Acta Oncologica Vol. 30 No. 4 1991

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DIAGNOSTIC CRITERIA OF CLASSICAL CARCINOIDS

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Abstract

The classical (mid-gut) carcinoids of the intestinal tract display a characteristic light microscopic morphology. However, sometimes intestinal tumours are seen resembling carcinoids and differential diagnostic difficulties arise. In the present study silver stains and immunoreactivities to chromogranin A + B, cytokeratins and epithelial membrane antigen (EMA) were evaluated as diagnostic adjuncts in six classical carcinoids and six intestinal carcinomas with carcinoid-like features. All classical carcinoids were argentaffin and argyrophil and contained a majority cell population with chromogranin immunoreactivity while only one carcinoid-like carcinoma was chromogranin-immunoreactive and the stained cells in that case represented a minority of the tumour cell population. The cytokeratins were shown to be non-discriminatory. However, EMA expression occurred in five intestinal carcinomas and in the majority of the tumour cells of four of these cases, while only one classical carcinoid displayed a few EMA positive cells. Thus, silver stains in combination with chromogranin A + B and EMA appears to be of value to discriminate between classical carcinoids and carcinoid-like intestinal carcinomas. Further when intestinal carcinoids and carcinoid-like carcinomas are diagnosed with the aid of various tumour markers both qualitative and quantitative considerations must be made.

Key words: Classical carcinoids, gastrointestinal tract, histopathology, chromogranin, epithelial membrane antigen (EMA).

In 1907, Oberndorfer (1) coined the term 'carcinoid' to describe a separate tumour entity different to the more common intestinal adenocarcinoma. Nowadays it is well known that the carcinoids are neuroendocrine tumours that derive from the peripheral neuroendocrine cell system. Accordingly, carcinoids may occur at all levels of the gastrointestinal tract, in the lung, and at many other topographic sites of the body. However, within the group of carcinoids the tumours originally described by Oberndorfer possess almost unique biological properties. For instance, in addition to a characteristic morphology, they are argentaffin, contain considerable amounts of serotonin and they may give rise to the carcinoid syndrome (2–3). Such features are seldom present in carcinoids in other topographic areas and for that reason the small intestinal (or mid-gut) carcinoids are often designated 'classical carcinoids' (4).

Despite the fact that the classical carcinoids display a typical insular growth pattern, and mostly are easily identified, discriminatory difficulties may arise since intestinal carcinomas with carcinoid-like structures sometimes occur. In the present study six classical carcinoids and six carcinoid-like intestinal carcinomas diagnosed by routine light microscopy were evaluated with respect to silver staining properties and immunoreactivity to chromogranin A + B, cytokeratins and epithelial membrane antigen (EMA).

Material and Methods

In patients admitted to the Department of Internal Medicine, University Hospital, Uppsala, for anti-tumour therapy due to malignant neuroendocrine tumours with metastatic spread, the original histopathological specimens, on which the diagnosis was based, are reexamined. For that purpose the original paraffin blocks are collected from different pathology departments in Sweden. The blocks are sectioned and then re-evaluated after application of special stains for identification and characterization of neuroendocrine tumours.

Between 1985 and 1989, six intestinal carcinoid-like tumours appeared at routine re-examinations. They had primarily been diagnosed as possibly or probably carcinoids or carcinoid related. In the present investigation

Presented at the meeting on Recent Advances in Diagnosis and Treatment of Neuroendocrine Gut and Pancreatic Tumors held in Kebnekaise, Sweden, June 13–16, 1990.

Accepted for publication 10 December 1990

Table 1

Antisera/antibodies used in the study

Antigen specificity	Working dilution	Code No.	Source
Serotonin	1:800	YC5-45HLK	Sera Lab. Ltd., Crawley Down, Sussex RH10 4FF, England
Cytokeratin human 8, 18, 19	1:100	6400200	Labsystems Inc., P.O. Box 131970, Research Triangle Park, 27709 Raleigh, NC, USA
Cytokeratin-19	1:200	M772	Dako Corp., 6392 Via Real, Carpinteria, Ca, USA
Epithelial membrane antigen (EMA) human	1:30	M013	Dako Corp., 6392 Via Real, Carpinteria, Ca, USA
Chromogranin A + B	1:800	126G	Dr. B Eriksson, Dept Internal Medicine, University Hospital Uppsala, Sweden Eriksson B et al. Acta Endocrinol. 1990; 122: 145–55.

these cases and six additional tumours with the appearance of classical carcinoids were compared with regard to various silver and immunohistochemical stainings.

All paraffin blocks were sectioned in about 4 μm thin sections and stained with van Gieson's stain, hemotoxylin-eosin and an argentaffin (5) and argyrophil (Grimelius) (6–7) stain. The material was further examined immunohistochemically with different antibodies/antisera characterized in Table 1. For the immunohistochemical procedure the Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) was used with diaminobenzidine (DAB) as chromogen. In each assay normal serum was substituted for primary antibodies as a negative control.

Results

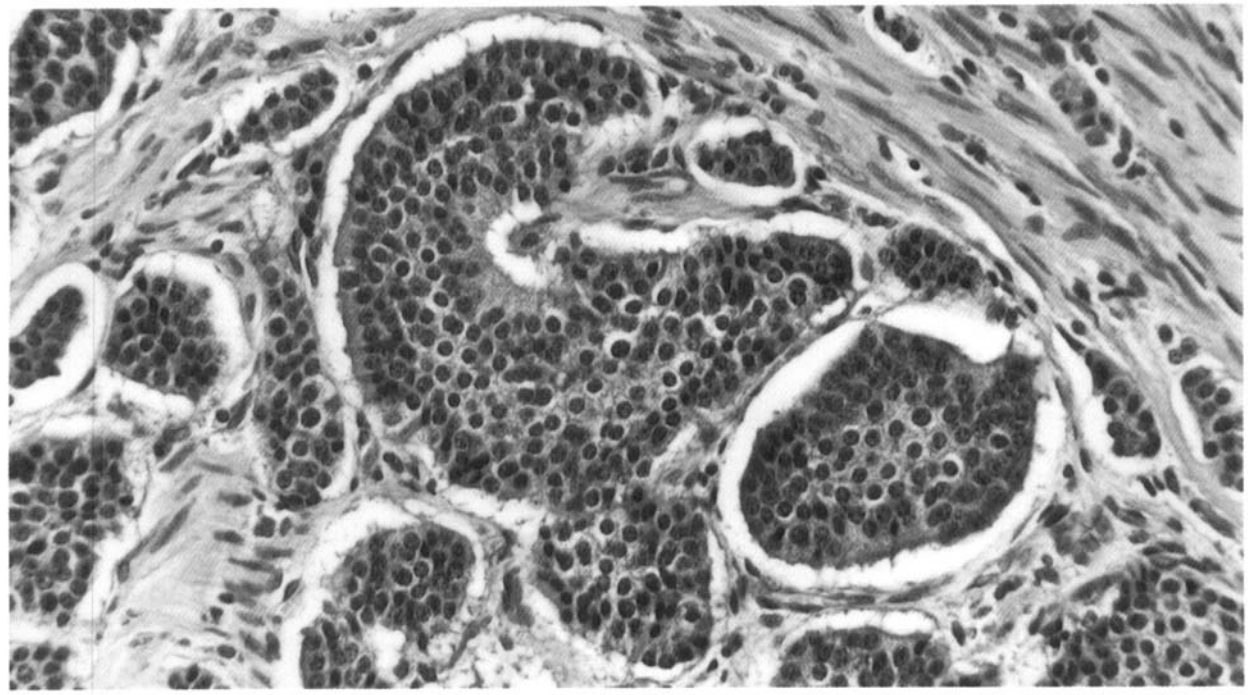
The six tumours primarily diagnosed as classical carcinoids were mainly built up of monomorphous tumour cells, growing in anastomosing cords or in an insular pattern (Fig. 1a). Of the six carcinoid-like tumours, two were highly differentiated and the growth pattern was mainly solid or insular although one of them also contained a few tubular structures (Fig. 2a). Four tumours were relatively low differentiated and displayed either a more solid picture or insular-like areas. In four of these tumours small areas of necrosis were evident and two were relatively rich in mitoses (Fig. 3).

Silver stains. The classical carcinoids all contained argentaffin cells. The number of argentaffin cells varied from a few scattered ones in one case to a majority cell population in two cases. In all six carcinoids more than 50% of the tumour cells were argyrophil (Table 2). The carcinoid-like tumours were both non-argentaffin and non-argyrophil although the silver reaction was not possible to evaluate in one case (No. 11).

Immunochemistry. The results of the immunohistochemical stainings are summarized in Table 2. Four of the classical carcinoids displayed serotonin immunoreactivity and all six contained a majority of chromogranin immunoreactive cells (Fig. 1b). The carcinoid-like intestinal carcinomas were all serotonin-negative. However, one case (No. 11) showed chromogranin immunoreactivity in less than 50% of the tumour cells (Fig 2b). Relatively few cytokeratin-positive cells occurred in some carcinoids and carcinomas. An obvious difference was seen with regard to EMA immunoreactivity within the two tumour types. Only one carcinoid contained EMA positive cells and they were few, while all carcinomas except one were EMA positive and in four of these cases the EMA staining was present in a majority of the tumour cells (Fig 2c).

Discussion

During the last two decades the knowledge about the clinical and pathobiological features of gastrointestinal carcinoids have increased considerably. Nowadays most of them can be identified and fairly well characterized. Since many of these tumours are now treated with various



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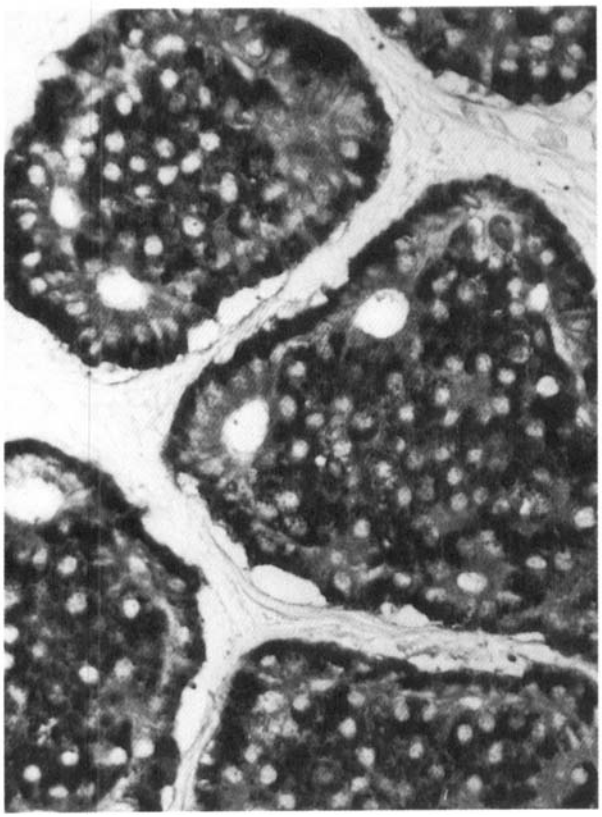

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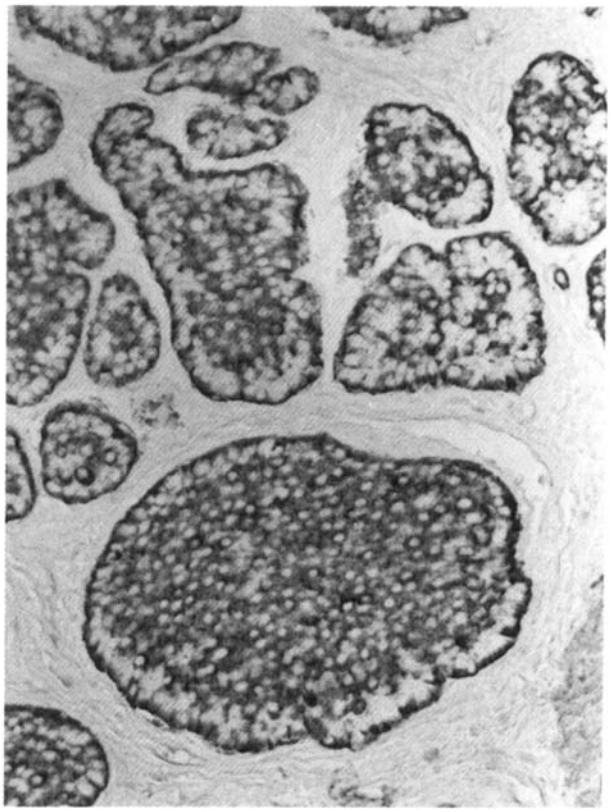
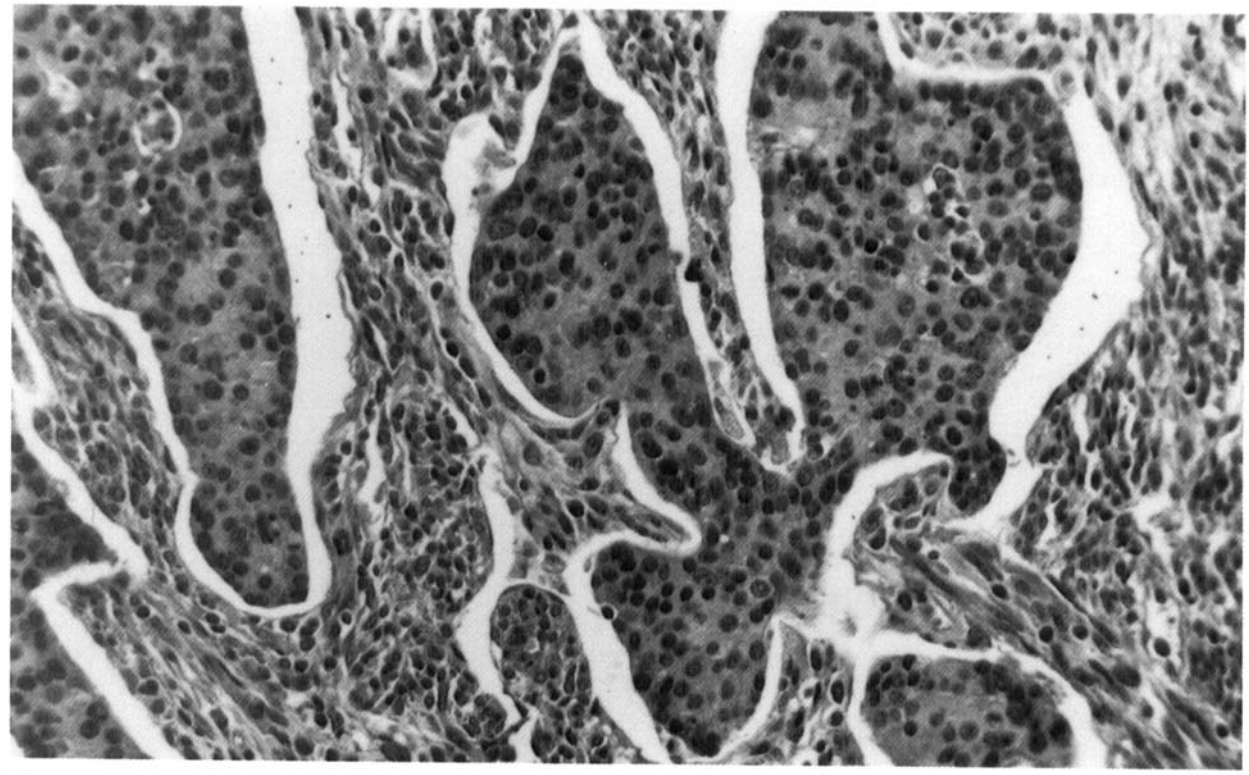


(c)

Fig. 1. a) Light microscopic picture of a classic carcinoid stained with hematoxylin-eosin × 400. An insular growth pattern is seen. b) Almost all tumour cells are chromogranin A + B positive, ABC-stain × 400, and c) argyrophil, Grimelius stain. × 260.



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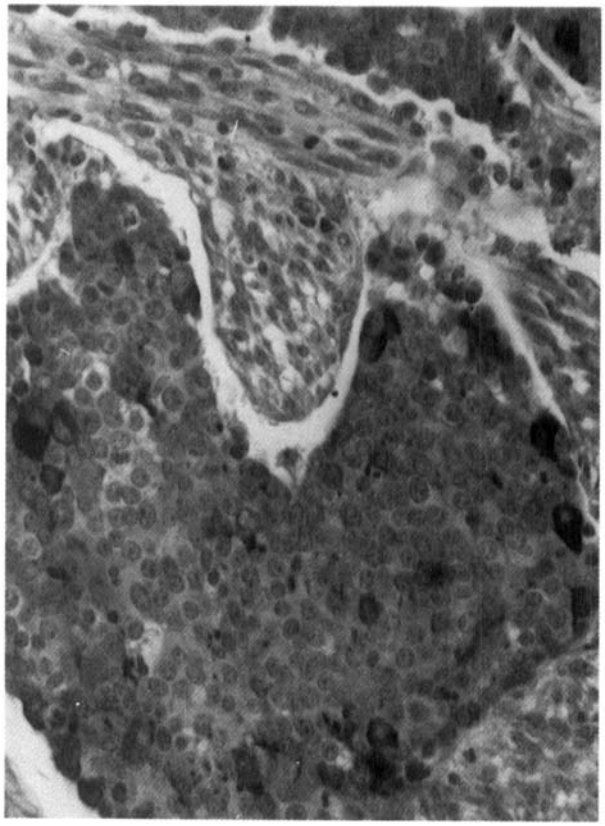

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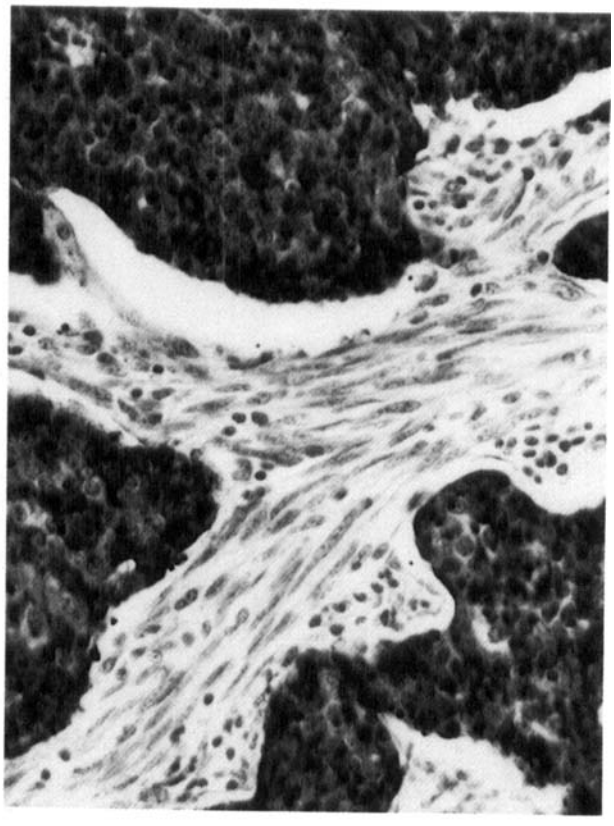


(c)

Fig. 2. a) Light microscopic picture of a carcinoid-like intestinal carcinoma (case No. 11) with insular-like structures stained with hematoxylin-eosin. b) Several ($<50\%$) tumour cells display chromogranin immunoreactivity, and c) almost all ($>50\%$) tumour cells are EMA positive, ABC-stain. × 400.

Table 2

Staining pattern of classical carcinoids (Nos. 1–6) and intestinal (carcinoid-like) carcinomas (Nos. 7–12)

Case No.	Location	Age Years	Sex	Masson	Grimelius	Serotonin	Chromogranin	Cytokeratin	Keratin-19	EMA
1	Small intestine	49	M	+ +	+ + +	+ +	+ + +	—	—	—
2	Caecum	48	F	+ +	+ + +	—	+ + +	—	—	—
3	Small intestine	45	M	+ + +	+ + +	+	+ + +	—	+	—
4	Small intestine	52	M	+ +	+ + +	+ +	+ + +	+	+	—
5	Small intestine	60	M	+ + +	+ + +	—	+ + +	+	+	—
6	Small intestine	77	M	+	+ + +	+ +	+ + +	+ +	+ +	+
7	Small intestine	32	F	—	—	—	—	—	+	—
8	Caecum	48	M	—	—	—	—	—	—	+
9	Colon transv.	64	F	—	—	—	—	—	—	+ + +
10	Small intestine	36	F	—	—	—	—	—	—	+ + +
11	Colon ascend.	58	F	*	*	—	+ +	+ +	+ +	+ + +
12	Caecum	57	M	—	—	—	—	+ +	+ +	+ + +

\+ = few positive cells.
\+ + = positive cells < 50%.
\+ + + = positive cells > 50%.
* = Indeterminable.

anti-tumour agents with some success, a proper diagnosis of each individual case is desirable.

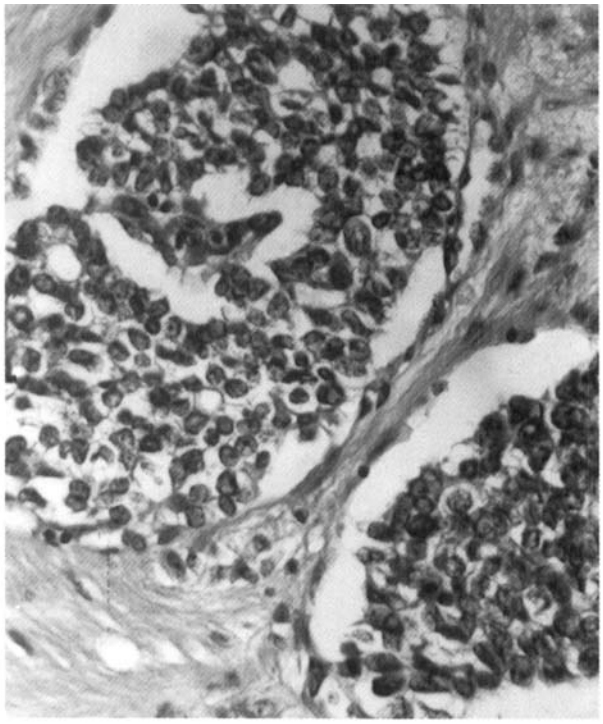
Although most of the classical carcinoids can be easily recognized in routinely processed light microscopic slides, diagnostic pitfalls exist, partly since intestinal carcinomas are sometimes seen with carcinoid-like features. It must be pointed out that carcinoids and adenocarcinomas have several characteristics in common. For instance, both tumour types may produce mucous material (8), express carcinoembryonic antigen (CEA) and various types of cytokeratins (9–12). Further, a neuroendocrine differentiation of subpopulations of tumour cells is relatively frequent in ordinary adenocarcinomas (13–16). Consequently, when carcinoids and other intestinal tumours are examined with the aid of various tumour markers both qualitative and quantitative considerations must be made.

Already in 1914, classical carcinoids were demonstrated to be argentaffin (17). The silver reaction is due to the presence of serotonin in the tumour cells (18). Serotonin can also be visualized immunocytochemically with antibodies to serotonin and with formalin-induced fluorescence. Of these alternative methods the argentaffin reaction appears to be most frequently positive, although various modifications of the argentaffin stain may give discrepant results (19). The proportion of argentaffin cells in classical carcinoids has been calculated to be between 20 and 74%, a finding which is in agreement with the present results (20).

The argyrophil reaction of Grimelius is caused by an accumulation of small silver particles on the secretory granules. The method is non-discriminatory and neuroendocrine-differentiated cells are mostly argyrophil despite their content of different types of peptide hormones and/or biogenic amines (7). Characteristically in classical carcinoids the number of argyrophil tumour cells exceeds the number of argentaffin tumour cells as in the present study (20).

Recently chromogranin, the main protein component of the secretory granules of many neuroendocrine cell types, has been shown to display argyrophil properties, and a pronounced agreement has been observed between the arygrophil reaction and chromogranin immunoreactivity in normal neuroendocrine cells and carcinoid tumours (21, 22). In the present study the classical carcinoids contained a majority cell population of chromogranin immunoreactive cells, while only one of the intestinal adenocarcinomas was chromogranin-positive with a reaction that occurred in less than 50% of the tumour cells.

As seen in the present and in previous studies, CEA and different cytokeratin antibodies are not useful to discriminate between classical carcinoids and carcinoid-like intestinal carcinomas (9–12). However, EMA appears to be of value since a majority of the tumour cells were EMA positive in four of the intestinal carcinomas and in none of the classical carcinoids. Similar results have been reported before (12, 23).



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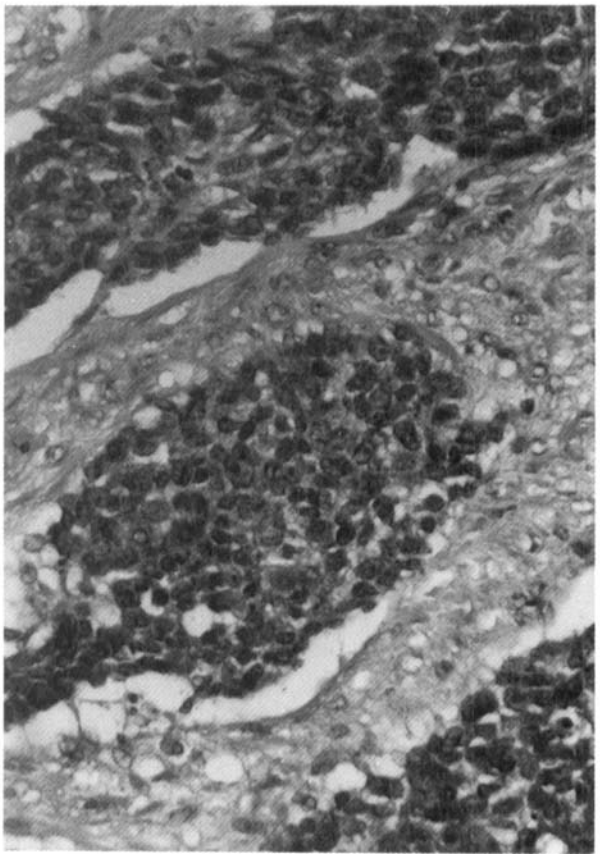

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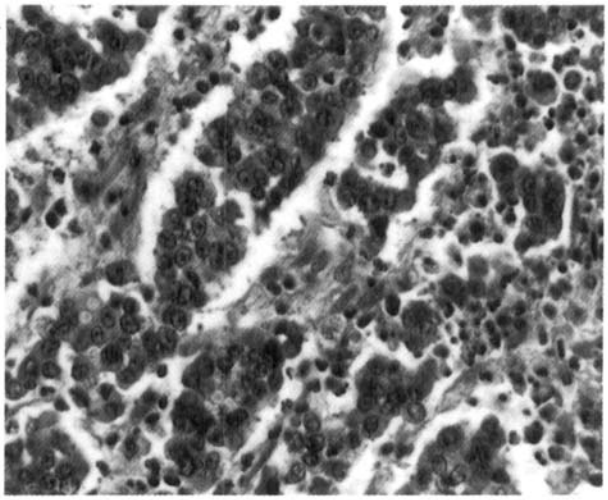

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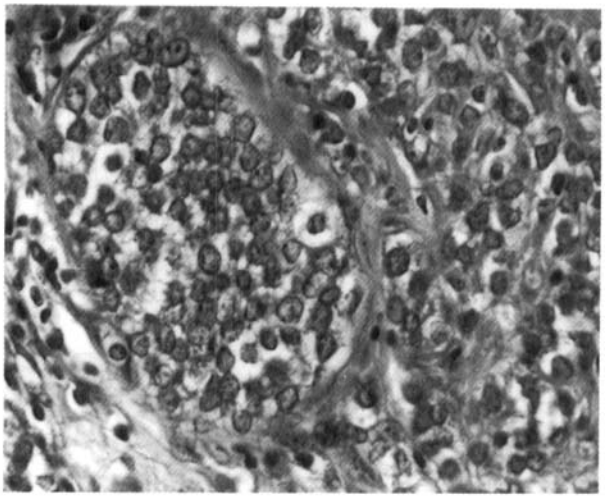

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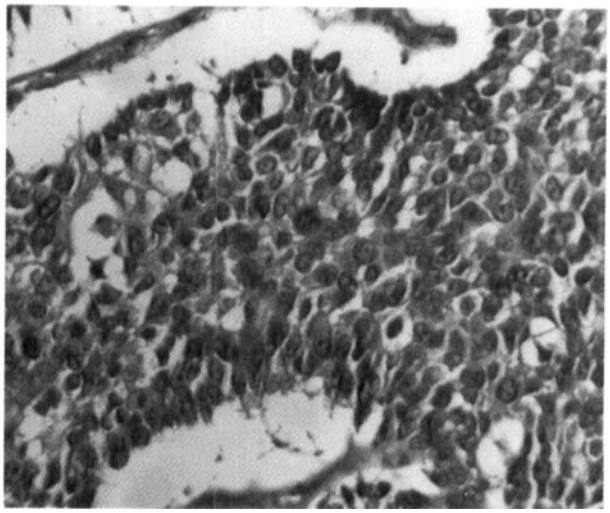

(e)

Fig. 3. Pictures of intestinal carcinomas primarily identified as possibly or probably carcinoids or carcinoid-related. a) case No. 7, b) case No. 8, c) case No. 9, d) case No. 10, and e) case No. 12. case No. 7 a) and 12 e) appear to be relatively highly differentiated tumours while case No. 8 b), 9 c) and 10 d) display signs of lower differentiation. Hematoxylin-eosin stain. × 400.

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish Medical Research Council (Project No. 6817).

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