Gastroenterologia Japonica
Vol. 8, No. 1 —1973—

—Original Article—

MUCIN METABOLISM IN INTESTINAL METAPLASIA OF GASTRIC MUCOSA

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Summary:

The incorporation of $^{35}SO_4$ and Glucose-^3H has been autoradiographically investigated in human gastric mucosa, especially in intestinal metaplasia, applying in vivo local labelling method during the operation of gastro-intestinal tract. The fact that sulfated mucin is actively secreted in gastric mucosa of various animals is already known, but little about the mucin metabolism of human stomach itself. In intestinal metaplasia of human gastric mucosa, markedly high metabolism of sulfated mucin have been observed compared with its low metabolism in normal gastric mucosa. In detail observation of intestinal metaplasia, columnar cells, which were considered to be absorptive cells, play an active and primary roll in the production of mucin. The goblet cells are to be considered rather as retentive cells with a slow turnover of mucin. Intestinal metaplasia occuring in the pyloric gland are, is suggestive of adaptation ability of gastric mucin for the disturbed mucin metabolism of pyloric glands to protect the gastric mucosa.

It is generally agreed that gastric mucin, including sulfated mucin is very important as a deffensive factor, as Hollander[1] described in 1953. The fact that sulfated mucin is actively secreted in gastric mucosa of various animals is already reported[2)3)] and we also confirmed experimentally of the marked incorporation of $^{35}SO_4$, in the generative cell zone of rat and dog gastric mucosa.[4)5)6)] Little is known, however, about the mucin metabolism of human stomach itself. We have investigated the incorporation of $^{35}SO_4$, applying in vivo local labelling method to the human gastrointestinal tract, in normal and cancerous tissue and reported that the metabolism of sulfate mucin of human gastric mucosa is markedly different from that of animals, i.e. weak incorporation of $^{35}SO_4$ in normal human stomach.[4)5)6)] Strong incorporation is found at the site of intestinal metaplasia, which is frequently seen around the various pathologic lesions. Magnus stated in 1937 that "the presence of intestinal epithelium in the stomach is the result the faulty regeneration of surface epithelium in a mucosa repeatedly damaged by gastritis".[7)] However, it was infered from the standpoint of mucin metabolism that intestinal metaplasia played some roll as the deffensive factor of gastric mucosa.

Materials and Methods

1) **Materials:**

Seventy-seven cases of surgical specimens of gastro-intestinal tract labelled either with $^{35}SO_4$ or Glucose-^3H were collected. Both colon and rectal specimens were used for the comparative study of mucin metabolism in intestinalized gastric mucosa versus that of the colon and rectum.

The specimes were as follows.

	$^{35}SO_4$	Glucose-^3H
Gastric Cancer	29 cases	6 cases
Gastric Ulcer	19	3
Normal Mucosa of Stomach	13	3
Colon Carcinoma	4	...
Normal Mucosa of Colon	3	...
Normal Mucosa of Rectum	6	...

2) **Methods:**

Applying the local labelling method devised by us in 1964,[8] we labelled the human gastro-intestinal mucosa at operation and investigated the labelling pattern of the resected tissue micro-autoradiographically. The local labelling method is superior in respects of safety that various injuries of isotopes to the human body is avoidable and of effective labelling in small amount of isotopes, compared with the generalized way to give isotopes intravenously or intramuscularly. The details of this method is as follows: During operation, we injected 250-500μCi of $^{35}SO_4$ or Glucose-^3H into the gastric mucosa after performing gastrotomy. The isotope should be injected not deeper than tunica muscularis mucosa, but rather more superficially producing vesicle at the injection site. The specimens were obtained 10-120 minutes after the injection, except for the rectum. Resected specimens were fixed in Carnoy's Fluid or in 10% Formalin, embedded in paraffin, and sectioned at about 4μ. Sections were washed to remove free sulfate or Glucose-^3H, then coated by dipping in liquid NTB 3 or Sakura NR-M2 emulsion for autoradiography. After exposure for one month, we developed and fixed by a FD III fixer, then stained with Hematoxylin-Eosin, Alcian Blue (pH. 2.6) and PAS.[9] Sections of tissue labelled with Glucose-^3H were treated with saliva for 10 minutes at 37°C for the removal of labelled glycogen before dipping.[10][11][12]

Results

1) Labelling pattern in the intestinalized mucosa

The intestinal metaplasia of gastric mucosa was most frequently seen in the pyloric gland area. In most cases of gastric cancer and gastric ulcer, intestinal metaplasia was observed at pyloric gland region and surrounding area of the lesion to a varied degree. Epithelium of intestinal metaplasia consisted of columnar cells and goblet cells with mucin granules positively stained by PAS and/or Alcian Blue, similar to the proper intestinal epithelium. In cases of regularly formed tubules, the Paneth's cell is located at its base.

The intestinalized epithelium was strongly labelled with $Na_2{}^{35}SO_4$ and/or Glucose-^3H in comparison with the weakly labelled proper gastric glands (**Table 1**). The difference in the labelling was distinct in the marginal area of the proper

Table 1. Incorporation of $^{35}SO_4$ and Glucose-^3H in the stomach epithelium and relation with histochemical reaction

Tissue and cell	Histochemical reaction		Incorporation of label	
	PAS	Alcian Blue	$^{35}SO_4$	Gl-^3H
Fundic region				
Surface epithelial cell	++	−	0–1	0–1
Foveolar cell	+~++	−	0–1	0–1
Generative cell	−~+	−	0~1	0~1
Parietal cell	−	−	0	0
Mucous neck cell	+	−	0	0
Chief cell	−	−	0–1	0–1
Pyloric region				
Surface epithelial cell	++	−	0–1	0–2
Foveolar cell	+~++	−	0–1	0–2
Generative cell	−~+	−	0–1	0–2
Pyloric glandular cell	−	+	1–2	1–2
Intestinal metaplasia				
Goblet cell	++	++	2	2
Columnar cell	−	−	3	3
Paneth's cell	−	−	0	0

* 0: negative, 1: mild, 2: moderate, 3: marked

Fig. 1. Strong incorporation of radioactive sulfate at the site of intestinal metaplasia of human stomach. The labels abruptly decrease where the metaplastic change has not take place. (Autoradiograph stained with H. & E. × 100)

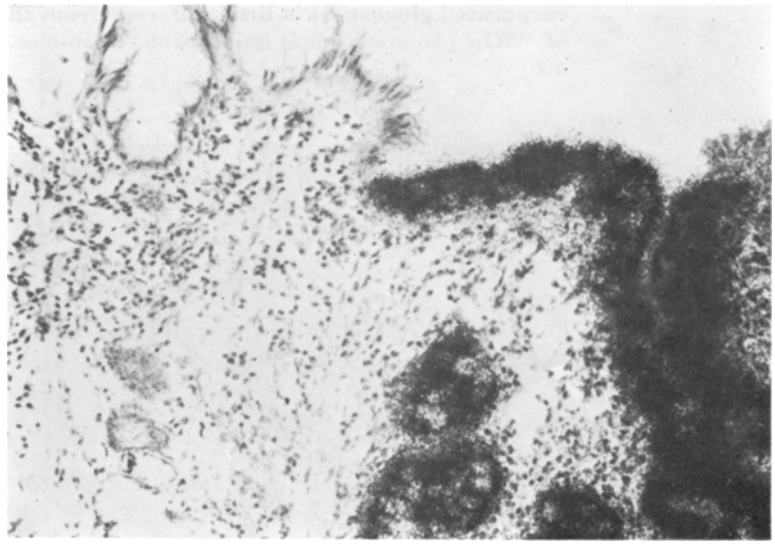


Fig. 2 Normal colonal crypt. The label of $^{35}SO_4$ is marked in the columnar cell which appears to hold no mucin and rather scant in mucin droplet in the goblet cell. (Autoradiograph stained with Alcian-blue × 400)

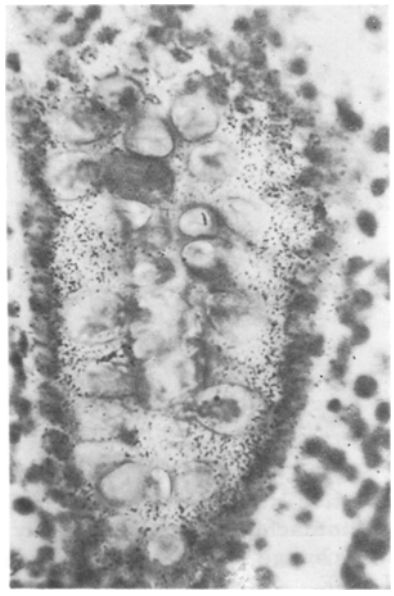


Fig. 3 Strong incorporation of $^{35}SO_4$ seen in intestinal metaplasia, especially in columnar cells situated between goblet cell. (Autoradiograph stained with Alcian-blue × 400)

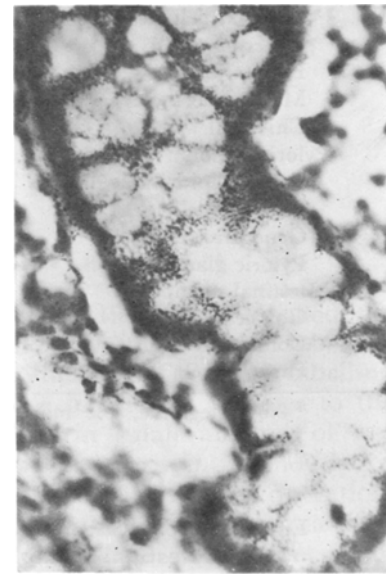
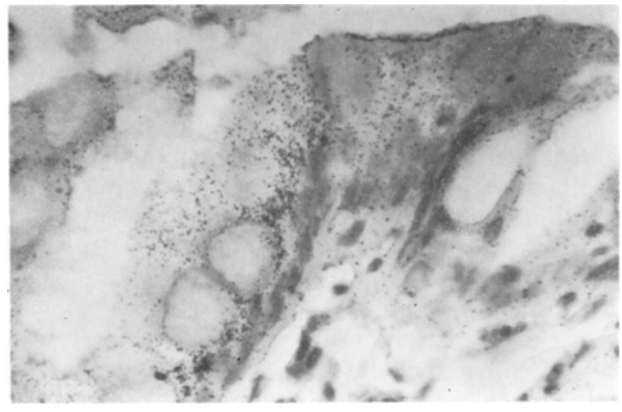


Fig. 4. Strong incorporation of Glucose-^{3}H seen in intestinal metaplasia. Distribution pattern of incorporated glucose-^{3}H is little different from that of $^{35}SO_4$. (Autoradiograph stained with Alcian-blue × 400)



and intestinalized gastric mucosa (**Fig. 1**) The labelling pattern of intestinalized mucosa is quite similar to that of colon (**Fig. 2**) with no difference in the labelling between $Na_2{}^{35}SO_4$ and Glucose-^3H (**Fig. 3,4**). The labelling was marked in the goblet cells and the cytoplasm of columnar cells, especially in the supranuclear area, where mucin granules were not recognizable with ordinary staining. The labelling was denser around the mucin droplet in the goblet cells than in the center of aggregate of the mucin droplets. Labelling was observed in the tubular lumen but not in Paneth's cells. Sometimes, lables of $Na_2{}^{35}SO_4$ and/or Glucose-^3H were observed in incompletely intestinalized epithelium, which was considered histologically as a surface epithelium of foveolar cell in the pyloric gland region (**Fig. 5**). At the same time, moderate labelling was seen in the morphological pyloricglands, located in the neighbourhood of the intestinalized glands (**Fig. 6**).

Histochemically there was no straight correlation between the labelling and staining by PAS or Alcian-blue of columnar cells (**Table 1**).

2) Turnover of $^{35}SO_4$ in the rectal mucosa

To examine the turnover of mucin in the intestinalized gastric mucosa, we investigated rectal mucosa in which the labelling pattern was similar to the intestinalized gastric mucosa, and a longer follow-up was possible in the rectum. Using $Na_2{}^{35}SO_4$, we investigated the movement of mucin at 5.5, 8 and 21 hours after injection. The rectal glandular surface was strongly lebelled at 5.5 and 8

Fig. 5. The labels of $Na_2{}^{35}SO_4$ were intensely observed in incompletely intestinalized epithelial cells, which was morphologically considered as a surface epithelium of proper pyloric gland. (Autoradiograph stained with H. & E. × 400)

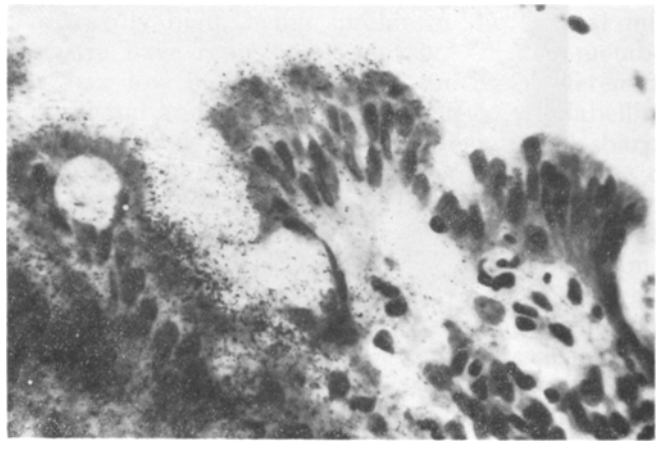


Fig. 6. Moderate labelling of $Na_2{}^{35}SO_4$ was seen in the morphological pyloric glands, located in the neighbourhood of the intestinalized cells.

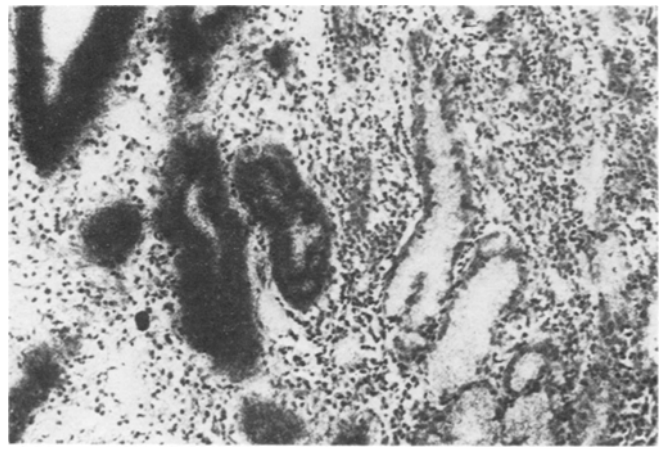
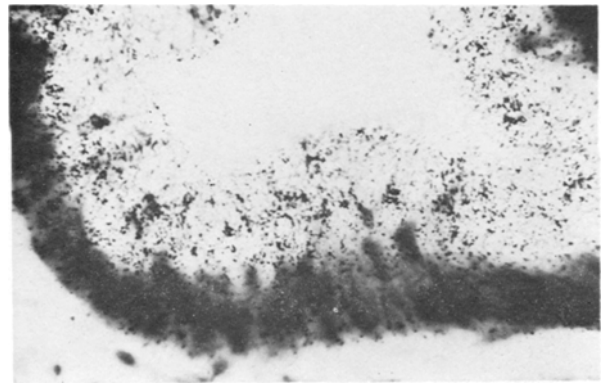
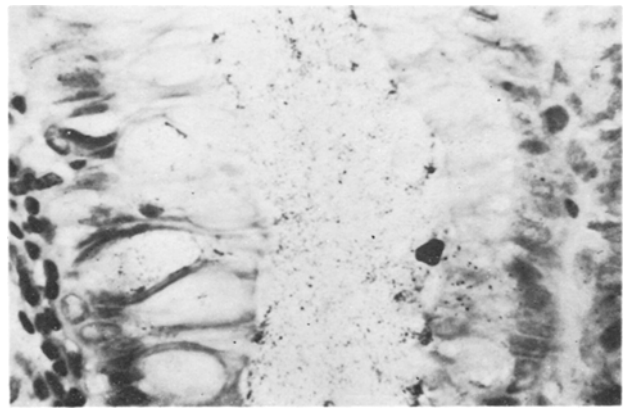


Fig. 7. Human rectal epitahelium. 8 hours after local labeling of $^{35}SO_4$ shows intense incorporation of the label in both columnar and goblet cell. (Autoradiograph stained with H. & E. × 400)



hours showed almost equal labelling in the goblet cells and columnar cells in both cases (**Fig. 7**). At 21 hours after injection, almost all labelled mucin was observed in the extra-ductal area, except for minute amount in the cytoplasm of goblet cells (**Fig. 8**).

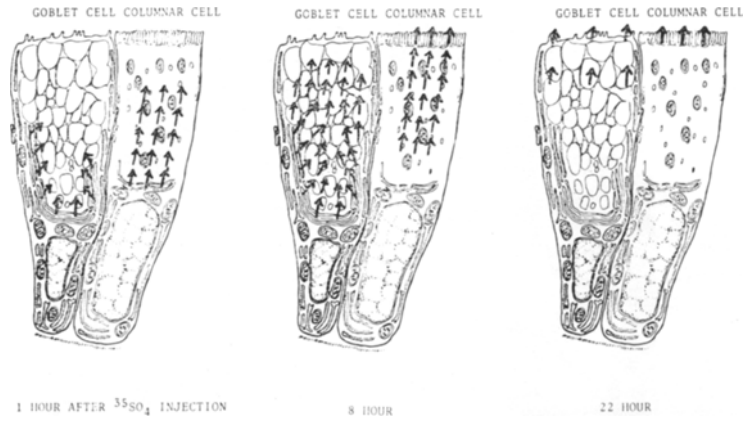
Fig. 8. Human rectal epithelium 22 hours after local labeling of $^{35}SO_4$. Labeled mucin was almost discharged in the crypt-lumen but some still remained in the mucin droplet in the goblet cell. (Autoradiograph stained with H. & E. × 400)



Discussion

Abnormal gastric mucosa, similar to intestinal mucosa, in the stomach has been said to be a congenital abnormality of the mucosa.[13] Recently, however, this finding has been considerd to be pathognomonic of chronic gastritis.[14] We investigated the problem from the standpoint of mucin metabolism in the intestinalized mucosa by autoradiography. The mucin metabolism of the intestinalized gastric mucosa is markedly high, being similar to the normal mucosa of the colon. However, as we have reported previously,[4)5)6)] the metabolism of the sulfated mucin was very low in human gastric mucosa, different from a high metabolism in experimental animals. Therefore the intense labelling at the site of intestinalized gastric mucosa was, in general, demarcated sharply from the minimal labelling in proper gastric mucosa. In further minute observations, the labels of $^{35}SO_4$ were recognizable on every cell of metaplastic epithelium showing intestinalization, that is, the labels were observed even on the columnar cells which seemed not to hold mucin histochemically, as well as or more intensely than the goblet cells. Originally, the goblet cell had been considered to produce mucin primarily, with no mucin producing ability residing in the columnar cells, they being considered absorption cells.[15] However, it was clear from our investigation by flash labelling in the intestinalized gastric mucosa and by long-term labelling in the rectal mucosa that the most important role of mucin production is played not by goblet cells but by the columnar cells. The goblet cells are considered to have a tendency for retention of mucin with a slow turnover (**Fig. 9**). Sulfated mucin is considered to be secreted not only from the goblet cells, but continuously, from almost all cells of metaplastic epithelium. In some cases, however, dense $^{35}SO_4$ labelling was observed in the proper gastric epithelium or pyloric

Fig. 9. Schema of comparative movement of mucin in goblet cell and in columnar cell.



glands contiguous to the intestinalized mucosa. These proper gastric epithelium or pyloric glands near the aberrant mucosa are considered functionally to be almost intestinalized. If this change suggests an adaptation ability of gastric mucin for the disturbed mucin metabolism of pyloric glands to protect the gastric mucosa, it seems likely from the standpoint of mucin metabolism, that the presence of intestinalized gastric mucosa is not necessarily the "faulty regeneration of mucoda"[7)16)17)]

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Received 3rd Oct. 1972
Accepted 23rd Oct. 1972