Glandular duodenal carcinoid—a somatostatin rich tumour with neuroendocrine associations

DFR GRIFFITHS, B JASANI, GR NEWMAN, ED WILLIAMS, GT WILLIAMS

From the Department of Pathology, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN

SUMMARY The clinical and pathological features of four cases of duodenal carcinoid tumour are presented. All four tumours showed a glandular pattern, and in three cases this was associated with psammoma bodies. In three tumours somatostatin was identified by immunocytochemistry in most tumour cells. In two cases the duodenal tumours were associated with von Recklinghausen's disease and phaeochromocytoma. The importance of these unusual features is discussed, and it is suggested that these glandular carcinoids are a specific subgroup of endocrine cell tumours which appear to have potentially important clinical and pathological associations.

Carcinoids are a heterogeneous group of tumours, defined by the World Health Organisation[1] as tumours of the diffuse endocrine system, excluding C cell and islet cell tumours. They have been variously classified according to the site in which they occur,[2][3] the hormones they produce[4] and their histological pattern.[5][-7] Carcinoids arising in the duodenum are rare; they are among the least common primary malignant tumours of the duodenum,[8] and form only 2% of all gastrointestinal carcinoids.[6] In the past the most commonly reported functional link has been with gastrin secretion and the production of the Zollinger Ellison syndrome.[9]

Over the past three years we have seen four duodenal carcinoids with some unusual pathological and clinical features. We believe that recognition of this subgroup of tumours as a separate entity is of both pathological and clinical importance.

Material and methods

Formalin fixed tissue in paraffin blocks was available in all cases and wet fixed tissue in case 1. Light microscopic study was performed on haematoxylin and eosin, alcian blue, and periodic acid Schiff stained sections and after Sevier-Munger and Grimelius argyrophil methods and the Hamperl argentaffin and von Kossa silver impregnation techniques. For electron microscopy, wet formalin fixed tissue from case 1 was refixed in buffered glutaraldehyde, postfixed in osmium tetroxide, and "block stained" in uranyl acetate. This tissue was then embedded in

Epon 812, and the ultra-thin sections stained on the grid with lead citrate. Tissue from cases 3 and 4 was recovered from paraffin blocks, dewaxed, rehydrated, and then post-fixed and processed for electron microscopy as in case 1. Immunocytochemistry was performed on paraffin sections by the hapten labelled antibody bridge method of Jasani *et al*[10][11] using antisera to insulin, glucagon, somatostatin, human pancreatic polypeptide, gastrin, calcitonin, and neurone specific enolase. The results are shown in the Table.

CASE 1 (Fig. 1)
A 49-year-old woman with generalised neurofibromatosis underwent an exploratory operation for suspected phaeochromocytoma. An encapsulated tumour (7 cm diam) was removed from the region of the right adrenal gland and confirmed histologically as a phaeochromocytoma. During the operation a nodule in the duodenal wall near the end of the common bile duct was removed. The cut surface of the nodule showed a hard whitish oval mass, 1 cm in diameter, and sections showed a tumour ulcerating the mucosa and infiltrating the muscularis. The tumour consisted of regular cells with eosinophilic granular cytoplasm forming acini or glands with central lumina, some containing dense calcified bodies from 5–25 μm in diameter, occasionally concentrically laminated, with the characteristic appearance of psammoma bodies. In other areas glandular structures were absent and the cells were arranged in sheets. The tumour elements

Immunoperoxidase staining results

Antisera to:	Insulin	Glucagon	Somatostatin	HPP	Gastrin	Calcitonin	NSE
Case 1	+	−	+++	−	−	−	++
Case 2	−	−	+++	−	+	−	++
Case 4	−	−	+++	+	−	−	++

+++ = positive staining in most tumour cells.
++ = positive staining in moderate number of tumour cells.
+ = positive staining in occasional tumour cells.
Case 3 was an autopsy case unsuitable for immunolocalisation studies.
HPP = human pancreatic polypeptide.
NSE = neurone specific enolase.

were separated by stroma containing smooth muscle. The overlying mucosa was partly ulcerated and showed dilatation and elongation of crypts. Structures resembling normal pancreatic ducts were also present in the centre of the tumour.

Periodic acid Schiff and alcian blue stains showed positive staining in the acinar lumina and also in the epithelium of the ductal elements. The results of argentaffin impregnation were negative as were those by the Grimelius method. The Sevier-Munger technique showed argyrophil granules in the tumour cells. Immunoperoxidase staining showed the presence of somatostatin and neurone specific enolase in most tumour cells. Some cells contained insulin. Ultrastructurally, many tumour cells examined contained numerous spherical intracytoplasmic electron dense granules of about 450–700 nm diameter, with no discernible halo.
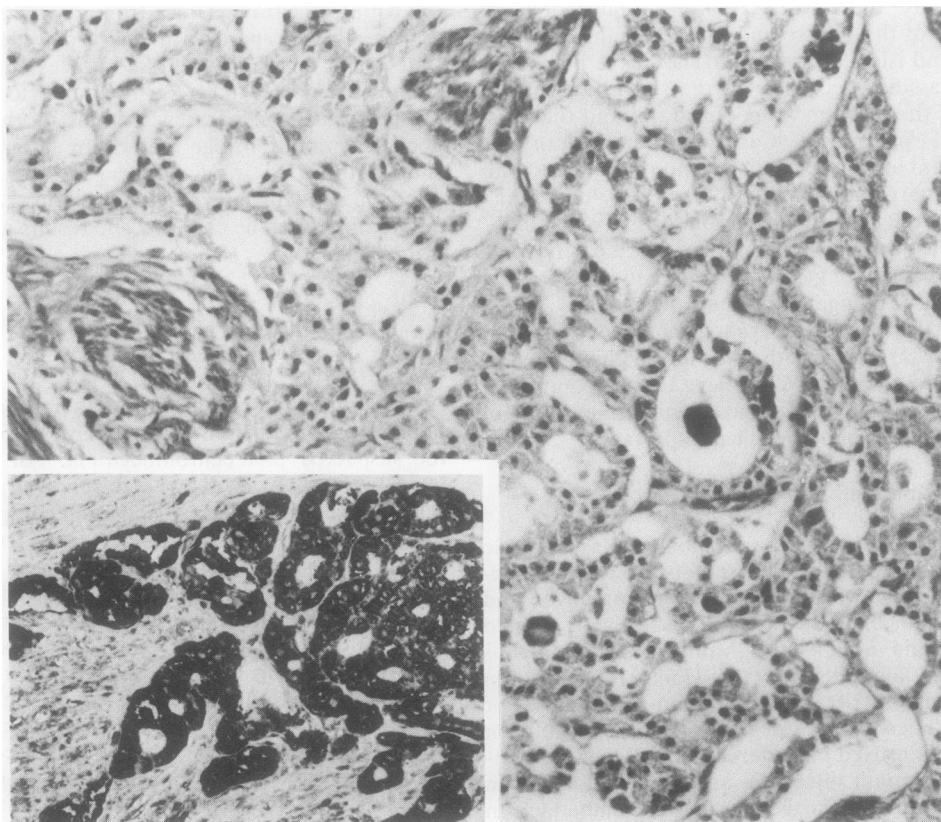

Fig. 1 *Tumour from case 1 showing glandular spaces and infiltration of muscle. Haematoxylin and eosin × 150. Inset: Immunolocalisation with anti-somatostatin. DNP hapten sandwich technique × 200.*
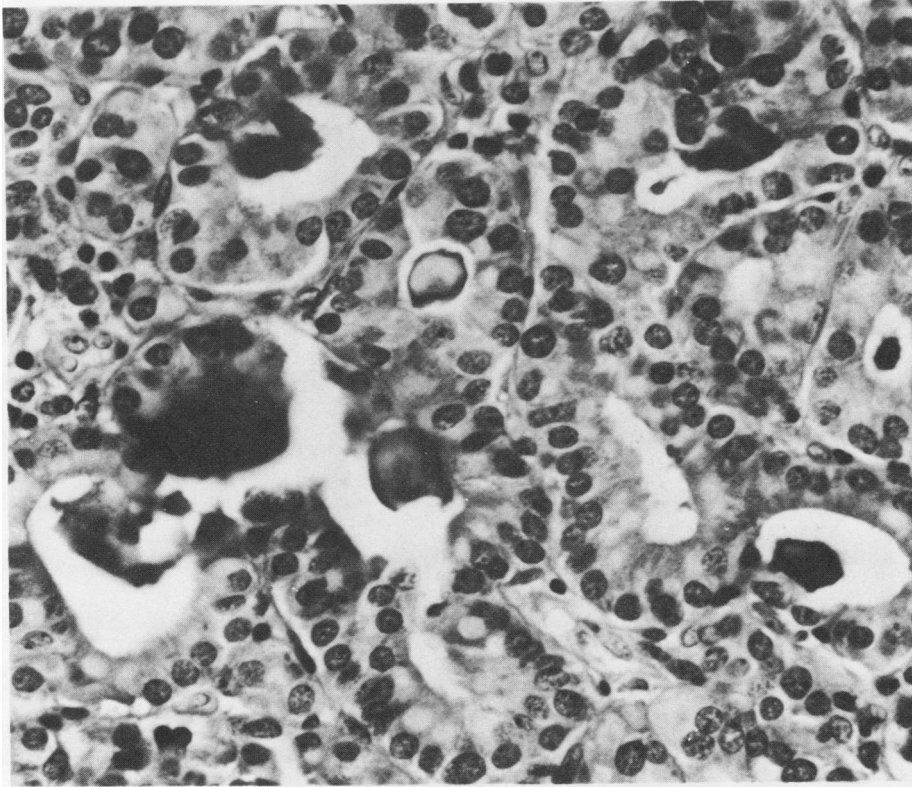
Fig. 2   *Tumour from case 2 showing regular tumour cells forming glands with many intraluminal psammoma bodies, in one area associated with disruption of the section. Haematoxylin and eosin × 500.*

CASE 2 (Fig. 2)

A 60-year-old woman with generalised von Recklinghausen's neurofibromatosis underwent a laparotomy to remove bilateral phaeochromocytoma. A firm nodule found in the duodenal wall close to the common bile duct was biopsied. Sections of the nodule showed a tumour within the mucosa without ulceration, the general microscopic appearances being very similar to these of case 1. The tumour cells had eosinophilic cytoplasm, regular nuclei, and were forming glands with lumina, most containing mucin and many containing psammoma bodies. The tumour stroma contained some fine bundles of smooth muscle and some hyaline material that did not stain as amyloid.

Argyrophilia could not be shown in the tumour cells. No material was available for electron microscopy, but immunohistochemistry showed neurone specific enolase and somatostatin in most tumour cells and a few scattered cells stained for gastrin.

CASE 3 (Fig. 3)

This duodenal tumour was an incidental finding at postmortem examination of a 79-year-old retired labourer who died of cor pulmonale and emphysema. Twelve years before his death he had had a partial gastrectomy for a chronic gastric ulcer, followed by a laparotomy for intestinal obstruction due to adhesions from the previous operation, at which a Meckel's diverticulum was also removed. During his last months he had lost weight and suffered from chronic diarrhoea. There was no evidence of tumour elsewhere.

Macroscopically there was a firm 2 cm diameter mass in the medial wall of the second part of the duodenum close to, but not affecting, the ampulla of Vater. Sections showed a circumscribed but not encapsulated tumour that was largely of a regular pattern, consisting of pale cells forming acini with a central lumen. The lumina contained mucin; in some there were psammoma bodies. Towards the centre
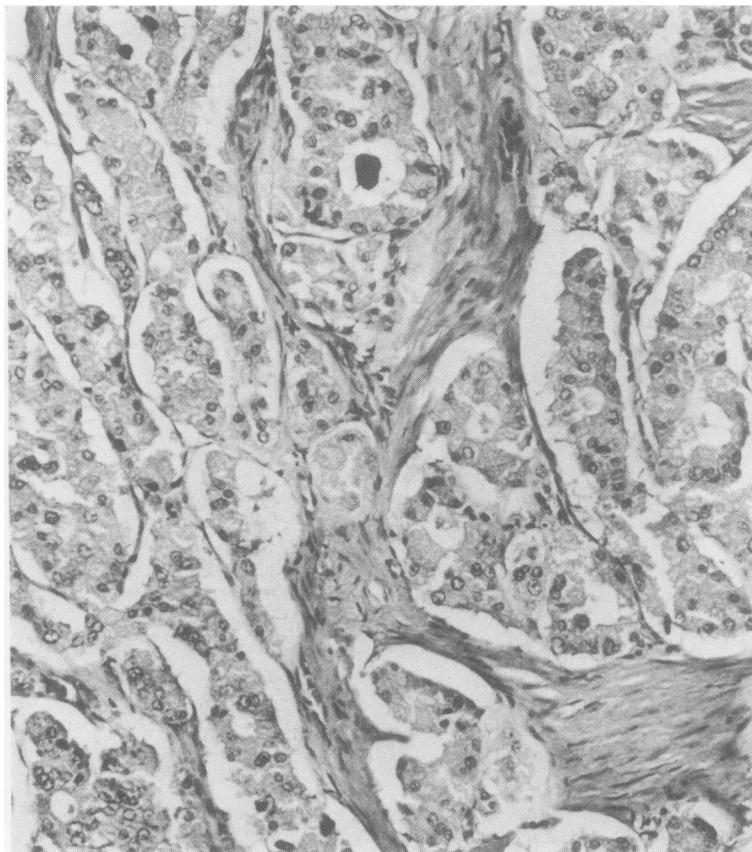
Fig. 3 *Tumour from case 3 showing neoplastic glands infiltrating smooth muscle. An intraluminal psammoma body is present. Haematoxylin and eosin × 175.*

of the tumour there were benign duct like elements intermingled with a denser stroma containing smooth muscle.

Results of argentaffin techniques were negative, but the Grimelius and Sevier-Munger methods showed patchy impregnation of intracytoplasmic granules in the cells lining the acini. Ultrastructurally the tumour cells were poorly preserved, but many intracytoplasmic membrane bound granules, compatible with endocrine granules, could be identified.

CASE 4 (Fig. 4)

A 42-year-old woman underwent a laparotomy to remove a 10 cm diameter cystadenoma of the ovary. She had complained of episodic watery diarrhoea and had previously undergone cholecystectomy for gall stones. No stigmata of neurofibromatosis were noted. A nodule at the duodenal-jejunal flexure was discovered during a routine search of the abdomen and removed. The duodenal tumour was a pale firm 1 cm diameter nodule within the bowel wall. His-

tology showed a well circumscribed but non-encapsulated tumour, which infiltrated the overlying mucosa. It consisted mainly of "nests" or "packets" of regular tumour cells with pale cytoplasm and regular round nuclei. In some areas, however, the cells formed acini, some with a central lumen containing mucin. No psammoma bodies were seen, but, as in previous cases, there were smooth muscle bundles and pancreatic duct like structures within the tumour.

The tumour cells were negative with both argentaffin and argyrophil techniques, but some cells within the ductal elements contained argyrophil positive granules. Immunoperoxidase staining showed that almost all tumour cells were immunoreactive to antibodies to somatostatin and neurone specific enolase, with a few cells staining for human pancreatic polypeptide.

Electron microscopy of tissue recovered from paraffin blocks showed that the tumour cells contained many spherical intracytoplasmic membrane bound electron dense granules of approximately 170–350 nm diameter.
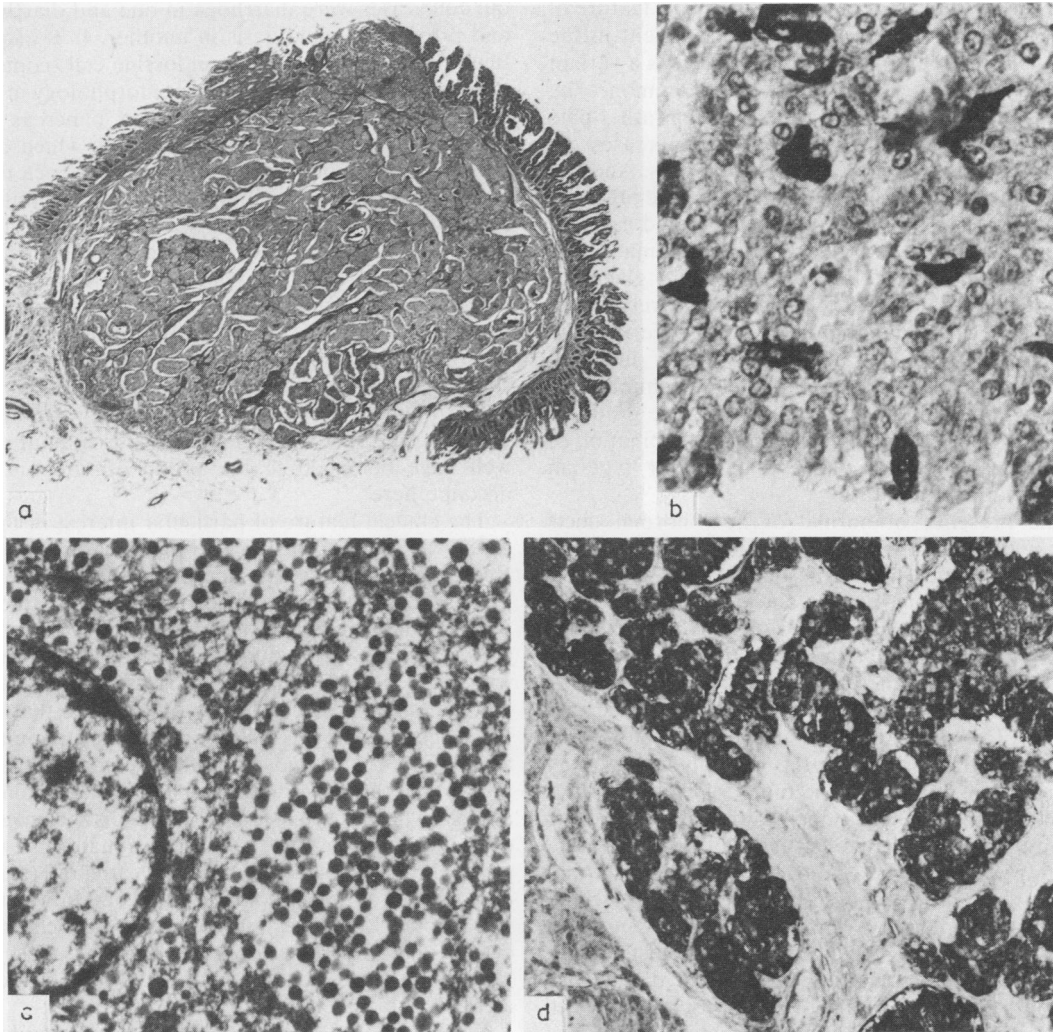
Fig. 4   *Tumour from case 4. (a) Low power view showing tumour affecting the mucosa and submucosa of the duodenum. Haematoxylin and eosin × 10. (b) Immunolocalisation with anti-human pancreatic polypeptide showing occasional strongly positive cells. DNP hapten sandwich technique × 500. (c) Numerous dense core secretory granules are present in the cytoplasm of a tumour cell in tissue recovered from a paraffin block, Epon embedded, and uranyl acetate/lead citrate stained × 10 500. (d) Immunolocalisation with anti-somatostatin. DNP hapten sandwich technique × 175.*

Discussion

These tumours are clearly carcinoids as they are composed almost exclusively of cells which show evidence of endocrine differentiation by silver impregnation techniques, by electron microscopy, and by immunolocalisation. We regard them as of low grade malignancy on the basis of local invasion; none of them showed evidence of metastases, the nuclear morphology was regular, and mitoses were scanty. There were several interesting features which merit discussion, the unusual pathological pattern, the hormone content, and the clinical associations being the main points.

The major pathological feature of interest in these tumours was the true glandular pattern of the tumour, which could lead to confusion with exocrine adenocarcinoma or with a Brunner's gland tumour.

This acinar appearance was the dominant feature of three tumours and was a minor component in the fourth. A glandular pattern is described as a variant in carcinoid tumours,[5][7] but it is uncommon. The association of a glandular pattern with a rather pale cytoplasm in some of these tumours increases the possibility of confusion with other tumours. Another interesting histological observation was the presence in three of our four tumours of laminated calcified basophilic structures resembling psammoma bodies occurring mainly in the lumina of the glandular structures. These are not a commonly recognised feature of carcinoids, although we believe that the case reported by Murayama *et al*[12] of a duodenal carcinoid showing both glandular lumina and psammoma bodies clearly belongs to the group of tumours that we are reporting. This combination of histological features makes this tumour type an easily identifiable entity.

The presence of normal or near normal ducts within three of these tumours deserves comment, but we are uncertain of its importance. The ducts were cytologically benign and resembled normal ampullary ducts. Such inclusions of normal ducts have been previously described in endocrine cell tumours of the pancreas[13][14] and have been previously noted by one of us (EDW) in a gastric carcinoid. The ducts may be normal or heterotopic structures trapped within the tumour or they may form an integral part of the tumour. The ultrastructural features of these tumours were not diagnostic of any single endocrine cell type, but preservation, fixation, and technique differed in each case, and none was ideally prepared for electron microscopy.

The second major feature of interest in these tumours was the presence of immunohistochemically identifiable somatostatin in three of the four tumours (Table). The fourth was a postmortem case, and a negative result is of no importance as we have found that somatostatin is not well preserved in postmortem material. Occasional somatostatin cells are found in a wide variety of endocrine tumours, but tumours composed largely of somatostatin containing cells, as in these cases, are rare and have sometimes been recognised as a result of excess somatostatin production. In our cases the granular morphology was variable, but the presence of somatostatin in most cells would justify the subclassification of D cell carcinoid. We have not used the term somatostatinoma to describe these cases as we believe that this should be reserved for cases in which there is evidence of a high circulating somatostatin concentration produced by a tumour. The somatostatinoma syndrome consists of diabetes mellitus, cholelithiasis, and diarrhoea;[15] the only features possibly related to this syndrome noted in

our four cases were diarrhoea in one and diarrhoea and previous cholelithiasis in another. It is of particular interest to note that endocrine cells containing somatostatin show a different morphology in different tissues. In the stomach and pancreas the somatostatin cells show long processes which contact other cell types and presumably influence their function. In the duodenum the somatostatin containing cells show a flask like shape, do not possess long processes, but do contact the lumen.[16] This difference in morphology may well correlate with this unusual glandular pattern of endocrine tumours of somatostatin cells in the duodenum. It is of interest that the first report of a duodenal "somatostatinoma" was described microscopically as an adenocarcinoma because the tumour contained glandular areas.[17] We believe that this tumour may well have belonged to the group of tumours we describe here.

The clinical feature of particular interest in these cases was the presence in two patients of both von Recklinghausen's disease and phaeochromocytoma. This link is unlikely to be coincidental; a recent report[18] and a review of the published work[19] show six other cases with duodenal tumours associated with von Recklinghausen's disease, phaeochromocytoma, or both conditions. We believe that this is a specific form of multiple endocrine neoplasia and suggest that if two of the three components are present the third should be sought. We have pointed out elsewhere the existence of two distinct neuroendocrine syndromes: neurofibromatosis with phaeochromocytoma and duodenal carcinoid (some, and possibly all, being somatostatin containing), and von Hippel-Lindau syndrome with phaeochromocytoma and pancreatic islet cell tumours. We have tentatively suggested that they should be referred to as MEN IIIa and b.[19]

We considered the possibility that the duodenal tumours, because of their association with von Recklinghausen's disease and phaeochromocytoma, might be of neural origin. We believe this to be unlikely because no intestinal endocrine cell has yet been shown to be derived from the neural crest and because of the occasional admixture of other hormone containing cell types. This pattern is seen in other carcinoids and islet cell tumours, for which the available evidence supports an endodermal origin.

We therefore report the occurrence of a distinctive group of duodenal carcinoids, usually occurring near the ampulla of Vater, which can be characterised histologically by their glandular pattern and somatostatin content, with, in addition, in some cases, luminal psammoma bodies. Some of the tumours are associated with von Recklinghausen's disease and phaeochromocytoma. The clinical

importance of these tumours requires further elucidation, but the presence of diarrhoea in two cases raises the possibility of a humoral effect. The histological recognition of the particular features of this group of tumours should enable them to be separated and allow the study of these various associated interesting features.

We thank the following for allowing us to study their material: Dr GD Powell of Rotherham District General Hospital (case 1), Dr ID Ansell and Dr DC Banks of the City Hospital, Nottingham (case 2), Dr WH Beasley of Bronglais Hospital, Aberystwyth (case 3), and Professor LE Hughes of the University Department of Surgery, Cardiff (case 4).

References

[1] Williams ED, Siebenmann R, Sobin LH. International histological classification of tumours No 23, *Histological typing of endocrine tumours*. Geneva: World Health Organisation, 1980.

[2] Williams ED, Sandler M. The classification of carcinoid tumours. *Lancet* 1963;i:238–9.

[3] Sanders RJ, Axtell HK. Carcinoids of the gastrointestinal tract. *Surg Gynecol Obst* 1964;119:369–80.

[4] Helmstaedter V. Clinical features of the 'new' endocrine tumours of the upper gastrointestinal tract. In: Friedman M, Ogawa M, Kisner D, eds. *Diagnosis and treatment of upper gastrointestinal tumours*. Amsterdam: Excerpta Medica, 1981:68–73.

[5] Soga J, Tazawa K. Pathological analysis of carcinoids, histological re-evaluation of 62 cases. *Cancer* 1971;28:990–8.

[6] Martin ED, Potet F. Pathology of endocrine tumours of the GI tract. *Clin Gastroenterol* 1974;3:511–32.

[7] Jones RA, Dawson IMP. Morphology and staining patterns of endocrine cell tumours in the gut, pancreas and bronchus and their possible significance. *Histopathology* 1977;1:137–50.

[8] Lillemoe K, Imbembo AL. Malignant neoplasms of the duodenum. *Surg Gynecol Obst* 1980;150:822–6.

[9] Weichert RF, Roth LM, Kvementz ET, Hewett RL, Drapanus T. Carcinoid-islet cell tumours of the duodenum; report of twenty one cases. *Am J Surg* 1971;121:195–205.

[10] Jasani B, Wynford-Thomas D, Williams ED. Use of monoclonal antihapten antibodies for immunolocalistion of tissue antigens. *J Clin Pathol* 1981;34:1000–2.

[11] Jasani B, Thomas ND, Newman GR, Williams ED. DNP-hapten sandwich staining (DHSS) procedure: design, sensitivity, versatility and applications. *Immunol Communications* 1983;12:51.

[12] Murayama H, Imai T, Kikuchi M, Kamio A. Duodenal carcinoid (Apudoma) with psammoma bodies; a light and electron microscopic study. *Cancer* 1979;43:1411–7.

[13] Sieracki J, Marshall RB, Horn RC. Tumours of the pancreatic islets. *Cancer* 1960;13:347–57.

[14] Reid JD, Yuh S, Petrelli M, Jaffe R. Ductuloinsular tumours of the pancreas: a light, electron microscopic and immunohistochemical study. *Cancer* 1982;49:908–15.

[15] Keejs GJ, Orci L, Conlon JM, et al. Somatostatinoma syndrome: biochemical, morphologic and clinical features. *N Engl J Med* 1979;301:285–92.

[16] Larsson L-I. Somatostatin cells. In: Bloom SR, Polak JM, eds. *Gut hormones*. Edinburgh: Churchill Livingstone, 1981:350–3.

[17] Kaneko H, Yanaihara N, Ito S, et al. Somatostatinoma of the duodenum. *Cancer* 1979;44:2273–9.

[18] Hough DR, Chan A, Davidson H. Von Recklinghausen's disease associated with gastrointestinal carcinoid tumours. *Cancer* 1983;51:2206–8.

[19] Griffiths DFR, Williams GT, Williams ED. Multiple endocrine neoplasia associated with von Recklinghausen's disease. *Br Med J* 1983;287:1341–3.