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Serotonin in tubular adenomas, adenocarcinomas and endocrine tumours of the stomach

An immunohistochemical study *

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Summary. Serotonin was examined immunohistochemically in seven tubular adenomas, 194 adenocarcinomas and 41 endocrine cell tumours of the stomach. In tubular adenomas, serotonin-containing cells showing argentaffinity were present in the lower portion of the adenomatous glands and were considered to be an expression of intestinal character. Scattered serotonin-containing tumour cells were found in 60 (30.9%) of 194 adenocarcinomas regardless of their histological type. Cell fusions between carcinoma and enterochromaffin (EC) cells might be a possible mechanism for the occurrence of serotonin-containing cells within the tumour. In 17 (54.8%) of 31 endocrine cell carcinomas, serotonin-containing tumour cells were observed in a variable degree in contrast to the absence of these cells in classical carcinoid. Moreover, diffuse serotonin reactivity was found in four cases of scirrhous endocrine cell carcinoma. The histogenesis and the occurrence of serotonin-containing cells in each type of gastric tumour is also discussed.

Key words: Serotonin – Gastric tumour – Immunohistochemistry

Introduction

Since the report of Hamperl (1927), it has been known that argentaffin cells are not infrequently seen in adenomas, ordinary adenocarcinomas and endocrine cell tumours of the human stomach (Honma et al. 1957; Azzopardi and Pollock 1963; Kubo and Watanabe 1971; Watanabe 1972; Tahara et al. 1975, 1982). They were considered to be an integral part of the tumour, but the role which argentaffin cells play in the development and growth of gastric tumours has not yet been elucidated. Argentaffin cells have been thought to be roughly identical to the serotonin-containing EC cells which are present throughout the gastrointestinal mucosa. Recently, we have demonstrated immunohistochemically that only 10–20% of EC cells show argentaffinity in gastric mucosa and that the majority of them are argyrophil (Ito et al. 1986a).

The purpose of this study was to examine the incidence and distribution of serotonin-containing cells in adenomas, adenocarcinomas and endocrine tumours of the stomach. The significance and histogenesis of the occurrence of these cells in gastric tumours is also discussed.

Materials and methods

A total of 242 surgically removed stomachs was examined. Seven cases of tubular adenoma which showed hyperplasia of serotonin-containing cells were selected from 49 adenomas (Ito et al. 1986b). The 194 cases of gastric adenocarcinoma were composed of 80 cases of early carcinoma, including 19 cases of the small mucosal carcinoma under 10 mm in diameter, and 114 cases of advanced carcinoma. Investigation was also made of 41 cases of endocrine tumours of the stomach. For each case, 5–20 tissue sections including the main lesions were cut longitudinally after fixing in 10% formalin and embedding in paraffin. Sections of these blocks cut 4 μm in thickness were stained with haematoxylin-eosin, the Grimelius silver nitrate technique for argyrophil reaction, and Fontanna-Masson's silver impregnation method for argentaffinity. Toluidine blue and naphthol-ASD-choloacetate esterase staining were also done in 15 cases comprising 5 adenocarcinomas and 10 endo-

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crine cell tumours with a good number of serotonin-containing tumour cells.

Histological classification of gastric adenocarcinoma was made according to the criteria of the World Health Organization (1977). Endocrine tumours of the stomach were defined and classified by Tahara's criteria (1986). Briefly, carcinoids showing classical histological pattern correspond to those of Soga type A and B (1971). Mucocarcinoid is analogous to that of Soga type C or the WHO classification (1980). Endocrine cell carcinoma was subclassified into two types, medullary and scirrhous. The former corresponds to that of Soga D type. The latter was diagnosed as carcinoma, consisting predominantly of neoplastic endocrine cells with a diffuse distribution throughout the fibrotic tumour as previously described in detail by Tahara (1982) et al. and Tahara (1986). Of these cases with endocrine tumours, no overt endocrine syndrome was noted clinically.

Immunohistochemistry

For the detection of serotonin in tumour cells, the avidin-biotin-peroxidase complex (ABC) method of Hsu et al. (1981) was used. Incubation with anti-serotonin antibody or rinsing in PBS at each step was performed at least for 30 minutes at room temperature. Endogeneous peroxidase activity was inactivated by immersing the specimen in 0.03% hydrogen peroxide in absolute methanol for 20 minutes. The sections were counterstained with 3% methyl green.

Anti-serotonin rat monoclonal antibody was purchased from Seralab, limited (Mas 055 clone YC5/45 HLK, Crowely Down, Sussex, U.K.), and employed at a 1:600 dilution. Preparation and characterization of the antiboy have been described previously (Consolazione et al. 1981; Cuello et al. 1982). Biotinylated anti-rat IgG and avidin-biotinylated horse raddish peroxidase complex (ABC) were purchased from Vector Laboratories, Inc. CA, U.S.A.

The specificity of immunostaining for serotonin was examined as described by Sternberger (1979): (1) anti-serotonin-antibody was previously absorbed with serotonin-creatinine sulfate complex (50 μg/ml diluted antibody); (2) normal rabbit IgG was used as the first layer; (3) omission of 3,3′-diaminobenzidin-tetrahydrochlorides or H_2O_2 from the incubation medium for peroxidase reaction. Control slides were invariably negative for immunostaining.

Results

In seven cases of tubular adenoma, a good number of serotonin-containing cells were present mainly in the lower portion of the glands of the adenoma (Fig. 1a). They showed a columnar appearance with elongated nuclei and strong immunoreactivity for serotonin in the basal area. They obviously had an open cell character with direct contact with the gland lumen. Most of them showed argentaffinity by Fontana-Masson's staining (Fig. 1b).

The frequency of serotonin-containing tumour cells in 194 gastric adenocarcinomas is shown in Table 1. All of the serotonin-containing tumour cells were scattered in or restricted to a small area of the adenocarcinoma (Figs. 2a and b). These

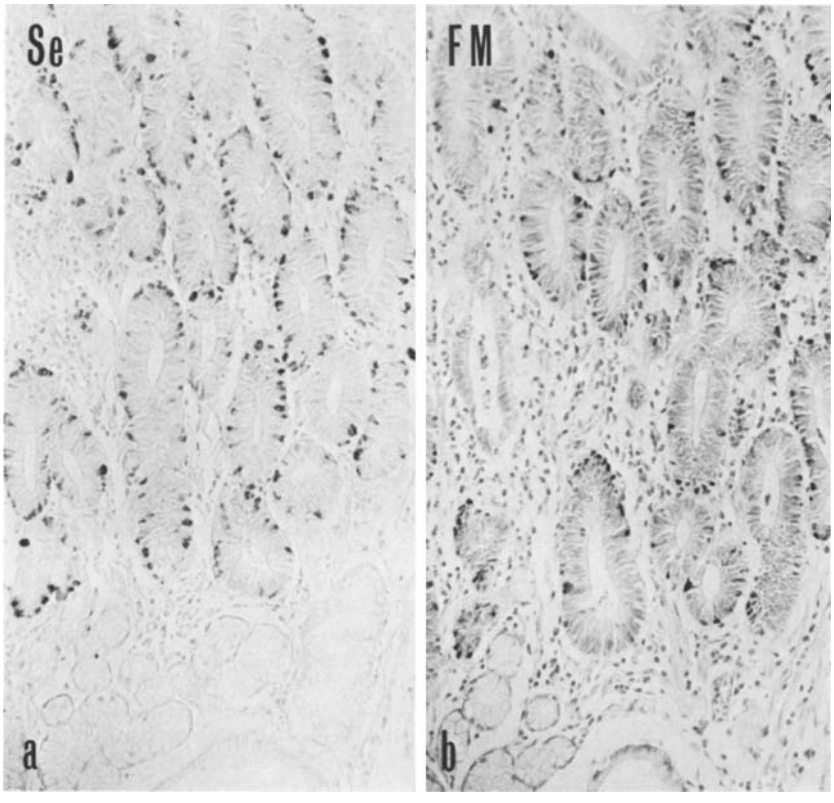

Fig. 1a, b. Tubular adenoma of the stomach. (**a**) A good number of serotonin-containing cells are preferentially present in the lower portion of the adenoma glands. Immunostaining with anti-serotonin antibody. (×160) (**b**) Most of the serotonin-containing cells show argentaffinity. Semi-serial section of a. Fontana-Masson staining. (×160)

cells were found in 37 (31.3%) of the 118 papillary or tubular adenocarcinomas, and in 23 (30.3%) of the 76 undifferentiated, mucinous or signet-ring cell carcinomas.

The relationship between the incidence of serotonin-containing cells and depth of invasion through the gastric wall, or to metastatic lesions is shown in Table 2. These tumour cells were found in 59 (30.4%) of 194 tumours in the mucosal layer, in 17 (12.0%) of 142 tumours in the submucosal layer, and in eight (7.2%) of 111 lesions in the muscularis propria and subserosa, respectively. Moreover, they were observed in four (8.5%) of 47 metastatic foci in the perigastric lymph nodes and in four of 10 metastatic tumours in the ovary or liver. In all cases with serotonin-containing tumour cells observed in submucosa, muscle layer, subserosa or in metastatic lesions, these cells were also found in the mucosal layer except in one case, in which mucosal infiltration of adenocarcinoma could not be adequately evaluated. Occasionally, adenocarcinoma involved enterochromaffin (EC) cells in the gastric mucosa adjacent to the tumour and EC could not be distinguished from serotonin-containing tumour cells (Fig. 2a). A majority of serotonin-containing tumour cells showed an argyrophil reaction by Grimelius silver nitrate (Figs. 3a and b), but argentaffinity by Fontana-Masson's technique was only demonstrated exceptionally.

The frequency of serotonin in each type of gastric endocrine tumour is shown in Table 3. Serotonin was not found in carcinoid tumours showing a classical histological pattern, but was frequently detected in scirrhous endocrine cell carcinomas. Of these, four had a large number of serotonin-containing tumour cells in the fibrotic submucosa and muscle layer. Hyperplasia of these cells was also detected in the mucosa in the vicinity of ulcerative primary lesion (Figs. 4a–c). They showed both argentaffinity and argyrophilia (Fig. 4d).

Mast cells were variably observed throughout the tumour tissue of 15 gastric tumours with many serotonin-containing cells. There was no correlation between the number of mast cells and that of serotonin-containing tumour cells.

Discussion

We have reported previously that among the several kinds of endocrine cells in the tubular adenoma of the stomach, serotonin-containing cells were

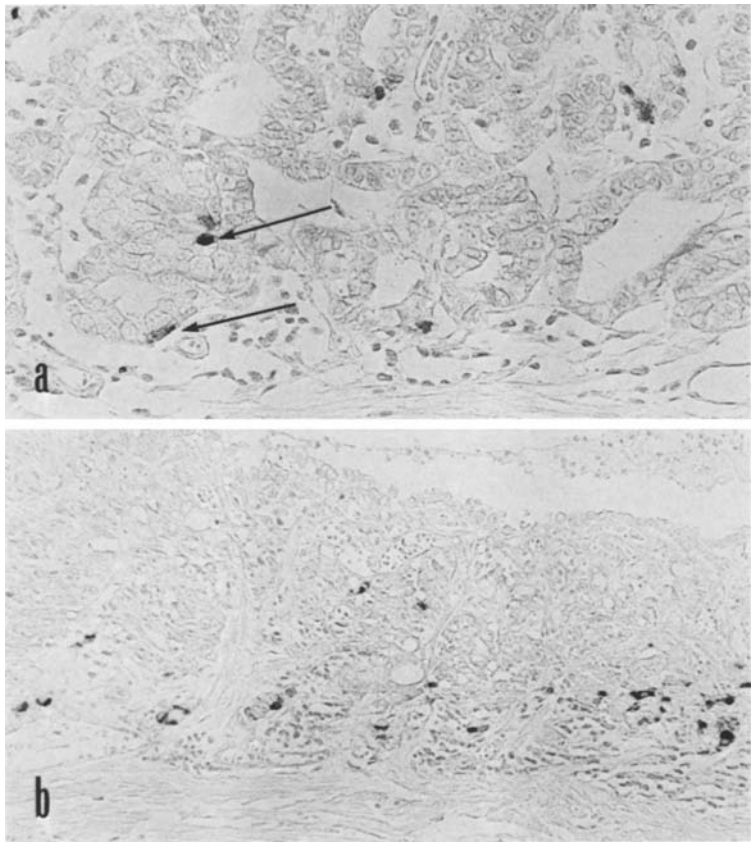

Fig. 2a, b. Serotonin-containing tumour cells in mucosal adenocarcinoma. (**a**) Scattered serotonin-containing cells are noted in well differentiated tubular adenocarcinoma. EC cells in pyloric glands adjacent to the adenocarcinoma (*arrows*) are also observed. Immunostaining with anti-serotonin antibody. (×250) (**b**) A fairly good number of serotonin-containing cells are distributed in the lower mucosal signet ring cell carcinoma. Immunostaining with anti-serotonin antibody. (×160)

Table 1. Histological type and the incidence of serotonin-containing tumour cells in 194 gastric adenocarcinomas

Histological Type[a]	Total Number of Cases	Cases with Serotonin-Containing Tumour Cells	
		Number	(%)
Papillary	18	4	(22.2)
tubular	100	33	(33.0)
	118	37	(31.6)
Undifferentiated	45	16	(35.6)
signet-ring cell	26	7	(26.9)
mucinous	5	0	(0)
	76	23	(30.3)

[a] According to the classification of World Health Organization

Table 3. Incidence of serotonin-containing tumour cells in endocrine cell tumour (ECT) of the stomach

Type of ECT[a]	Number of Cases	Number of Cases with Serotonin-Containing Tumour Cells			
		−	+	++	+++[b]
Carcinoid	9	9	0	0	0
Mucocarcinoid	1	0	0	1	0
Endocrine Cell Carcinoma					
A. Medullary	16	10	3	2	1
B. Scirrhous	15	5	1	5	4

[a] Classification and definition by Tahara (1986) and Tahara et al. (1982)

[b] These reaction are graded +, ++ and +++ on the basis of the frequency of positive staining tumour cells rather than on the intensity of staining of individual cells

Table 2. Incidence of serotonin-containing tumour cells related to depth of tumour invasion and metastasis in 194 adenocarcinomas of stomach

Depth of tumour	Early cancer		Advanced cancer		Total
	Mucosal 52 cases	submucosal 28 cases	metastasis (−) 67 cases	metastasis (+) 47 cases	
Mucosa	18 (34.6%)	6 (21.6%)	20 (29.8%)	15 (31.9%)	59/194 (30.4%)
Sub-mucosa	–	3 (10.7%)	7 (10.4%)	7 (14.9%)	17/142 (12.0%)
M. propria & subserosa	–	–	4 (6.0%)	4 (8.5%)	8/111 (7.2%)
Metastasis Lymph nodes	–	–	–	4 (8.5%)	4/47 (8.5%)
Ovary and Liver	–	–	–	4 (40.0%)	4/10 (40.0%)

highest in both frequency and distribution density (Ito et al. 1986b). Serotonin-containing tumour cells in tubular adenoma showed an open cell character which was normally present in the small intestine and metaplastic glands of the stomach. Therefore, the occurrence of serotonin-containing tumour cells in the adenoma was considered to be an expression of its intestinal character. We reported previously (Ito et al. 1986a) that only a few EC cells (estimated at 10–20%) showed argentaffinity in the metaplastic glands of the stomach. In the present study, most of the serotonin-containing tumour cells in the tubular adenoma showed argentaffinity. The discrepancy of argentaffinity between EC cells in gastric metaplastic glands and serotonin-containing cells in tubular adenoma might account for the greater number of intracytoplasmic secretory granules in tubular adenoma (Ito et al. 1986a).

Many reports have been published on the occurrence of argentaffin cells in gastric adenocarcinoma, but the incidence varies considerably by investigators, ranging from 1.7 to 14.5% (Honma et al. 1957; Azzopardi and Pollock 1963; Kubo and Watanabe 1971; Watanabe 1974; Tahara et al. 1975; Shinkai et al. 1984). As we have repeatedly stressed, the argentaffinity of EC cells is not so frequent in non-neoplastic gastric mucosa. Wilander et al. (1985) had also compared three different techniques, namely: argentaffin reaction, formalin-induced fluorescence according to Falck et al. (1962) and immunocytochemistry using the monoclonal antibody to serotonin also used in the present study. They concluded that the immunocy-

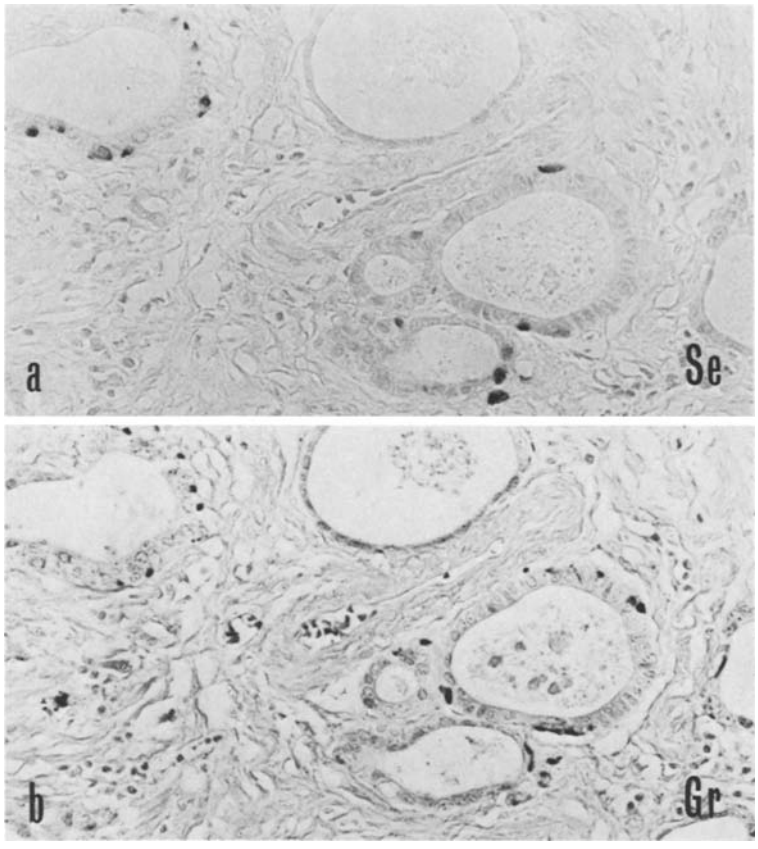

Fig. 3a, b. Serial sections of moderately differentiated tubular adenocarcinoma infiltrating in the submucosa of the stomach. **(a)** Scattered serotonin-containing cells are found. Immunostaining with anti-serotonin antibody. (×270) **(b)** Most of serotonin-containing cells show argyrophil reaction. Grimelius silver staining. (×270)

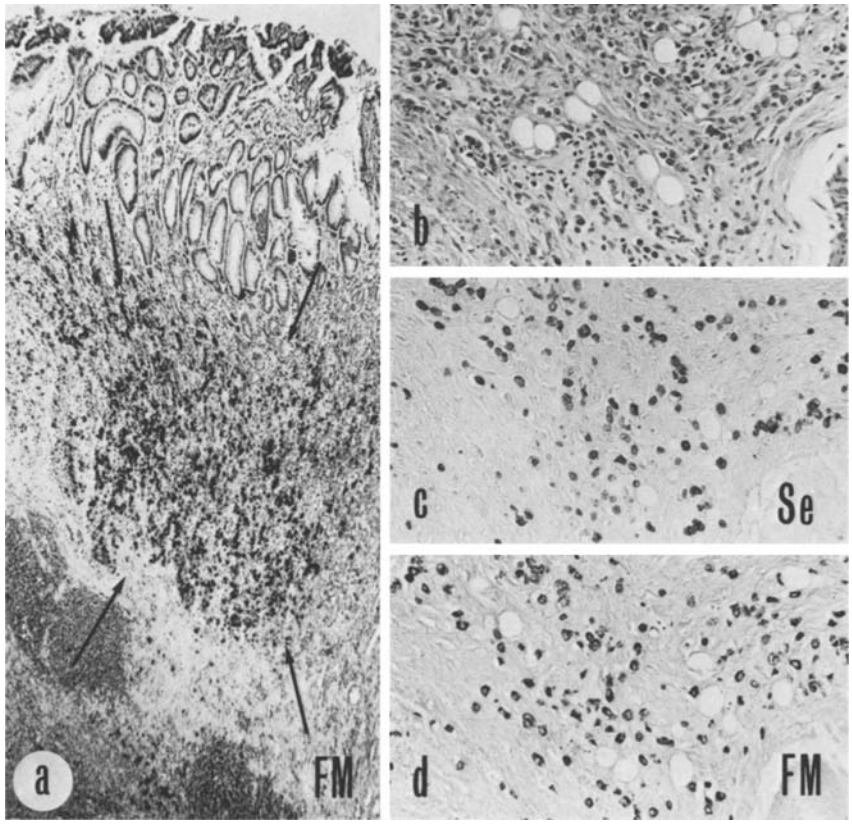

Fig. 4a–d. Serial sections of endocrine cell carcinoma, scirrhous type, of the stomach. **(a)** Hyperplasia of the neoplastic argentaffin cells surrounded by arrows is noted in the mucosa adjacent to the ulcertative primary lesions. Fontana-Masson staining. (×33) **(b)** Tumour cells resembling undifferentiated adenocarcinoma infiltrating the fibrotic submucosa. H & E. (×110) **(c)** A majority of the tumour cells show serotonin-immunoreactivity. Immunostaining with anti-serotonin antibdoy. (×110) **(d)** Most of the serotonin-containing tumour cells show argentaffinity. Fontana-Masson's staining. (×110)

tochemistry was most sensitive and specific for detecting serotonin among the three techniques. A higher incidence of serotonin-containing cells in adenocarcinomas than previously reported was therefore expected and we have observed these cells in more than 30% of all adenocarcinomas, although they were scattered or restricted to a small area of the tumour.

The varieties of morphological arrangement between endocrine the cells of the gastrointestinal tract and tumours has been already described by Tahara et al. (1975) as follows: (1) classical carcinoid, (2) endocrine cell carcinoma, often showing poorly differentiated adenocarcinoma, and (3) endocrine cell clones with a scattered appearance in a tumour. More recently, Tahara have reported on the detailed definition, morphological findings and biological behavior of endocrine tumours of the gastrointestinal tract. Serotonin-containing cells in adenocarcinoma of the stomach in the present study corresponded to (3), which had been considered to be attributable to the differentiation of the tumour (Cox and Pierce 1982; Bosman 1984). Meanwhile, Warner and Leo (1979) had proposed the theory of "cell hybridization", which implies that hybridization of a neoplastic epithelial cell with APUD cell will progress to a tumour with a mixed cell population. The theory is difficult to refute in consideration of the data obtained in the present study: (1) Serotonin-containing cells in the tumour showed argyrophilia with an almost complete scarcity of argentaffinity, as in EC cells in non-neoplastic gastric mucosa (Ito et al. 1986a). (2) The frequency of these endocrine cells was highest in the mucosal layer. Cases with serotonin-containing tumour cells in submucosa, muscle layer or metastatic lesion, also had serotonin-containing cells in the mucosal layer with one exception. This might imply that the tumour cells might fuse with EC cells in the gastric mucosa adjacent to carcinoma and then infiltrate into the submucosa as endocrine cell clones. Recently, Kovacs (1985) has successfully demonstrated the evidence for in vivo cell fusion of various human malignant tumours by cytogenetic analysis of premature chromosome condensation. He reported that cell fusion in vivo was not very rare in naturally-occurring human malignancies and that this might explain the heterogeneity of tumour cell populations.

It is of interest that serotonin-immunoreactivity was not found in classical gastric carcinoid tumour. We have not yet found any literature stating that serotonin-immunoreactivity was detected diffusely in gastric carcinoid despite the presence of EC cells in the gastric mucosa. Wilander et al. (1986) have found a few serotonin-containing tumour cells immunocytochemically in only three cases of 25 gastric carcinoids. However, serotonin-containing tumour cells were frequently found in gastric endocrine cell carcinomas, especially of the scirrhous type. In four cases, the majority of tumour cells showed serotonin immunoreactivity, and hyperplasia of the serotonin-containing cells was also detected in the mucosal layer. These cases might be regarded as diffuse serotoninomas, although they showed no overt endocrine syndromes. In view of these findings, expression of serotonin in endocrine cell tumours of the stomach seems to have a negative correlation between morphological and functional differentiation of tumour cells.

In the present study, we have demonstrated a higher incidence of serotonin-containing cells in gastric tumours than previously described. The histogenesis of these endocrine cells in tubular adenoma, adenocarcinoma and endocrine tumours of the stomach is apparently different, as stressed by Tahara et al. (1975). Attention should be paid to the existence of diffuse serotoninoma of the stomach, which might have been previously classified as undifferentiated or scirrhous carcinoma.

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