

Research Article

The histochemical assessment of sulpho-, sialo-, and neutral-mucosubstances in fetal gastric mucosa

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ABSTRACT

Background: Mucins are complex composition of carbohydrates and may be present as a mixture of different types. Normal distribution of mucin and its alteration in various inflammatory, benign and malignant lesions of gastrointestinal tract has aroused interest in the field of histochemistry. The main purpose of present work is to study the staining pattern and distribution of cells in different parts of fetal gastric mucosa and to correlate the nature of gastric mucins and its functional significance.

Methods: A total of 25 fetus stomach specimens (total 75 samples) one sample each from different parts of the stomach like fundus, body and pylorus, from fresh specimens. The samples were washed in normal saline, fixed in 2% calcium acetate in 10% formalin. These tissues were routinely processed and paraffin blocks were prepared. 6 μ sections of these blocks were taken for histological and different histochemical staining.

Results: Fetal fundic part of stomach shows increased neutral mucin in surface epithelium and foveolar cells. With combined AB pH 2.5 - PAS technique increased neutral mucin and small amount of acid mucins are observed. With AB pH 1, surface epithelium and deep glands show negative staining. Moderate alcinophilia is observed in deep foveolar cells and glandular cells. AB pH 2.5 shows alcinophilia in surface epithelium, foveolar cells and mucous neck cells indicating presence of sialomucin. Fetal pyloric part of stomach shows increased acid and neutral mucins. With pH 2.5 - PAS staining, purple staining is observed in surface epithelium, deep foveolar and pyloric glands.

Conclusion: All types of mucosubstances - neutral, sialo and sulpho-mucins, are secreted in relatively increased amounts by the surface epithelium and the glands of the stomach of the human fetus and neonate. Sulphomucin is seen mainly in the cells of the surface epithelium.

Keywords: Mucins, Histochemistry, Gastric glands, Mucosubstances, Foveolar cells

INTRODUCTION

Mucins of the human gastrointestinal tract can no longer be thought of merely as a mechanical lubricant or an inert protective barrier.¹ The histochemical difference between adult and fetal mucins are difficult to interpret since relationship between the chemical structure of epithelial glycoproteins in anatomical segment and their physiological role has not yet been established. The understanding of both nature and significance of mucin

changes in fetal development may be potentially useful in recognition of early neoplastic changes in adult and transitional mucosa adjacent to colorectal carcinoma.²

Although there have been many efforts of isolation and chemical characterization of mucin, lacunae still persist in many areas and the field is wide open for further exploration. Recently there has been considerable interest in detecting alterations of gastro intestinal mucin in various disease states with a view to using them as

criteria for differential diagnosis and prognosis. Most investigators have applied histochemical methods to this problem.³

The main purpose of the present work is to study the staining pattern of different parts of the fetal gastric mucosa and to correlate the nature of gastric mucins with its functional significance.

METHODS

Samples from full term still born fetuses of full term gestational age taken from the department of obstetrics and gynaecology, after obtaining a written consent from their parents. A total of 25 fetuses selected after a thorough embalming procedure and tissue samples were obtained within 4 to 6 hours after death of the fetus. The samples were taken from the different sites of the stomach like fundus, body and pylorus (total 75 specimens; 25 each from fundus, body and pylorus regions).

The samples were processed routinely and sections were stained with various histochemical techniques like - Alcian Blue (AB) staining at pH 1.0 and pH 2.5; Periodic Acid Schiff (PAS); Combined Alcian Blue and Periodic Acid Schiff (AB pH 2.5 - PAS); Aldehyde Fuscin (AF); Combined Aldehyde Fuscin and Alcian Blue (AF-AB pH 2.5) staining were employed to differentiate neutral, sulpho and sialo-mucins.

RESULTS

Thickness of fetal gastric mucosa is about one third of the adult gastric mucosa thickness. Gastric pits vary in different regions and lamina propria shows gastric glands lined by chief cells and parietal cells. Number of chief cells is reduced. Parietal cells have large rounded nuclei with basophilic cytoplasm. Cuboidal cells are seen lining the mucosal surface and the glands. The other types of cells, namely, mucous neck cells and enteroendocrine cells are not distinguished.

Fetal fundic part of stomach shows increased neutral mucin in surface epithelium and foveolar cells. With combined AB pH 2.5 - PAS technique increased neutral mucin and small amount of acid mucins are observed. Foveolar cells show purple staining while mucous neck cell show neutral and acid mucin.

With AB pH 1, surface epithelium and deep glands show negative staining. Moderate alcinophilia is observed in deep foveolar cells and glandular cells. AB pH 2.5 shows alcinophilia in surface epithelium, foveolar cells and mucous neck cells indicating presence of sialomucin.

AF-AB pH 2.5 shows glands purple staining, indicating increased sialomucin and sulphomucin.

Fetal pyloric part of stomach shows increased acid and neutral mucins. With pH 2.5 - PAS staining, purple staining is observed in surface epithelium, deep foveolar and pyloric glands. Alcinophilia with AB pH 2.5 is observed in gastric mucosa.

With AF-AB pH 2.5 technique, predominant sialomucin and small quantity of sulphomucin are observed. Deep foveolar and mucus neck cells in pyloric region show abundant neutral and acid mucin.

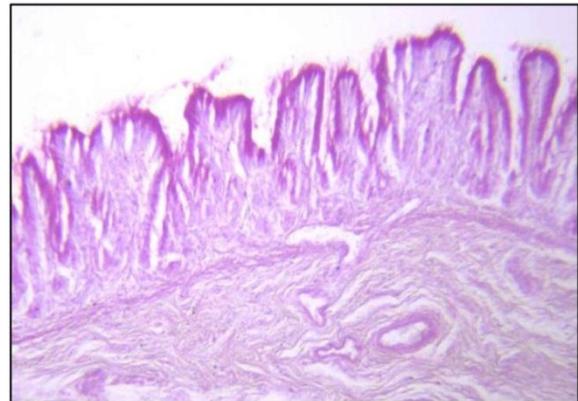


Figure 1: Fetal gastric mucosa (body) with PAS x100.

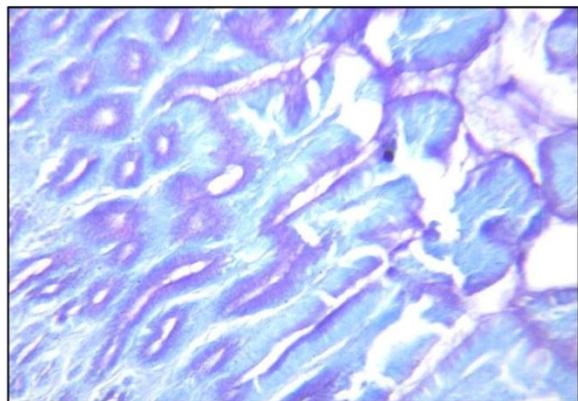


Figure 2: Fetal pyloric glands with AB pH 2.5 - PAS x400.

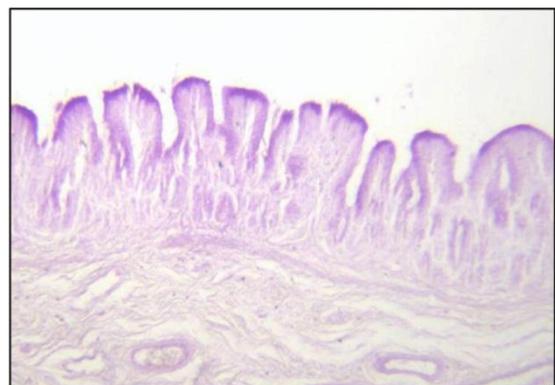


Figure 3: Fetal gastric mucosa (body) with AF x100.

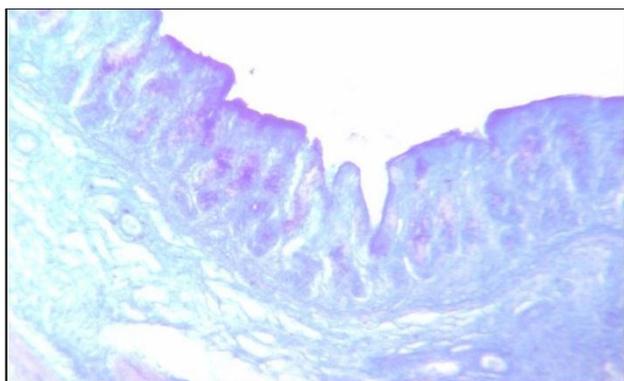


Figure 4: Fetal gastric mucosa with (pylorus) AF-AB pH 2.5 x100.

According to visual estimation of the intensity of colour reactions of the histochemical results were interpreted as follows:

Table 1: Interpretation of intensity of colour reactions.

Sign	Interpretation
-ve	Negative staining
±	Weak or variable staining
+	Slight staining
++	Moderate staining
+++	Strong staining

The distribution of mucins in epithelial elements of fetus stomach fundus, body and pylorus regions were noted as depicted in Table 2, 3 and 4 respectively.

Table 2: Distribution of mucins in epithelial elements of fetal stomach (fundic part).

Histo. Tech.	Sup. zone		Neck zone	Deep zone
	Surface epithelium	Foveolar cell	Mucous neck cell	Fundic gland
AB pH 1	+ B	++ B	+ B	+ B
AB pH 2.5	++ B	++ B	+ B	++ B
PAS	+++ M	++ M	+ M	++ M
AB pH 2.5 - PAS	++ MP	++ MP	+ B	++ BP
AF	± PP	± PP	-ve	± PP
AF-AB pH 2.5	++ BPP	+ BPP	+ BPP	++ BPP

- M-Magenta, P-Purple, MP-Magenta-purple, B-Blue, BP-Blue-purple, PP-Pink-purple
- BPP: AB positive mucin predominant over AF positive (Mixture of sulpho and sialo-mucins)
- MP: Mixture of PAS and AB positive with PAS positive predominant (Mixture of acid and neutral mucins)
- BP: Mixture of AB & PAS positive mucin with AB positive predominant (Mixture of acid and neutral mucins)

Table 3: Distribution of mucins in epithelial elements of fetal stomach (body part).

Histo. Tech.	Sup. zone		Neck zone	Deep zone
	Surface epithelium	Foveolar cell	Mucous neck cell	Body gland
AB pH 1	-ve	+ B	± B	+ B
AB pH 2.5	++ B	++ B	+ B	++ B
PAS	+++ M	++ M	++ M	++ M
AB pH 2.5 - PAS	++ MP	+ MP	+ B	+ BP
AF	± PP	+ PP	-ve	+PP
AF-AB pH 2.5	+ BPP	+BPP	+ BPP	± BPP

Table 4: Distribution of mucins in epithelial elements of fetal stomach (pyloric part).

Histo. Tech.	Sup. zone		Neck zone	Deep zone
	Surface epithelium	Foveolar cell	Mucous neck cell	Pyloric gland
AB pH 1	+ B	+ B	++ B	+ B
AB pH 2.5	+ B	++ B	++ B	+ B
PAS	+++ M	++ M	+ M	+++ M
AB pH 2.5 - PAS	++ MP	+++ BP	+++ BP	++ BP
AF	++ PP	+ PP	± PP	± PP
AF-AB pH 2.5	++ BPP	++ BPP	++ B	+++ BPP

DISCUSSION

Gastric mucosa in fetal life shows different pattern of mucin secretion as compared to adult gastric mucosa.⁴⁻⁶ All three types of mucins are secreted in relatively increased amount in fetal life by surface epithelium and glands of fetal stomach - Neutral mucin, sialomucin and sulphomucin in varying proportion.⁴

Stauffer et al demonstrated that gastric mucosa showed mixed neutral/acidic secretion during 2nd term. The neutral mucin increased during 3rd term when acid mucin failed and represented only 20% of the secretions by the end of gestation as during the neonatal period.⁷

Surface epithelium of fetal gastric mucosa shows intense magenta staining with PAS technique. AB pH 2.5 - PAS shows purple staining indicating predominance of neutral and acid mucin. Sulphomucin is seen mainly in the cells of the surface epithelium, which is confirmed by AF and AB pH 1 techniques.

With AF-AB pH 2.5 technique, surface epithelium, foveolar cells and mucous neck cells show sialo and

sulphomucin in varying proportion in fundic part of stomach. There is increased sialomucin and small amount of sulphomucin in deep foveolar and mucous neck cells.

Pyloric part shows purple staining indicating mixture of acid and neutral mucin in surface epithelium, foveolar cells and mucin neck cells. With AF-AB pH 2.5 surface epithelium and pyloric gland show bluish purple staining indicating presence of predominant sialomucin and small amount of sulphomucin.

Mucus neck cells and deep foveolar cells with increased section of neutral and acid mucin (sulphomucin and sialomucin) resemble with intermediate columnar cells of intestinal metaplasia showing sialomucin and sulphomucin in varying proportion.⁷

With AF-AB pH 2.5 technique, surface epithelium, foveolar cells and mucous neck cells show sialo and sulphomucin in varying proportion in fundic part of stomach. There is increased sialomucin and small amount of sulphomucin in deep foveolar and mucous neck cells.

Pyloric part shows purple staining indicating mixture of acid and neutral mucin in surface epithelium, foveolar cells and mucin neck cells. With AF-AB pH 2.5 surface epithelium and pyloric gland show bluish purple staining indicating presence of predominant sialomucin and small amount of sulphomucin.

Mucus neck cells and deep foveolar cells with increased section of neutral and acid mucin (sulphomucin and sialomucin) resemble with intermediate columnar cells of intestinal metaplasia showing sialomucin and sulphomucin in varying proportion.⁷

It was consistently observed that histologically normal mucosa adjacent to malignant tumours of gastrointestinal tract, be it gastric, small intestinal or colic, secretes mucins which differ histochemically and biochemically from normal but resemble those described in fetal gut.⁸⁻¹⁰

An interesting analysis of sulphoglycoprotein during fetal development has been performed by Hakkinen et al. who have found at least one fetal sulphoglycoprotein, which also occurs in gastric cancer cells. The meaning of this malignant dedifferentiation and its importance from clinical viewpoint was not clearly mentioned.¹¹

It was suggested that such changes could therefore be further evidence of the reversion to an embryonic state which may be a characteristic of the early stage of carcinogenesis.

CONCLUSION

All types of mucosubstances - neutral, sialo- and sulpho-mucins, are secreted in relatively increased amounts by

the surface epithelium and the glands of the stomach of the human foetus and neonate. Sulphomucin is seen mainly in the cells of the surface epithelium.

Carcinomas of gastrointestinal tract are now classified according to their cellular and morphological features, but it may be possible in future to classify mucin secreting tumours according to composition of their mucins.

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Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Gad A. A histochemical study of human alimentary tract mucosubstance in health and disease I: Normal and tumours. *B J Cancer*. 1969;23(1):52-63.
2. Singh R, Gorton AWP. Orcein-alcian blue staining a new technique demonstrating acid mucins in gastrointestinal tract. *J Clin Pathol*. 1989;881-4.
3. McFadden DE, Owen DA, Reid PE, Jones EA. The histochemical assessment of sulphated and non-sulphated sialomucin in intestinal epithelium. *Histopathology*. 1985;9:1129-37.
4. Gad A. Pathophysiology of gastrointestinal mucins. *Adv Physiol Sci*. 1982;29:161-83.
5. Lev R. A histochemical study of glycogen and mucin in developing human fetal epithelia. *Histochem J*. 1968;1:152-65.
6. Lev R, Orlic D. Histochemical and radiographic studies of normal human fetal colon. *Histochemistry*. 1974;39:301-11.
7. Stauffer A, Lallamand A, Gaillard D. Mucin histochemistry of digestive tract in human foetuses. *Gastroenterol Clin Biol*. 1990;14(6-7):561-6.
8. Lev R, Siegel HR, Bortman J. Histochemical studies of developing human fetal small intestine. *Histochemistry*. 1972;29:103-19.
9. Filipe MI, Branfoot AC. Abnormal patterns of mucus secretion in apparently normal mucosa of large intestine with carcinoma. *Cancer*. 1974;34:282-90.
10. Culling CFA, Reid PE, Worth AJ, Dunn WL. A new technique of use in the interpretation and diagnosis of adenocarcinoma and villous lesions in the large intestine. *J Clin Path*. 1977;30:1056-62.
11. Hakkinen I, Javvi O, Gronroos J. Sulphoglycoprotein antigens in the human alimentary canal and gastric cancer. *Int J Cancer*. 1968;3:572-81.

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