Virchows Arch. A Path. Anat. and Histol. 374, 275–284 (1977)

Virchows Archiv A

Electron Microscopic Study of Signet-Ring Cells in Diffuse Carcinoma of the Human Stomach

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Summary. The signet-ring cells seen in gastric carcinoma of the diffuse type were studied by light and electron microscopy. A classification of these cells into Types A, B and C was developed, based on intracellular mucous granules, nuclei and organelles, and by reactions to PAS and Alcian-blue staining. The gradual transition of Type A cells to Type B and the subsequent change of Type B to Type C suggest successive stages in maturation. These cells, especially those of Type B, resemble the mucous neck cells of the corpus and the glands of the pylorus.

Key words: Signet-ring cell – Scirrhous carcinoma – Gastric carcinoma – Electron microscopy – Stomach cancer.

Introduction

Currently, human gastric carcinomas are classified histologically into intestinal and diffuse types since the proposal by Laurén (1965). Intestinal type carcinoma forms well or moderately differentiated tubules or glands composed of columnar epithelium and is accompanied by severe intestianl metaplasia of the gastric mucosa. These findings strongly suggest that gastric carcinomas of this type may be derived from metaplastic mucosa. Ultrastructural similarity between intestinal type carcinoma and metaplastic epithelium has been described (Ming et al., 1967; Goldman and Ming, 1968).

Diffuse carcinoma, in which glandular formation is extremely poor, is characterized by infiltration of solitary or small clusters of cancer cells into the stroma. In the mucosa the tumor cells often produce large amounts of intracellular mucin and in the advanced stage, infiltrating cancer cells are quite often accompanied by dense fibrous connective tissue, giving a scirrhous appearance.

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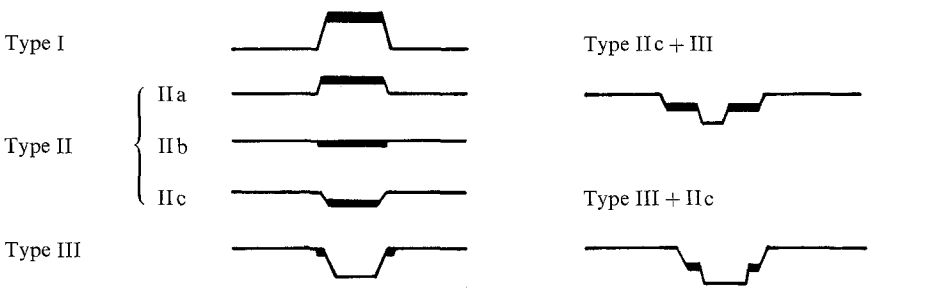
Table 1. Macroscopic classification of early gastric carcinoma. When a carcinoma shows diverse morphological patterns, 2 or more types are described together



In early stage of gastric carcinoma of the diffuse type, the characteristic macroscopic feature is a shallow depression or mucosal erosion with a sharp transition from normal mucosa without adjoining mucosal elevation. This type was classified as IIc (superficial depressed type) by the Japan Gastrointestinal Endoscopy Society in 1962 (Table 1) and most of the type shows histologically a picture of poorly differentiated adenocarcinoma or signet-ring cell carcinoma.

In recent years gastric cancer, diagnosed histologically either as poorly differentiated adenocarcinoma or signet-ring cell carcinoma, has increased in Japan. Histogenetic evidence and new methods of studying diffuse gastric carcinomata are therefore necessary in an attempt to asses the reasons for this increase. This paper report a light and electron microscopic study of the histogenesis of diffuse carcinoma. Cases of colloid carcinoma or mucinous adenocarcinoma with signet-ring cells were not included in the present study.

Materials and Methods

The material was obtained from surgically resected stomachs diagnosed histologically as gastric carcinoma. It included 21 cases of poorly differentiated adenocarcinoma (scirrhous type) and 6 cases of signet-ring cell carcinoma (mucosal cancer). The specimens were obtained in the operating room immediately after excision and fixed in 3% glutaraldehyde in 0.05 M phosphate-buffer at pH 7.5 for 10 min. After fixation they were cut into two pieces. One piece was further cut into several smaller fragments and fixed again in 3% phosphate-buffered glutaraldehyde for 3 to 24 h. The fragments were washed for 1 to 3 days in 0.1 M phosphate buffer containing 7.3 g of sucrose/100 ml and then post-fixed in 1% osmium tetroxide in 0.05 M phosphate buffer containing 0.2 M sucrose for 4 h. Subsequently the fragments were dehydrated in a graded series of ethanol concentrations and embedded in Epon according to the method of Luft (1961). Ultrathin sections were made using a LKB ultratome with glass knives and stained with uranyl acetate and lead. The stained sections were examined with a Hitachi HU-12 electron microscope. One micron thick sections were cut from each Epon-embedded fragment and stained by Huber's method (1968). Another one-micron thick section was stained with PAS and Alcian-blue (pH 2.5) after removal of Epon (Lane and Europa, 1965). Both sections were used to correlate light and electron microscopic appearances.

The other piece of the specimen was fixed in 10% formalin for conventional light microscopic sections stained with hematoxylin and eosin, PAS and Alcian-blue (pH 2.5).

Results

The signet-ring cells of diffuse carcinoma were classified into three main types – A, B and C – on the basis of the electron microscopic appearance of the intracellular mucous granules, the nuclei and cellular organelles, and their reactions to PAS and Alcian-blue staining in the preparations for light microscope (Table 2).

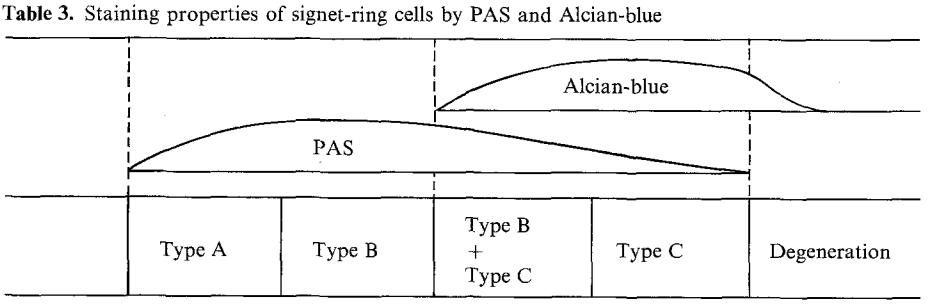
1. Type A Signet-Ring Cell (Fig. 1)

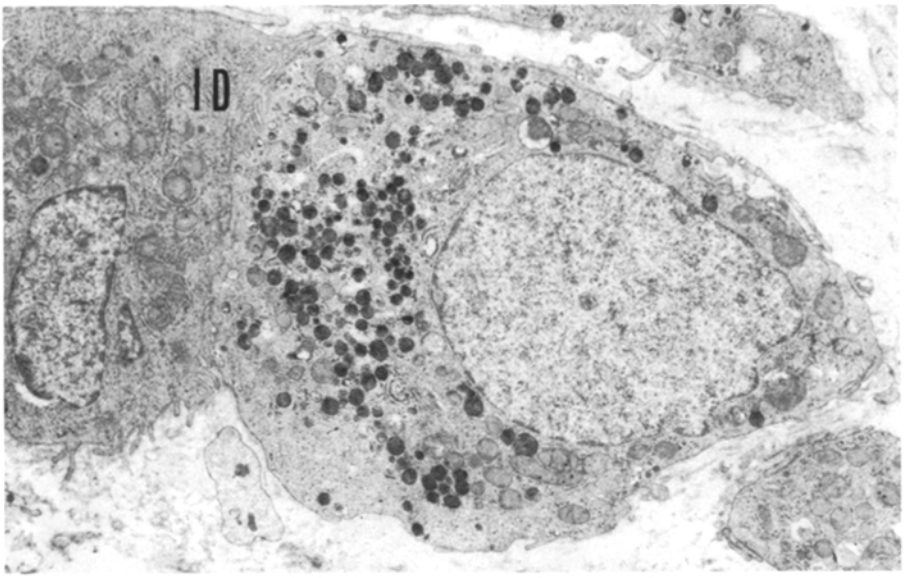
The ratio of the nucleus to the cytoplasm was largest in this type of cell. The nucleus, which was round or elliptical or occasionally indented, was seen

Table 2. Electron microscopic classification of signet-ring cells in diffuse carcinoma

	Type A	**Type B**	**Type C**
Nucleus	near center round or slightly elliptical in shape	eccentric elliptical in shape	periphery crescent in shape
Cell organelles	abundant	decreased	extremely decreased
Mucous granules	high density small (0.3 ~ 0.5 μ) coalescence (−) core (−)	moderate density medium (0.5 ~ 1 μ) coalescence (±) core (+)	low density large (2 μ or more) coalescence (+) core (±)
Staining behavior	PAS only	mainly PAS	mainly Alcian-blue

Table 3. Staining properties of signet-ring cells by PAS and Alcian-blue





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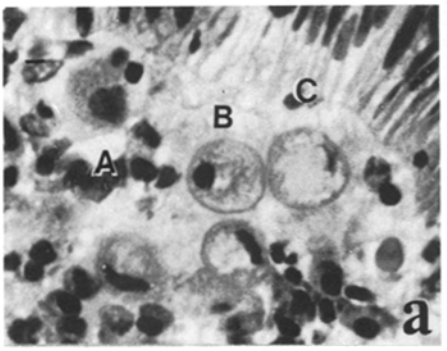

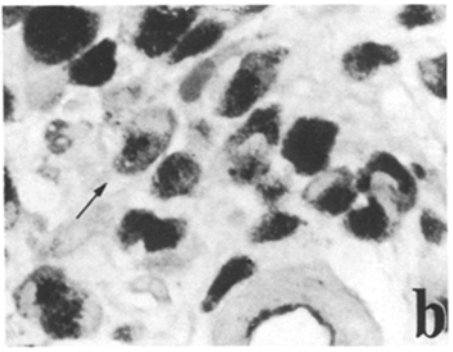

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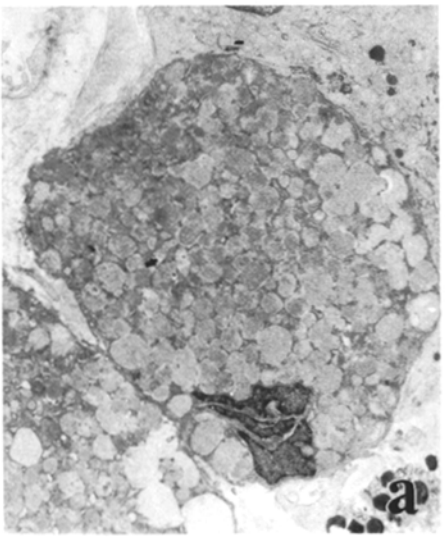

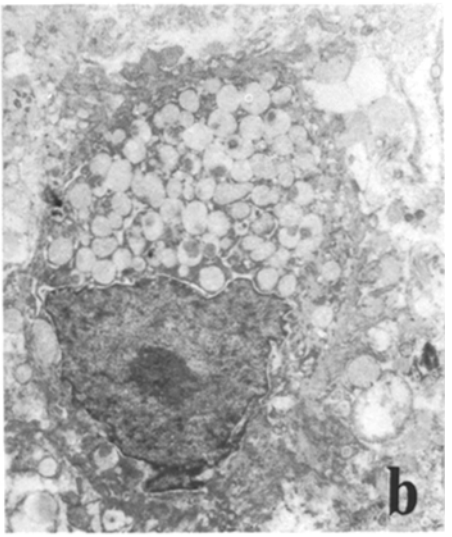

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in the center of the cell. Electron microscopically, signet-ring cells of this type possessed electron dense, small mucous granules measuring 0.3 to 0.5 μ. These granules were sparsely distributed, but were most numerous near the indented side of the nucleus. They were discrete. Abundant smooth and rough endoplasmic reticulum, mitochondria, Golgi complex, and free ribosomes were observed in the cytoplasm. Cells of this type observed by light microscopy were relatively small, with eosinophilic cytoplasm and a round nucleus (Fig. 2a). In both Epon embedded one-micron-thick sections and paraffin sections staining with PAS, tiny granules in the cytoplasm showed PAS-positive (Fig. 2b). These granules were Alcianblue negative.

2. Type B Signet-Ring Cell (Fig. 3)

The nucleus was located eccentrically and was occasionally compressed by the mucous granules and displaced towards the periphery of the cell. Signet-ring cells of this type had moderately electron-dense mucous granules measuring 0.5 to 1 μ. These granules were usually separated, but when the size and number of the granules increased they tended to coalesce. Highly electron dense cores were occasionally observed within these granules, and were similar in density to mucous granules of Type A cells. The cytoplasmic organelles were decreased and could be seen at the periphery of the cell or between mucous granules. Cells of this type had slightly eosinophilic, foamy cytoplasmic granules by light microscopy, which were PAS positive and Alcian-blue negative.

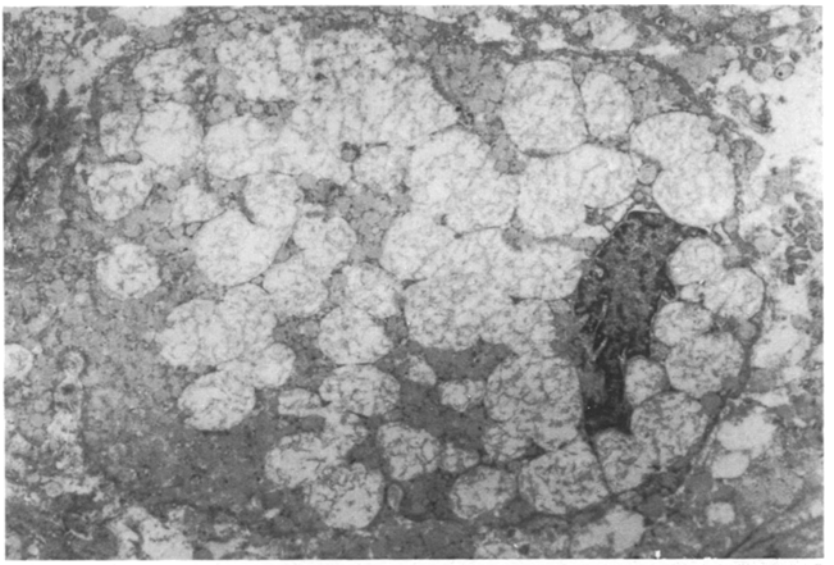
3. Type C Signet-Ring Cell (Fig. 4)

The nucleus was seen at the periphery of the cell compressed by abundant large mucous granules. Signet-ring cells of this type possessed low density, large mucous granules measuring 2 μ or more. These granules were coalescent and usually lacked electron dense cores. The cytoplasmic organelles were greatly decreased. The cells had clear, foamy cytoplasm and a crescentic nucleus by

Fig. 1. Type A signet-ring cell. A round nucleus situated in the center of the cell and small, spherical, dense mucous granules are sparsely distributed. The cytoplasmic organelles are abundant and interdigitation (*ID*) between cells are present. ×7380

Fig. 2. **a** Hematoxylin and eosin staining of paraffin embedded section. Type A cell (*A*), Type B cell (*B*), and Type C cell (*C*) are shown. ×1600. **b** PAS and Alcian-blue double staining of one-micron-thick section (after removal of epon). PAS positive granules are sparsely distributed in the cytoplasm of Type A cells (arrow). Nuclei were not stained in the photograph. Same case as Figure 1. ×1750

Fig. 3. **a** Type B signet-ring cell. The nucleus is compressed by abundant mucous granules toward the periphery of the cell. In the cytoplasm, moderately electron dense mucous granules are seen and cell organelles are decreased. ×4400. **b** Type B signet-ring cell. Density of granules with core are slightly low, but these granules are sharply distinguishable from granules of Type C. ×4700



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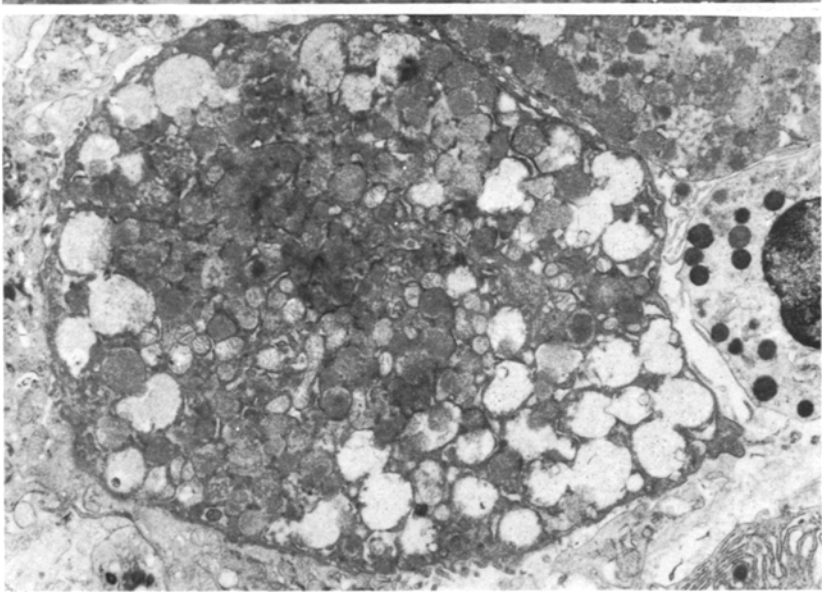

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Fig. 4. Type C signet-ring cell. The cell has large mucous granules of low density and appear to contain flocculent material. Some of them have coalesced. The nucleus is compressed peripherally by abundant, large mucous granules. Cell organelles are markedly decreased. Usually cells of this type have no interdigitations between adjacent cells. ×4800

Fig. 5. Intermediate type from Type B to Type C (Type B with Type C cell). Moderately dense mucous granules of Type B cells and low density, large mucous granules of Type C cells are intermingled within the cytoplasm. A gradual transition from the former to the latter appears to occur. ×6600

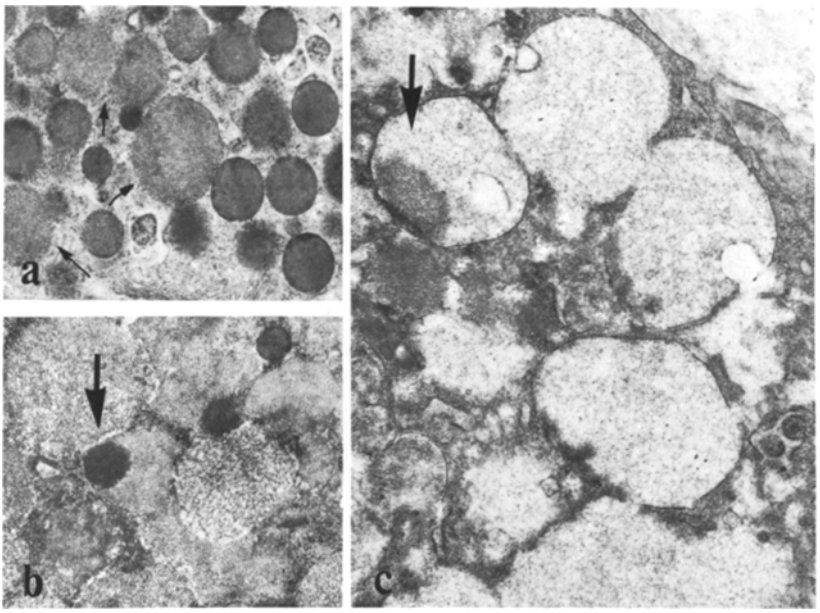


Fig. 6. **a** Mucous granules of Type A cell and of Type B cell (arrows). The former are small, spherical and dense, but the latter as relatively large and have a moderate electron density. ×17,000. **b** Mucous granules of Type B cell. One of these granules possesses a dense core (arrow) which is similar to the mucous granules of Type A cells. ×17,000. **c** Mucous granules of Type C cell. The granules are of low density and appear to contain floccurent material. One of these possesses a moderately dense core (arrow), which is similar to the mucous granules of Type B cells. ×17,000

light microscopy (Fig. 2a). The cytoplasmic foamy granules were visible in both Epon-embedded one micron thick sections and in paraffin embedded sections. These granules were PAS and Alcian-blue positive.

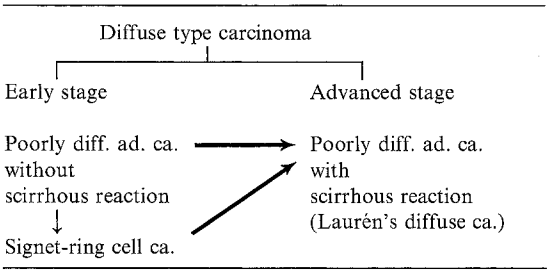
Cells intermediate in form between Type A and Type B and between Type B and Type C, were also observed. Type B cells or Type B with Type C cells were most frequently seen in our material (Fig. 5). The mucous granules of each cell-type are shown in Figure 6.

In addition, cells having an "intracellular lumen" lined by microvilli were observed in ten cases of diffuse carcinoma. We have also encountered this change in four cases of intestinal type carcinoma however, and have concluded that this structure is not specific for cells of diffuse carcinoma.

Discussion

Histological classification of gastric carcinoma is a perplexing problem, since the tumors give a varying picture from case to case and not infrequently even within the same case. Laurén (1965) classified stomach carcinomas into two main groups based upon histological characteristics: The intestinal and the

Table 4. Different nomenclature of histologic appearances of early and advanced gastric carcinoma of diffuse type



diffuse type. In an attempt to improve the classification of gastric cancer, the Japanese Research Society for Gastric Cancer proposed, several years ago a classification based on the predominant histological pattern. In their classification, papillary adenocarcinoma and well differentiated tubular adenocarcinoma correspond to the intestinal type of carcinoma, and poorly differentiated adenocarcinoma and signet-ring cell carcinoma correspond to the diffuse carcinoma in Laurén's classification (Table 4).

It is generally believed that some gastric carcinomas arise from intestinal metaplastic epithelium and others from non-metaplastic epithelium. Morson (1955) suggested that at least 30% of gastric carcinomas arose from mucosa with intestinal metaplasia, and Laurén (1965) claimed that the frequency was about 50%. Nagayo (1975) defined chronic gastritis as a series of chronic changes of the gastric mucosa progressing to atrophy of pyloric glands, with ultimately the appearance of intestinal metaplasia. From this definition, therefore, atrophy of pyloric glands appears more essential for the development of gastric carcinoma than intestinal metaplasia.

Nevertheless, it is true that intestinal type carcinoma is almost always seen in combination with severe intestinal metaplasia of the gastric mucosa, and that the histological features of intestinal type carcinoma are similar to intestinal metaplasia. From these observations it is generally accepted that gastric carcinoma of the intestinal type arises from gastric mucosa with severe intestinal metaplasia.

With regard to the histogenesis of diffuse carcinoma, Nagayo (1974) indicated that the mucosal cancers were found frequently in antrum or angulus of younger patients and in women of middle age. These diffuse cancers also developed in the anterior or posterior wall of the body and had the histological feature of poorly differentiated adenocarcinoma containing numerous signet-ring cells. In these cases, intestinal metaplasia was mild or not observed. In addition, microscopic foci of poorly differentiated adenocarcinoma were usually found in the middle layer of the non-metaplastic gastric mucosa rather than in the pyloric glands beneath them. On the basis of these observations he suggested that gastric carcinoma of the diffuse type arises from indifferent cell zone of non-metaplastic gastric mucosa (Nagayo, 1974, 1975; Nagayo and Komagoe, 1961; Nagayo et al., 1965).

Nakamura et al. (1968) examined 33 foci of primary mucosal cancer of the stomach less than 5 mm in greatest diameter and came to the conclusion from the study of these early lesions that intestinal type carcinoma originated

Table 5. Hypothesis for histogenesis of signet-ring cell of diffuse carcinoma

Generative cell layer of non-metaplastic gastric mucosa with atrophy of gastric gland
↓
Type A signet-ring cells
↓
Type B signet-ring cells
↓
Type C signet-ring cells
⋮
Degeneration

from metaplastic epithelia, whereas diffuse carcinoma originated from ordinary gastric mucosa.

In our light and electron microscopic study, it has been shown that carcinoma of the diffuse type consists mainly of a series of signet-ring cells, which can be divided into three types. Cases composed of only one cell-type, however, were rare, and intermediate cell types were often seen. The combination of Type A and Type C cells without cells of Type B was not observed. Both PAS and Alcian-blue positive mucous granules were visible in the Type B with C cells despite the absence of Alcian-blue positive mucous granules in Type B cells. The existence of these intermediate types strongly suggests that Type A, B and C cells arise from the same cell system, that there is a gradual transition from Type A to Type B and from Type B to Type C, and that Type A cells may be the precursor for Type B and Type C cells.

Light microscopically, PAS positive cancer cells were the predominant type in diffuse carcinoma, but Alcian-blue positive cancer cells were relatively infrequent. This finding indicates that PAS positive cancer cells are more active and more immature than Alcian-blue positive cancer cells and indeed, electron-microscopically these PAS positive cells correspond to Type A or Type B signet-ring cells. The mitotic cells had slightly eosinophilic cytoplasm and had features resembling Type A or Type B cells by light microscopy. We believe that signet-ring cells usually mature through the course as indicated in Table 5, but we cannot deny that mitotic activity was absent in Type C cells.

The staining properties of signet-ring cells with PAS and Alcian-blue seemed to reflect the nature and degree of maturity of mucous granules in each cell type (Table 3). It seemed that the nuclear shape, quantity of cellular organelles, and nature of mucous granules was related to the rapidity of maturation and mucous producing activity of the cancer cells. Regarding the origin of signet-ring cells, it appeared that there was a similarity between Type B cells and mucous neck cells in the corpus and pyloric gland cells in the pylorus.

These histological and electron microscopic findings of diffuse carcinomas indicate that the appearance of Type A signet-ring cells can be regarded as an initial malignant change in the non-metaplastic but atrophic gastric mucosa.

Extracellular mucin was not observed in diffuse carcinoma. It was thought that the cells of diffuse carcinoma, filled with many mucous granules, might have lost their secreting ability. In particular, Type C cells appeared to be in the process of degeneration. These observations and opinions are summarized in Table 5.

Acknowledgements. The authors wish to thank Drs. S. Hosoda, M. Matsuyama, and M. Ito of Aichi Cancer Center Research Institute for helpful advice, and Drs. G.N. Stemmermann and S.M. Cohen for inspection of the manuscript. The author also wish to thank Porf. M. Akagi of the 2nd Department of Surgery, Kumamoto University School of Medicine, for his encouragement in the study. We are deeply indebted to Dr. H. Yokoyama, Yokoyama Gastro-intestinal Hospital, for supplying the surgical material for the study.

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Received February 19, 1977