Histol Histopath (1991) 6: 317-323

Histology and Histopathology

Endocrine profile in gastric carcinomas An immunohistochemical study

Aasmund Berner[1] and Jahn M. Nesland[1,2]

[1]Department of Pathology and [2]Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway

Summary. 22 gastric carcinomas (13 intestinal type and nine diffuse type) were immunostained for neuron specific enolase, chromogranin, Leu-7 and a panel of fifteen different peptide hormones. Five out of the 13 tumours of intestinal type and four out of the nine diffuse carcinomas expressed immunoreactivity for one or more of the pan endocrine markers. Seven out of the 13 tumours of intestinal type and five out of the nine diffuse carcinomas also expressed immunoreactivity for gastrin (3), ACTH (3), serotonin (7) and calcitonin (7). Immmunoreactivity for somatostatin (1) and substance P (1) were also seen in two tumours of intestinal type. Seven out of 18 cases with benign mucosa adjacent to the tumours expressed a focal immunoreactivity for chromogranin (6), serotonin (6), gastrin (5) and calcitonin (1). All hormone-producing tumours also expressed immunoreactivity for carcino-embryonic antigen. Our results confirm that a high proportion of gastric carcinomas are hormone producing.

Key words: Immunohistochemistry, Endocrine markers, Peptide hormones, Gastric lesions

Introduction

Gastric carcinomas have differentiation patterns that span from adenocarcinomas through mixed endocrine-glandular differentiation to carcinoid tumours (Oota and Sobin, 1977). The proportion of carcinomas with neuroendocrine differentiation varies considerably in individual investigations, ranging from 3.1% (Kubo and Watanabe, 1971) to 26% (Bonar and Sweeney, 1986). Very few reports deal with demonstration of hormones produced by the carcinomas (Alumets et al., 1982; Prade et al., 1982; Tahara et al., 1982; Dao-Nian and Elias, 1983; Bonar and Sweeney, 1986; Graham et al., 1987). In this study we have examined the occurrence of endocrine cells in gastric adenocarcinomas and in benign mucosa next to the tumours by using a panel of antibodies raised against different hormones and neuroendocrine screening markers.

Offprint requests to: Dr. Aasmund Berner, Department of Pathology, The Norwegian Radium Hospital, 0310 Oslo, Norway

Materials and methods

22 cases with resection specimens of gastric carcinoma were taken from our files. 13 were located in the antrum and canalis and 9 in the corpus. All specimens were fixed in 10% buffered formalin and embedded in paraffin. 5 μm thick sections were stained with haematoxylin and eosin for light microscopical evaluation. The carcinomas were classified into diffuse and intestinal type according to Lauren (1965).

Sections from formalin-fixed and paraffin-embedded material were stained with the avidin-biotin-peroxidase complex (ABC) method using the antisera listed in Table 1. After removal of paraffin, the sections were treated for 30 minutes with 0.3% hydrogen peroxide in methanol to block endogenous peroxidase before incubation for 20 minutes with normal serum diluted 1:75 in 0.01 M saline (PBS), pH 7.4, containing 5% bovine serum albumin (BSA) to eliminate nonspecific staining. They were then incubated at 4° C with the primary antibodies followed by 30 minutes incubation with a 1:2000 dilution of the biotin-labelled second layer antibody and a 60 minute incubation with ABC. After further incubation for 5 minutes in freshly prepared 0.05% 3,3' diaminobenzidine-tetrahydrocloride in 0.05 M Tris buffer, pH 7.6 containing 0.01% H_2O_2, the sections were counterstained with haematoxylin, dehydrated and mounted.

Control studies included: 1) relevant positive control sections; 2) the use of non-immune serum of IgG fractions as first layer; and 3) incubation with primary antibody preabsorbed with relevant antigen.

The immunostained sections were examined

independently by two pathologists. Localization of the staining product was noted and the number of positive cells evaluated semi-quantitatively as follows:

No positive cells	0
Less than 1/3 of cells positive	+
1/3 - 2/3 of cells positive	++
More than 2/3 of cells positive	+++

Results

Macroscopical features

All carcinomas were solitary and measured from 1.5 to 8 cm in diameter, with a mean size of 5 cm. Five were polypoid and protruding into the lumen, while 17 were ulcerating the gastric mucosa.

Light microscopy

13 of the carcinomas were of the intestinal type with tubular, acinar and/or papillary growth patterns. The cells were polygonal with central nuclei and prominent nucleoli. The nine carcinomas of diffuse type consisted of poorly cohesive cells with eccentric nuclei and varying amount of intracytoplasmic mucin. Most of them appeared as signet-ring cells which infiltrated diffusely into the gastric wall. Adjacent gastric mucosa was included in sections from 18 of the carcinomas. Seven cases showed intestinal metaplasia without atypia. Epithelial dysplasia was found in one case, while ten cases showed gastric mucosa with minimal inflammatory reaction.

Immunohistochemistry

A: Neuroendocrine screening markers

Immunostaining for one or more of the three pan-endocrine markers neuron specific enolase (NSE), chromogranin and Leu-7 were obtained in five out of the 13 tumours of intestinal type (Table 2). Immunoreactivity for NSE was seen in four out of the 13 carcinomas, while chromogranin and Leu-7 were

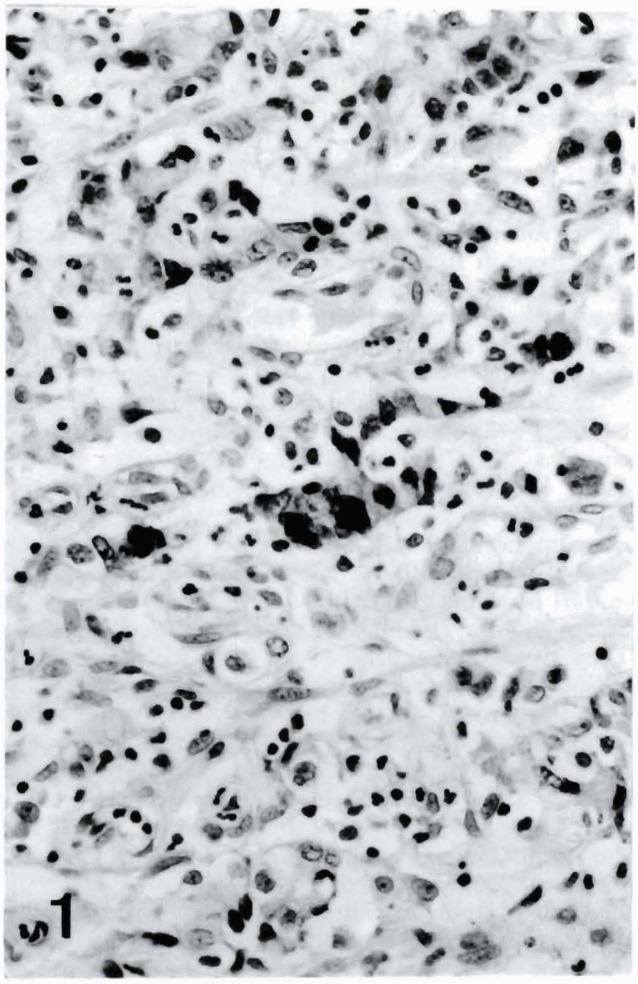

Fig. 1. Diffuse type of carcinoma. Immunostaining with chromogranin antibody. Tumour cells with a strong intracytoplasmic immunostaining. × 400

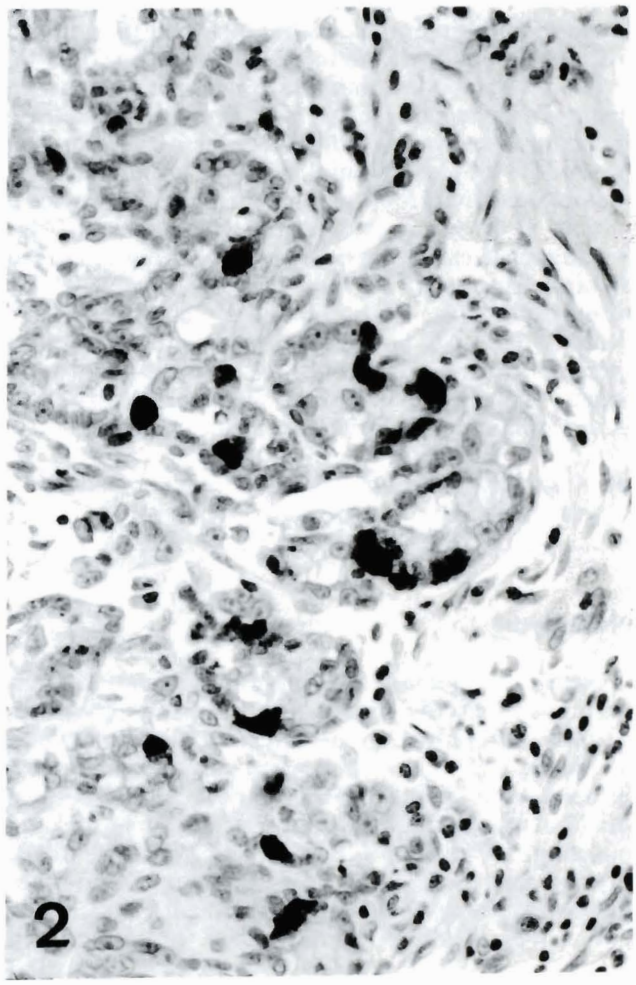

Fig. 2. Intestinal type of carcinoma. Immunostaining with serotonin antibody. × 400

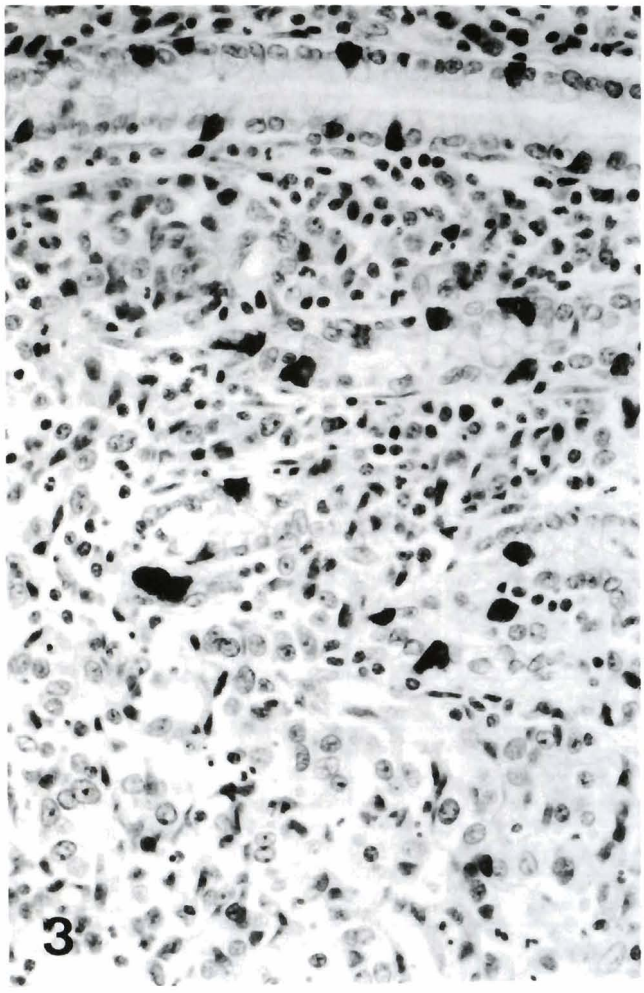

Fig. 3. Gastric mucosa next to tumour. Diffusely distributed immunoreactivity for gastrin. × 400

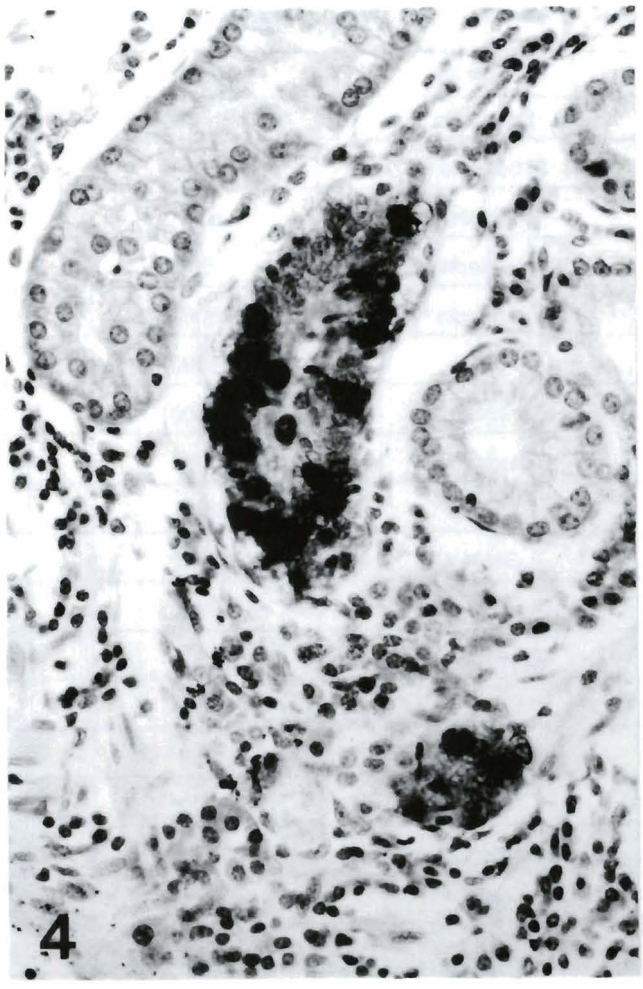

Fig. 4. Gastric mucosa expressing focal immunoreactivity for serotonin. × 400

seen in three and one tumour, respectively. Three of the carcinomas expressed immunoreactivity for two out of the three pan-endocrine markers while two carcinomas were positive for NSE alone.

Four out of the nine diffuse carcinomas expressed positive immunostaining for the secreening markers (Table 2). Chromogranin immunoreactivity was present in two cases, NSE and Leu-7 in one case only. The immunostaining was found diffusely distributed in the cytoplasm and only few to less than 25% of the neoplastic cells were positive (Fig. 1).

B: Peptide hormones

Seven out of the 13 tumours of intestinal type and five out of the nine tumours of diffuse type expressed immunoreactivity for one or more of the peptide hormones (Tables 2, 3). Tumour cells positive for serotonin, gastrin, adrenocorticotropic hormone (ACTH) and calcitonin were present in both tumour types. Five out of the 13 tumours of intestinal type were positive for two or more hormones (serotonin five cases, calcitonin three cases and ACTH two cases) compared to three out of the nine tumours of diffuse type (serotonin one case, calcitonin four cases and gastrin two cases). Two tumours of the intestinal type also reacted with somatostatin (one case) and substance P (Sub P) (one case). Only few neoplastic cells were immunoreactive in the hormone expressing tumours (Fig. 2).

C: Gastric mucosa next to tumour

In 18 cases benign gastric mucosa adjacent to tumour was available for examination (Table 5). A diffusely distributed immunostaining (Fig. 3) was observed for chromogranin (18 cases), Leu-7 (ten cases), NSE (eight cases), serotonin (16 cases), somatostatin (ten cases), gastrin (seven cases) and calcitonin (one case). Seven cases expressed focal immunostaining (Fig. 4) for chromogranin (six cases), serotonin (six cases), gastrin (five cases) and calcitonin (one case) in mucosa immediately adjacent to tumours (within 3 mm) (Table 4). The mucosa expressed

Table 1. Antisera, sources and conditions applied

antiserum	dilution	source	incubation time	temperature
Bombesin	1:800	Immunonuclear Corp., USA	18-22 h.	4°C
Substance P	1:1000	"	"	"
Insulin	1:10000	"	"	"
Leu-enkephalin	1:500	"	"	"
Serotonin	1:5000	"	"	"
VIP[a]	1:1000	"	"	"
Somatostatin	1:2000	Dako Corp., USA	"	"
Glucagon	1:2000	"	"	"
ACTH[b]	1:25	"	"	"
Gastrin	1:2000	"	"	"
Prealbumin	1:800	"	"	"
Neurotensin	1:1000	Amersham Intern., UK	"	"
CGRP[c]	1:2000	"	"	"
beta-endorphin	1:700	"	"	"
PP[d]	1:700	Milab, Sweden	"	"
Chromogranin	1:10000	BRG Boehringer Mannhein	"	"
NSE[e]	1:700	Dako Corp., USA	"	"
Leu-7	1:20	Becton. Dickinson	"	"
CEA[f]	1:320	*	"	"

a : Vasoactive Intestinal Peptide
b : Adrenocorticotrophic hormone
c : Calcitonin Gene-Related Peptide
d : Pancreatic Polypeptide
e : Neuron specific enolase
f : Carcino-embryonic Antigen
* : generous gift from Dr. O. Børmer, The Norwegian Radium Hospital

almost the same pattern of immunoreactivity regardless of the tumour type. The positive cells were of medium size with a centrally located nucleus and clear cytoplasm. Positive serotonin staining was observed in two cases of epithelium with intestinal metaplasia.

Discussion

Next to the central nervous system, the gastrointestinal tract is the largest endocrine organ in man. Until now, eight different types of gastric neuroendocrine cells have been described (Lewin, 1986; Chejfec et al., 1988).

NSE, chromogranin, Leu-7 and synaptophysin are known as screening markers for neuroendocrine differentiation (Bishop et al., 1988). The specificity as well as the sensitivity has been questioned, but at present they are among the best available for screening of formalin-fixed and paraffin-embedded material. In our study synaptophysin was not applied due to the fact that expression of synaptophysin immunoreactivity is more sensitive to formalin fixation than the other markers. Six peptide-producing adenocarcinomas did not reveal immunoreactivity for any of the three neuroendocrine screening markers.

Hormone immunoreactivity was found in 12 out of the 22 carcinomas. Like others (Prade et al., 1982; Tahara et al., 1982; Dao-Nian and Elias, 1983; Alumets et al., 1982; Bonar and Sweeney, 1986; Graham et al., 1987) we also observed tumours with more than one peptide hormone. Seven out of the 22 carcinomas expressed two or more hormones. Both the diffuse and the intestinal type of tumour immunostained for serotonin, gastrin, ACTH and calcitonin. All the immunoreactive cells looked similar to the neighbouring neoplastic cells.

Bonar and Sweeney (1986) reported a more frequent immunoreactivity for hormones in diffuse

Table 2. Immunoreactivity in gastric carcinomas

A: Intestinal type										
	Pan endocr. markers			Hormones						CEA
no	Chr	Leu-7	NSE	Ga	ACTH	Se	So	Ca	Sp	CEA
1	+	–	+	–	–	–	–	–	–	++
2	–	–	+	–	–	–	–	–	–	–
3	+	–	+	–	–	+	+	–	–	+++
4	+	+	–	–	–	++	–	–	–	++
5	–	–	+	+	–	++	–	–	–	–
6	–	–	–	–	–	–	–	–	–	++
7	–	–	–	–	+	–	–	++	+	+
8	–	–	–	–	–	+	–	–	–	–
9	–	–	–	–	–	+	–	+	–	–
10	–	–	–	–	–	–	–	–	–	++
11	–	–	–	–	–	–	–	–	–	+++
12	–	–	–	–	+	–	–	+	–	+
13	–	–	–	–	–	–	–	–	–	+
B: Diffuse type										
14	–	–	–	+	+	–	–	+	–	+++
15	+	–	–	–	–	–	–	–	–	++
16	+	–	–	–	–	+	–	+	–	++
17	–	–	–	–	–	–	–	–	–	+++
18	–	–	–	–	–	–	–	–	–	++
19	–	–	–	–	–	–	–	+	–	+++
20	–	–	–	–	–	–	–	+	–	++
21	–	–	+	+	–	+	–	–	–	++
22	–	+	–	–	–	–	–	–	–	+++

Chr: Chromogranin, **NSE:** neuron specific enolase, **Ga:** gastrin, **ACTH:** adrenocorticotrophic hormone, **Se:** serotonin, **So:** somatostatin, **Ca:** calcitonin, **Sp:** substance P, **CEA:** carcinoembryonic antigen

Table 3. Summary of immunostaining results

	no. of cases with hormone expression	no. of cases with CEA immunoreactivity
Intestinal type of tumour (n = 13)	7	9
Diffuse type of tumour (n = 9)	5	9

Table 4. Gastric mucosa with focal immunostaining next to tumour (n = 7)

serotonin	6
chromogranin	6
gastrin	5
calcitonin	1

Table 5. Immunoreactivity* in normal gastric mucosa from 18 cases.

	+	++	+++	total pos
Leu-7	6	4	–	10
chromogranin	4	11	3	18
gastrin	7	–	–	7
calcitonin	1	–	–	1
somatostatin	10	–	–	10

\+ : less than 1/3 of cells positive
++ : 1/3 - 2/3 of cells positive
+++ : more than 2/3 of cells positive

* None of the case expressed immunoreactivity for the following antigens: PP; sub P; bombesin; insulin; serotonin; enolase; glucagon; VIP; PRL; CGRP; neurotensin; leu-enkephalin; beta-endorphin; prealbumin.

carcinomas than in the intestinal type of tumour. However, his study was done on argyrophilic carcinomas and the differences were not statistically significant. In our study the intestinal type of tumour expressed a broader hormone immunoreactivity and more cells were stained than the diffuse type. This may be a reflection of the fact that the intestinal carcinomas are more heterogeneous (Hockey et al., 1984). Like Bonar and Sweeney (1986) and Graham et al., (1987), we observed a partly focal distribution.

Bonar and Sweeney (1986), Lewin (1986) and others also observed focal endocrine hyperplasia in mucosa adjacent to tumours, while Dao-Nian and Elias (1983) found a diffuse hyperplasia. In our study mucosa next to the carcinomas (within 3 mm) expressed a predominantly focal immunoreactivity for chromogranin, serotonin, gastrin and calcitonin, similar to the expression in the tumours. At some distance (more than 3 mm) the reactivity was mainly diffuse. The focal reactivity supports the concept of a local trophic effect of peptides produced by the neoplastic cells (Tutton and Barkla, 1987). The diffuse hyperplasia may also be a neuronal response (Cooke, 1986). As reported by Bonar and Sweeney (1986) and Dao-Nian and Elias (1983), the carcinomas expressed a low gastrin reactivity compared with serotonin, chromogranin, NSE and calcitonin. As expected, two cases expressed serotonin immunoreactivity in metaplastic intestinal epithelium.

In our study CEA reactivity was more frequent in diffuse carcinomas which is in agreement with previous reports (Berner et al., 1990). The CEA reactivity was present both in hormone expressing tumors and in tumours lacking neuroendocrine features, and illustrated multidirectional differentiation in gastric carcinomas.

One of the most intesting and controversial problems of neoplastic endocrine cells in carcinomas is whether their hormonal products play an important role in growth and differentiation. The trophic effect of peptide hormones on neoplastic cells may to some extent explain the aggressive behaviour by some tumours (Tutton and Barkla, 1987). Nesland et al. (1985) reported that NSE-positive breast carcinomas were more often estrogen receptor positive, but it has not been settled that these carcinomas behave less aggressively. In prostate carcinomas with neuroendocrine differentiation, Turbat-Herrara et al. (1988) documented a poor prognosis with a high frequency of metastases. Contrary to our findings, Bonar and Sweeney (1986) reported that gastric carcinomas with neuroendocrine differentiation were most frequently of the diffuse type. However, it still remains doubtful whether gastric adenocarcinomas

expressing neuroendocrine differentiation have a different prognosis compared with non-endocrine carcinomas. Unlike carcinoid tumours gastric adenocarcinomas do not express clinical symptoms caused by their peptide production.

Acknowledgements. We thank Inga Finseth, Ellen Hellesylt, Liv Inger Håseth, Mette Myhre and Elisabeth Emilsen Mølsted for skillful technical assistance.

References

Alumets J., Sundler F., Falkmer S., Ljungberg O., Håkanson R., Mårtensson H., Nobin A. and Lasson Å. (1982). Neurohormonal peptides in endocrine tumors of the pancreas, stomach, and upper small intestine: I. An immunohistochemical study of 27 cases. Ultrastruct. Pathol. 5, 55-72.

Berner A., Børmer O., Marton P.F. and Nesland J.M. (1990). Distribution of the carcinoembryonic antigen CEA in gastric lesions. Immunohistochemical testing of three novel monoclonal antibodies. Histol. Histopath. 5, 199-204.

Bishop A.E., Power R.F. and Polak J.M. (1988). Markers for neuroendocrine differentiation. Pathol. Res. Pract. 183, 119-128.

Bonar S.F. and Sweeney E.C. (1986). The prevalence, prognostic significance and hormonal content of endocrine cells in gastric cancer. Histopathology. 10, 53-63.

Chejfec G., Falkmer S., Askensten U., Grimelius L. and Gould V.E. (1988). Neuroendocrine tumors of the gastrointestinal tract. Pathol. Res. Pract. 183, 143-154.

Cooke H.J. (1986). Neurobiology of the intestinal mucosa. Gastroenterology. 90, 1057-1081.

Dao-Nian L. and Elias J.M. (1983). The detection of serotonin and gastrin in gastric carcinoma. Am. J. Clin. Pathol. 80, 436-440.

Graham A.R., Payne C.M. and Nagle R.B. (1987). The role of immunohistochemistry, electron microscopy, and ultrastructural cytochemistry in the diagnosis of mixed carcinoma-neuroendocrine neoplasms. Pathol Res. Pract. 182, 22-33.

Hockey M.S., Stokes H.J., Thompson H., Woodhouse C.S., MacDonald F., Fielding J.W.L. and Ford C.H.J. (1984). Carcinoembryonic antigen (CEA) expression and heterogeneity in primary and autologous metastatic gastric tumours demonstrated by monoclonal antibody. Br. J. Cancer. 49, 129-133.

Kubo T. and Watanabe H. (1971). Neoplastic argentaffin cells in gastric and intestinal carcinomas. Cancer 27, 447-464.

Laurén P. (1965). The histological main types of gastric carcinoma: diffuse and so-called intestinal type carcinoma. Acta Pathol. Microbiol. Scand. 64, 31-49.

Lewin K.J. (1986). The endocrine cells of the gastrointestinal tract. The normal endocrine cells and their hyperplasias. Pathol. Ann. 21, 1-27.

Nesland J.M., Memoli V.A., Holm R., Gould V.E. and Johannessen J.V. (1985). Breast carcinomas with neuroendocrine differentiation. Ultrastruct. Pathol. 8, 225-240.

Oota K. and Sobin L.H. (1977). Histological typing of gastric and oesophageal tumours. In: International Histological Classification of Tumors. No. 18. WHO. Geneva.

Prade M., Bara J., Gadenne C., Bognel C., Charpentier P., Ravazzola M. and Caillou B. (1982). Gastric carcinoma with argyrophilic cells: light microscopic, electron microscopic and immunochemical study. Human. Pathol. 13, 588-592.

Tahara E., Ito H., Nakagami K., Shimamoto F., Yamamoto M. and Sumii K. (1982). Scirrhous argyrophil cell carcinoma of the stomach with multiple production of polypeptide hormones, amine, CEA, lysozyme and HCG. Cancer 49, 1905-1915.

Turbat-Herrera E.A., Herrera G.A., Gore I., Lott R.L., Grizzle W.E. and Bonnin J.M. (1988). Neuroendocrine differentiation in prostatic carcinomas. Arch. Pathol. Lab. Med. 112, 1100-1105.

Tutton P.J.M. and Barkla D.H. (1987). Biogenic amines as regulators of the proliferative activity of normal and neoplastic intestinal epithelial cells (Review). Anticancer Res. 7, 1-12.

Accepted December 20, 1990