly sulphated A.M.P. and small amount of highly sulphated and neutral mucopolysaccharides.

#### IV. Discussion

In the present study the serous lingual glands in the vallate papillary region showed sero mucous characteristics invariably. Whereas in albino rat, small amount of highly and weakly sulphated and traces of neutral mucopolysaccharides were seen. None of the mucopolysaccharides was present in the rabbit, guinea pig and goat and only very small amount of weakly sulphated mucopolysaccharides was present in dog. On the basis of present finding, it may be concluded that only in **rabbit** the serous glands are purely serous, whereas in the rest of animals the serous alveoli are lined by seromucous cells with variable presence of different types of mucopolysaccharides. The presence of moderate amount of weakly sulphated mucopolysaccharides and neutral mucopolysaccharides may probably be due to different food habit of **dog** as compared to the rest of the animals. Posterior lingual glands had alveoli lined by pure mucus cells but in the rabbit, goat and dog, there were serous deminules also present, which were seromucous in nature. The mucus cells had mucin present in them which gave variable reaction indicating the presence of different types of mucopolysaccharides that is glycosaminoglycans. Highly sulphated mucopolysaccharides stained deep purple with toluidine blue at pH-1. This alcianophilia was abolished by active methylation. They were present in moderate amount in rabbit and guinea pig, in moderate amount in albino rat, rabbit and guinea pig probably indicate their common food habit, which is different from dog and goat. Weakly sulphated mucopolysaccharide was identified by histochemical reactions with toluidine blue and alcian blue. With toluidine blue; it stained light purple at pH -4.4 and light blue with alcian blue at PH- 2.5 and this alcianophilia was abolished by mild methylation. Weakly sulphated mucopolysaccharides were present in moderate to small amount in albino rat and rabbit, in moderate amount in goat and dog and in small amount to traces in guinea pig. It was the only mucopolysaccharides present in goat mucus cells. The significance of its presence variably in all these animals of different food habits is not explicable.

Neutral mucopolysaccharide gave a pink reaction with pas procedure; this staining reaction is liable to diastase digestion, if glycogen is present and to phenyl hydrazine treatment if neutral mucopolysaccharides are present. In the present series the neutral mucopolysaccharides were found in very small amount in rabbit and dog and were absent in albino rat, rabbit and goat.

Glycogen was found to be absent in the posterior lingual of all the animals except in rabbit, where it was present in traces, and has no significance.

The present work showed that in albino rat, rabbit and guinea pig highly sulphated mucopolysaccharides was predominant in mucus cell of albino rat, rabbit and guinea pig followed by weakly sulphated mucopolysaccharides; but in goat and dog the main mucopolysaccharides in the mucoid cells was weakly sulphated mucopolysaccharides; highly sulphated forms in these two animals were absent in goat and insignificantly present in dog. These findings are in keeping with the food habit of these animals. There was a significant observation, that in the same mucus alveolus in different animals except in goat, the same cells showed the presence of only weakly sulphated and highly sulphated form of mucin, while others showed both sulphated and neutral form in the same secretory cells.

## V. Conclusion

The histochemical reactions shown in Guinea pig are indicative of the presence of moderate amount of highly sulphated acid mucopolysaccharides and similar amount of weakly sulphated A.M.P. In posterior gland of webner, the mucus cells had mucin present in them which gave variable reaction indicating the presence of different types of mucopolysaccharides that is glycosaaminoglycans. In Guinea pig study results indicate the presence of good amount of highly sulfated A.M.P. and only traces of weakly sulphated A.M.P.

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