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Morphological Characteristics of the Columnar Epithelium Lining the Lower Oesophagus in Patients with Barrett's Syndrome

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Summary. Biopsies of five patients with Barrett's syndrome were examined by light microscopic, cytochemical and electron microscopic techniques. It was shown that the mucosa in the lower oesophagus lined by columnar epithelium was very similar to the mucosa lining the cardia of the stomach. It is supposed that such esophageal epithelium represents an upgrowth of cardia mucosa as a response to injury of the squamous epithelium of the oesophagus caused by chronic reflux of gastric contents. The occurrence of goblet cells and patches of villous columnar cells was interpreted as intestinal metaplasia.

The morphogenesis of the columnar epithelium lining the lower oesophagus has been debated since Barrett (1950) described the condition as congenital short oesophagus with intrathoracic stomach. Later Barrett (1957) agreed with the concept of Allison and Johnstone (1953) that the intrathoracic organ lined by columnar epithelium in fact was oesophagus and not stomach, and he stated that the presence of columnar epithelium was probably a result of a failure of the embryonic lining of oesophagus to achive normal maturity. Others (Goldman *et al.*, 1960; Mossberg, 1966; Bremer *et al.*, 1970) support the theory that the columnar epithelium developed secundarily to reflux oesophagitis and that the changes in the lining epithelium might have developed from proximal growth of cardia epithelium, or from extention of superficial cardia glands.

The present light- and electronmicroscopical study has been performed in an attempt to further clarify the nature of the columnar epithelium lining the lower oesophagus in patients with Barrett's syndrome.

Material and Methods

The material comprised biopsies from 5 patients admitted to the Department of Thoracic Surgery. All had oesophagoscopy, x-ray examination and manometry of the oesophagus performed. The various findings are listed in Table 1. By manometry it was possible to locate the sphincter in relation to the stricture and at the same time to characterize the motility pattern of the intervening area (Pedersen *et al.*, in press).

Biopsies were taken with biopsy forceps proximal to, at the level of, and distal to the stricture.

For *Light microscopy* studies the specimens wer fixed at 4° C for 24 hr in neutral buffed 10% formaldehyde, 5% glutaraldehyde buffed at pH 7.4 with 0.2 M Na-cacodylate and Bouin's fluid.

To paraffin sections the following methods were applied: A modification of Zimmermann's method (1925) for differential staining of gastric mucosa (Marks *et al.*, 1957) as a stain for

Table 1. Age, sex and X-ray findings in five cases of Barrett's syndrome

Case number	Age in years ♀	Age in years ♂	Site of stricture	Length of stricture (cm)	Hiatus hernia
1	52		T4	9	+
2	28		T7	1	+
3		59	T4–5	2	+
4	61		T9–10	1	+
5		56	T10	5	+

mucous cells, chief cells and parietal cells. Alcian blue at pH 2.5 followed by periodic-acid Schiff (PAS) as stains for Paneth cells (Spicer *et al.*, 1967) and goblet cells. Diazonium reaction with Fast Garnet GBC or Fast Black K and Masson's argentaffin reaction for biogenic amines (Solcia *et al.*, 1969b). As selective stains for endocrine cell granules HCl-toluidine blue ("masked metachromasia") (Solcia *et al.*, 1968), lead-haematoxylin (Pb-H) (Solcia *et al.*, 1969a) and Grimelius'argyrophilic silver impregnation (Grimelius, 1968) were used.

For *electron microscopy* studies the specimens were flattened cut surface down onto a cardboard and fixed 3 hr a 4° C in 5% glutaraldehyde buffed at pH 7.4 with 0.2 M Na-cacodylate. The specimens were cut into wedge-shaped blocks, post fixed in osmium tetroxid 1%, dehydrated and embedded on edge in epon in such a way that well-orientated 0.5 μ thick sections could be obtained (Pittman *et al.*, 1966) These sections were stained with toluidine blue and used to locate areas to be trimmed for ultrathin sectioning. Sections were cut on a Reichert OmU2 ultramicrotome and thin sections were examined with a JEM-T7 electron microscope after staining with Zn-uranyl-acetate 4% and Pb-citrate 0.4%.

For the purpose of comparison, gastric mucosa was obtained from 1 patient with gastric carcinoma, 1 patient with gastric ulcer and 1 patient with diffuse atrophic gastritis. These biopsies were fixed and embedded for electron microscopy as described above.

Result

Hiatal hernia, peptic oesophagitis with formation of stricture, and lining of lower oesophagus with columnar epithelium were the pathological findings in our patients.

Biopsies from above the stricture were all lined with squamous epithelium similar to that found in oesophagi of patients without Barrett's syndrome and were examined by light microscopy only. Biopsies obtained at the level of the stricture were devoid of epithelium or lined by columnar epithelium (Fig. 1a and b). In two patients transition from stratified squamous to columnar epithelium was demonstrated (Fig. 1b). All biopsies from below the stricture were lined by columnar epithelium. Biopsies lined by columnar epithelium were studied by histochemical methods and electron microscopy.

By *light microscopic* examination the mucosa lined by columnar epithelium was folded but not villous. Its deeper layer contained simple as well as branched tubular glands. Several types of cells could be identified (Table 2).

Most abundant were *mucus-producing columnar* cells situated in the surface epithelium as well as in the glandular epithelium. The apical cytoplasm of these cells stained intensely with PAS (Fig. 2) and toluidine blue but not with Alcian blue.

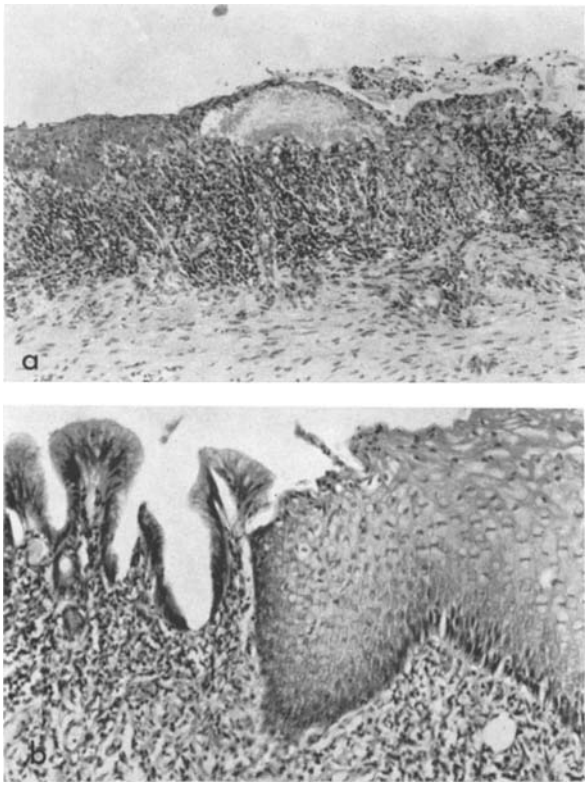


Fig. 1 a and b. Light micrograph of biopsies obtained at the level of the stricture. a Transition from mucosa lined by stratified squamous epithelium to ulcerated mucosa (to mucosa devoided of epithelium). Hematoxylin and eosin. ×80. b Transition from stratified squamous to columnar epithelium. Haematoxylin and eosin. × 180

Table 2. Cell types in the columnar epithelium lining the lower oesophagus in patients with Barrett'syndrome demonstrated by light- and electron microscopy

Case number	Mucous producing columnar cells	Parietal cells	Chief cells	Endocrine cells	Goblet cells	Villous columnar cells	Paneth cells
1	+	−	−	+	+	+	−
2	+	+	−	+	+	−	−
3	+	−	−	+	+	−	−
4	+	−	−	+	+	−	−
5	+	−	−	+	+	+	−

A variable number of *goblet* cells, most numerous in the surface epithelium was observed. The secretion product of the goblet cells stained brightly with PAS (Fig. 2) as well as with Alcian blue and metachromatically with toluidine blue.

Fig. 2. Light micrograph of the oesophageal mucosa lined by columnar epithelium, showing mucus producing columnar cells and goblet cells. Stained with PAS, ×500

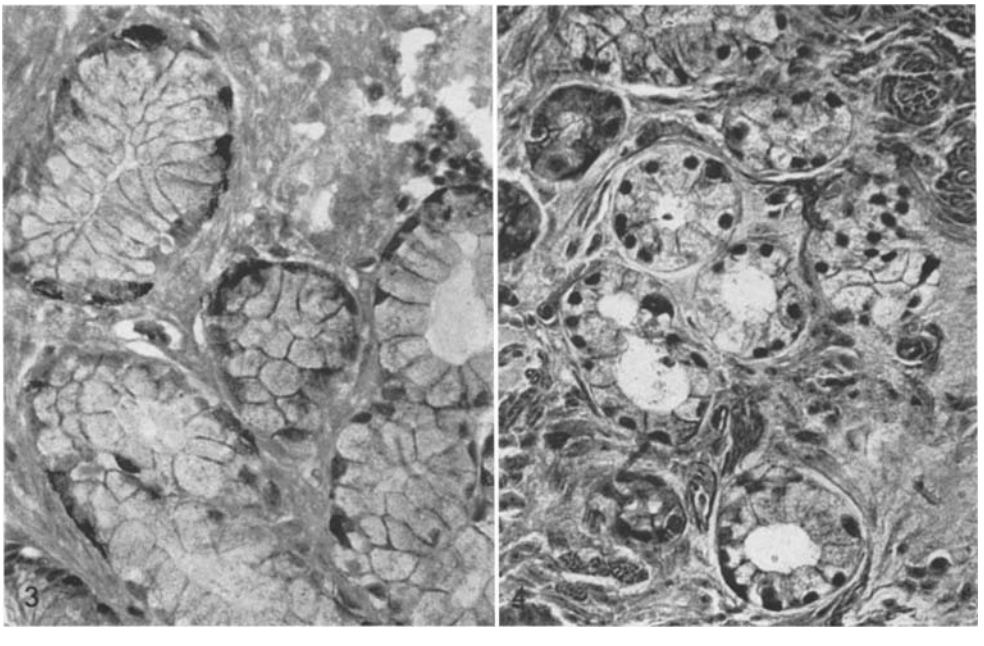


Fig. 3. Light micrograph of glands in the oesophageal mucosa lined by columnar epithelium, showing endocrine cells. Stained with Masson's argentaffin silver reaction, ×900 (reduced to 7/8)

Fig. 4. Light micrograph of glands in the oesophageal mucosa lined by columnar epithelium, showing endocrine cells. Stained with Pb-haematoxylin, ×750 (reduced to 7/8)

Endocrine cells were most numerous in the glandular epithelium (Fig. 3 and 4). By combining methods detecting biogenic amines with methods selectively staining endocrine cell granules (Vassallo *et al.*, 1971) several types could be distinguished (Table 3, Wiesbaden numeclature) (Pearse *et al.*, 1970).

Table 3. Staining patterns of endocrine cells in the columnar epithelium lining the lower oesophagus in patients with Barrett's syndrome. ECL = enterochromaffin-like, G = gastrin-secreting, D = equivalent to D cells in pancreas, A = equivalent to A cells in pancreas

Endocrine cell types		Masson Hamperl	Diazonium	Lead haematoxylin	Hcl-toluidine-blue	Grimelius
Enterochromaffin cell (EC)		+	+	dark blue	blue	+
Non enterochromaffin cell	(G, D, A)	—	—	dark blue	red-violet	+
	ECL	—	—	dark blue	—	+

Table 4. Electron microscopic characteristics of endocrine cells in the columnar epithelium lining the lower oesophagus in patients with Barrett's syndrome. EC = enterochromaffin, ECL = enterochromaffin-like, G = gastrin-secreting, D = equivalent to pancreatic D cell, A = equivalent to pancreatic A cell

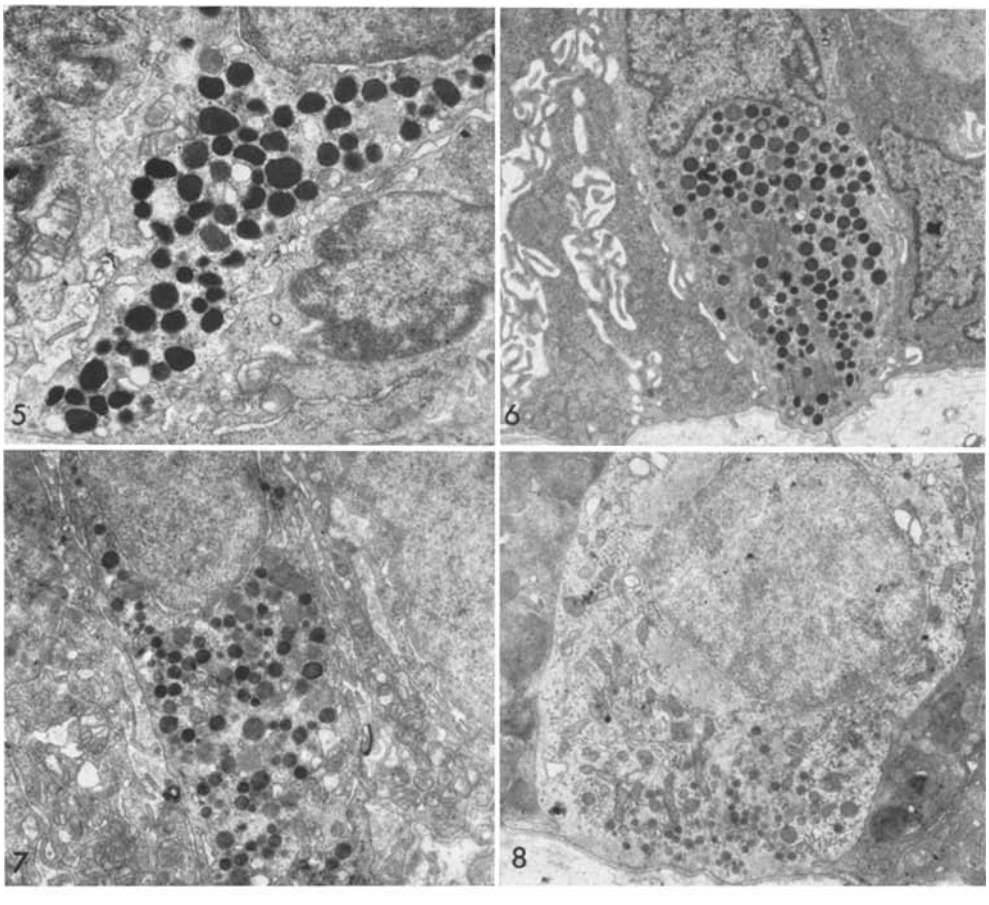
Endocrine cell type	Characteristics of secretory granules			other characteristics
	shape	diameter in nm	electron contrast	
EC-cell	irregular	325	high	basally located granules
ECL-cell	round	260	high to moderate	bubble-shaped granules
G-cell	round	180	low to moderate	variability in density of granules
D-cell	round	210	low to moderate	cytoplasmic microfilaments
A-cell	round	270	high	

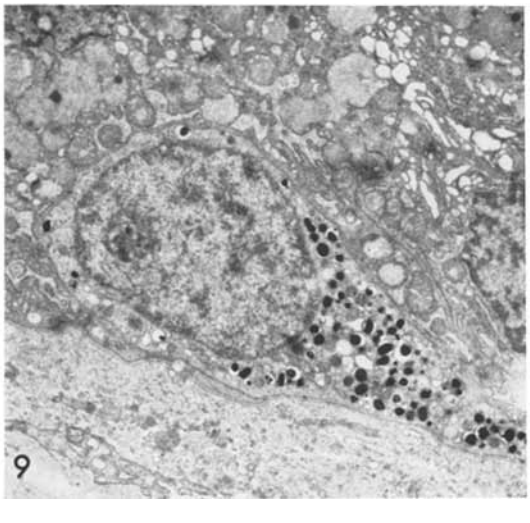
In order to differentiate between EC cells and non-EC cells, sections were stained by Pb-H, subsequently decolorated in dilute HCl and restained by diazonium Fast Black K. Among the Pb-H positive cells some stained black by diazonium showing EC cells. In sections stained by Grimelius silver technique some argyrophilic cells failed to react to the HCl-toluidine blue method and so must be ECl cells.

In some biopsies groups of columnar epithelial cells more elongated than the mucus producing columnar cells and with a few PAS positive granules in the apical cytoplasm could be observed. Groups of these cells were situated in the surface epithelium and within the glands and were given the name *villous columnar* cells because a brush border was lining the apical surface of the cells. A few scattered parietal cells could be demonstrated in the glandular epithelium of biopsies from one patient. Chief cells and Paneth cells were not observed.

By *electron microscopic* examination of the esophageal columnar epithelium distal to the stricture, the observations made with light microscopy were confirmed.

The apical cytoplasm of the *mucus producing columnar* cells, which on light microscopy were stained with PAS, contained, spherical or elipsoid droplets enclosed by a membrane. The granules occupied the entire apical cytoplasm and





Figs. 5—9

also were in the paranuclear, but not in the basal cytoplasm. The nucleus was located in the basal cytoplasm and a prominent Golgi complex was seen in a supranuclear site (Fig. 5). The lateral cell membranes were staight near the apical border but elaborately interdigitated towards the base. Microvilli were few and rudimentary. Mainly based on the ultrastructure of their secretory granules 5 types of *endocrine* cells have been detected (Table 4). All endocrine cells were in direct contact with the basal lamina of the epithelium. EC cells, G cells and D cells were observed to reach the glandular lumen and were on their apical surface provided with microvilli. EC cells and A cells were observed in biopsies from all patients (Figs. 6 and 7). G, D, and ECl cells were observed in biopsies from at least 3 patients (Figs. 8–10).

The *villous columnar* cells were characterized by microvilli covering their apical surface. Fine intracellular filaments could be observed in the central core of the microvilli. They did not end in a terminal web but penetrated deeply into the apical cytoplasm (Fig. 11). In the apical cytoplasm a few membrane bound granules, apparently secretory in nature, were observed. In between the villous columnar cells were goblet cells and occasionally mucus producing columnar cells.

The fine structure of the parietal cells and the goblet cells was similar to that of normal parietal cells and goblet cells.

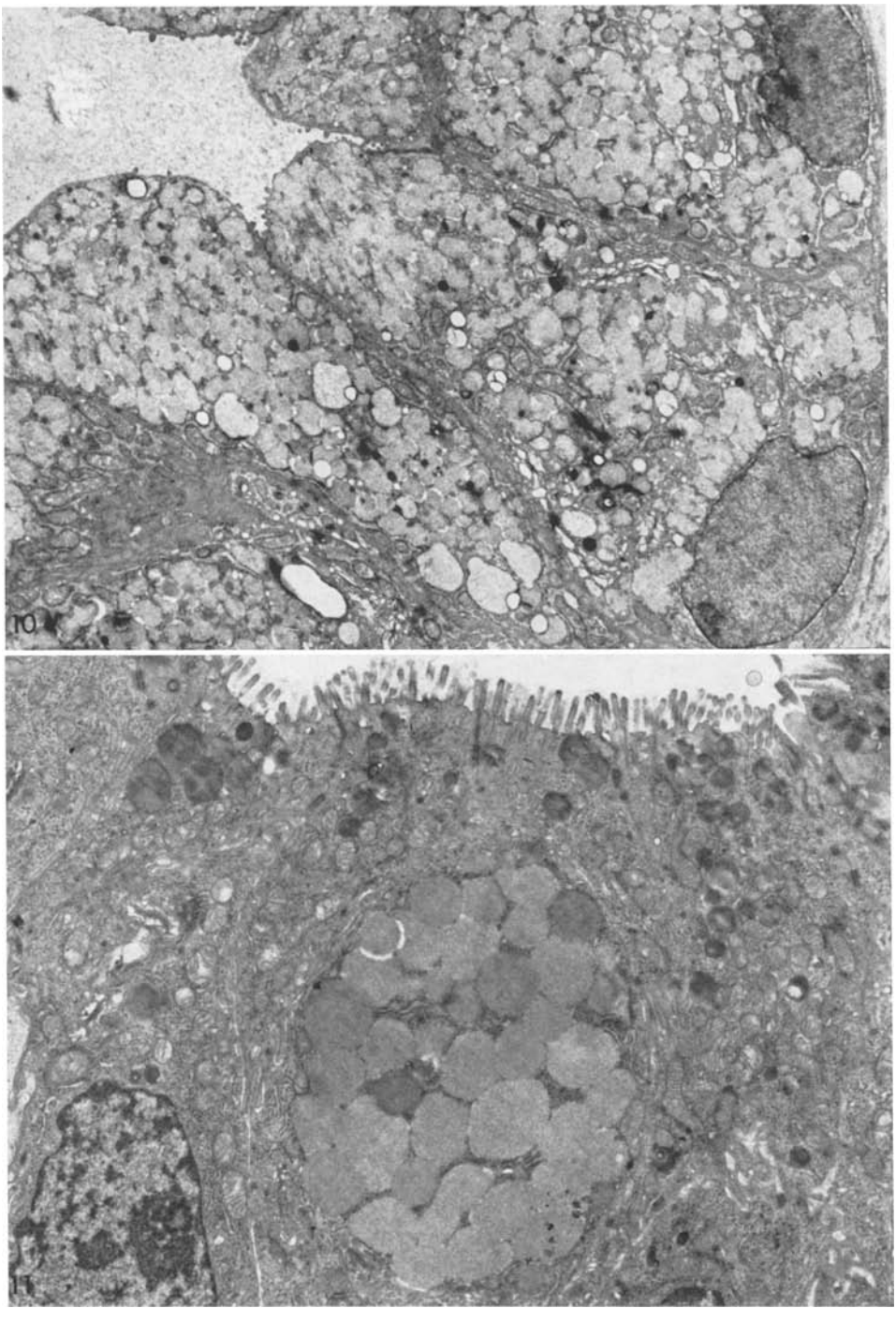
Chief cells and Paneth cells were not observed. Electron microscopy confirmed the morphological similarity between the columnar villous cells of oesophagus and the undifferentiated crypt cells in the metaplastic intestinal epithelium of gastric mucosa (Fig. 12a and b).

Discussion

In biopsies from five patients with Barrett's syndrome Trier (1970) described the luminal surface of the oesophageal mucosa lined by columnar epithelium to consist of villous-like folds. He pointed out that the columnar epithelial cells in the oesophagus differ from the mucous cells of the cardiac glands in housing numerous microvilli, a partially developed terminal web, and fewer granules in the apical cytoplasm. In the present study the mucosa was folded but not villous. The most abundant epithelial cell was a mucus producing columnar cell with few, rudimentary microvilli and numerous mucous granules in the apical as well as in the paranuclear cytoplasm. Moreover, in view of the finding of endocrine cells (type G, ECl and D) which have so far been described in gastric and duodenal glands only (Forssmann *et al.*, 1969; Vassallo *et al.*, 1971) we believed the columnar epithelium lining oesophagus as being of the same type as in cardia.

The occurrence of goblet cells and patches of villous columnar cells may be interpreted as intestinal metaplasia analogous to the findings in patients with gastric carcinoma, gastric ulcer and diffuse atrophic gastritis (Rubin *et al.*, 1966). Goblet cells among the mucus producing columnar cells and mucus producing

Figs. 5—9. Electron micrographs showing endocrine cells in the glandular epithelium of the oesophageal mucosa. 5: EC cell ×7300, 6: A cell ×4200, 7: G cell ×5700, 8: D cell ×5200 (reduced to 9/10), 9: ECL cell ×4200



Figs. 10 and 11

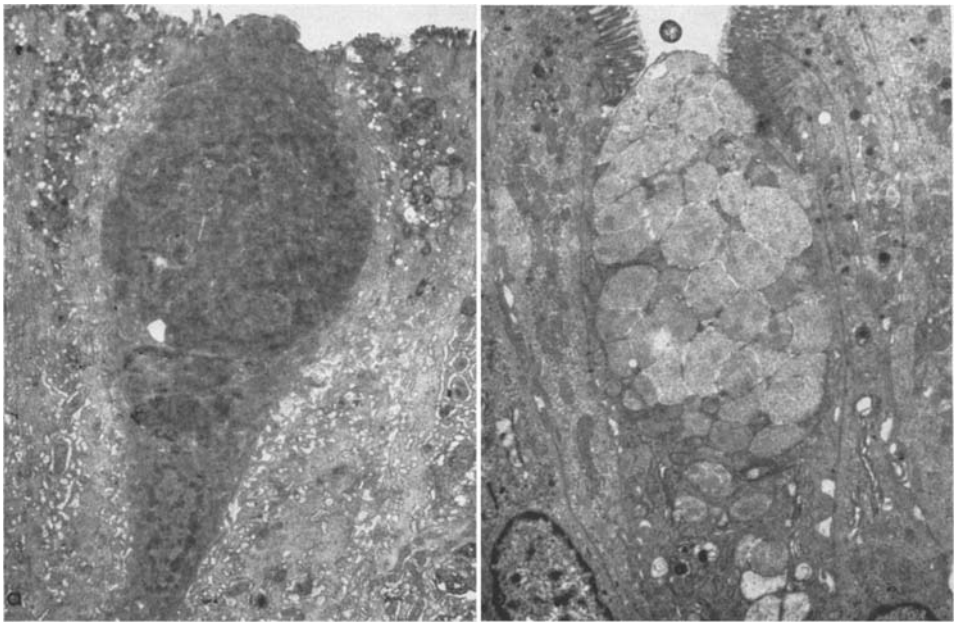


Fig. 12a and b. Electron micrographs of villous columnar cells and a goblet cell in a: the epithelium lining the luminal surface of oesophagus and b: the metaplastic intestinal epithelium of gastric mucosa, ×3600 (reduced to 9/10)

columnar cells among villous columnar cells might represent partial metaplastic transformation, as described by Si-Chun Ming *et al.* (1967) in cases of human gastric carcinoma.

Two hypotheses have been proposed for the origin of the columnar epithelium in the lower oesophagus. One suggests that the columnar epithelium derived from heterotopic tissues present at birth, the second that the epithelium is formed as response to injury of the squamous epithelium of the oesophagus caused by chronic reflux of gastric content. Recently, experimental studies of oesophageal mucosal regeneration in dogs made by Bremer *et al.* (1970) support the latter theory. In a description of regeneration of gastric mucosa Ito (1967) explained regeneration by proliferation and migration of relatively undifferentiated surface mucous cells from the margin of a damaged or excised area. This invaginates to form gastric pits and glands. The surface mucous cells appeared to become transformed to cells resembling mucous neck cells which may differentiate to parietal and later to chief cells. No transitional stage could be observed between

Fig. 10. Electron micrograph showing mucus producing columnar cells lining a gland in the oesophageal mucosa, ×11600 (reduced to 9/10)

Fig. 11. Electron micrograph showing villous columnar cells and a goblet cell in the epithelium lining the luminal surface of oesophagus, ×7400 (reduced to 9/10)

mucous neck cells and endocrine cells but the precursor cell of endocrine cells has not yet been identified (Ferreira *et al.*, 1971). Moreover, if this cell regeneration and differentiation is impaired by disease intestinal epithelium might develop.

Regeneration in this way of a damaged area of the very distal oesophagus caused by reflux of gastric content could explain why the columnar epithelium of the lower oesophagus has been so variably described and characterized as cardiac (Allison *et al.*, 1953) gastric (Heitman *et al.*, 1967; Hershfield *et al.*, 1965) and intestinal (Abrams *et al.*, 1965).

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