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Case report

Non-functional malignant paraganglioma of the stomach

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Summary. We report the second case of a malignant paraganglioma of the stomach in a 56-year-old female patient. However, our case is the first investigated by immunohistochemistry and electron-microscopy. The tumour was characterized immunohistochemically by the presence of neurofilament protein, glial fibrillary acidic protein, S-100 protein, neuron-specific enolase, chromogranin A, ACTH, leu-enkephalin and vasoactive intestinal polypeptide, and ultrastructurally by demonstration of neurosecretory granules and paranuclear intermediate filament whorls. Despite massive metastatic spread in the abdominal cavity, the patient is still alive 4 years after initial diagnosis.

Key words: Stomach – Paraganglioma – Immunohistochemistry – Electron microscopy

Introduction

Visceral autonomic paragangliomas have been reported to occur in association with many organs: heart (Johnson et al. 1985), hepatic duct (Sarma et al. 1980), gallbladder (Miller et al. 1972, Wolff 1973), kidney (Lagace and Tremblay 1968), urinary bladder (Davaris et al. 1986, Capella et al. 1988), spermatic cord (Eusebi and Massarelli 1971), uterus (Young and Thrasher 1982), thyroid gland (Mitsudo et al. 1987), prostate (Mehta et al. 1979), lung (Singh et al. 1977) and mesoappendix (Clark et al. 1985). Although paragangliomas of the small bowel, particularly the duodenum, do occur and have been described in many publications (Kheir and Halpern 1984; Kawaguchi et al. 1985; Perrone et al. 1985; Scheithauer et al. 1986), to our knowledge paraganglioma of the stomach has been described only once (Westbrook et al. 1972). This case is the first to be investigated by both immunohistochemistry and electron microscopy, and concerns a 56-year-old female patient in whom a paraganglioma of the stomach was removed surgically. Thirty-one months later massive metastatic spread to the lower abdominal cavity and the pelvis occurred.

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Case report

Clinical history

In March 1985 a 56-year-old female patient was admitted to the Surgical Department of the University Hospital in Graz because of a palpable mass in the left epi- and mesogastrium. On admission the patient was in good general condition, but a tender tumour, not fixed to its surroundings was evident. By sonography and computed tomography a solid, focally cystic tumour, 10 cm in diameter, was detected (Fig. 1). The tumour was situated between the tail of the pancreas, the posterior gastric wall and the transverse

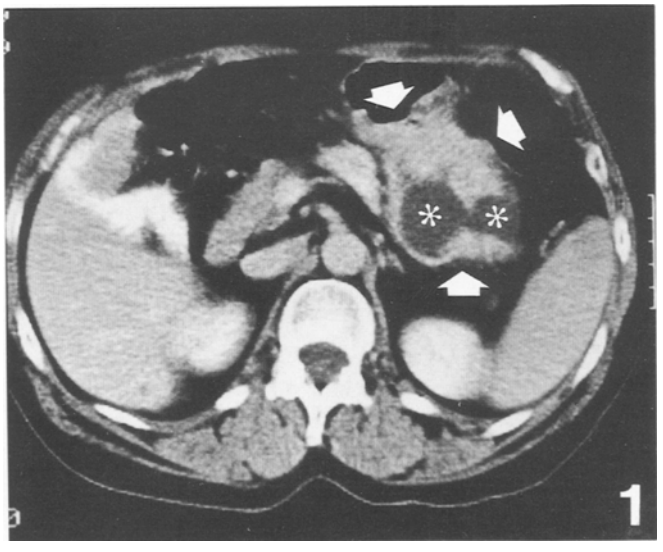
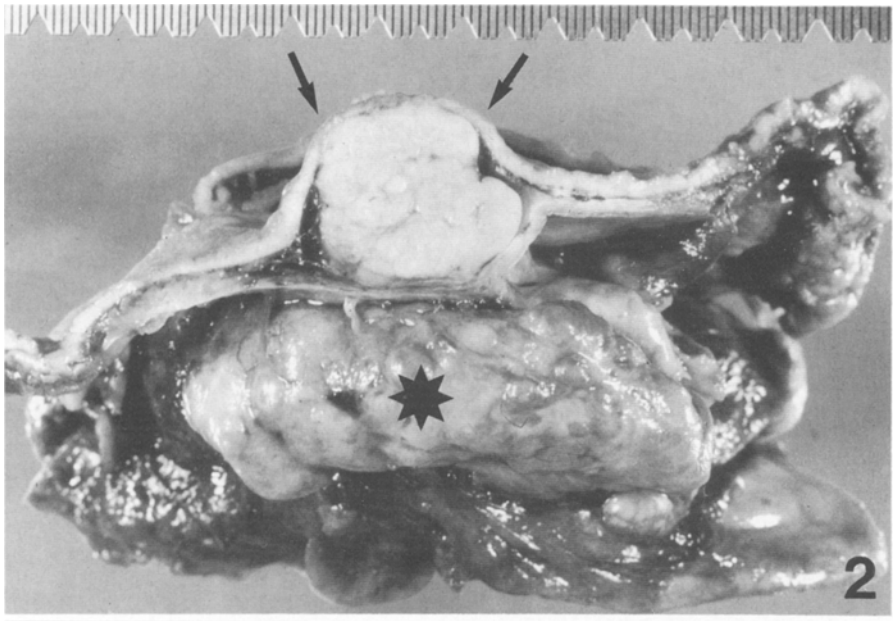


Fig. 1. Computer-assisted tomography of the upper abdomen. Solid, focally cystic (*asterisks*) tumour (*arrows*) localized in the posterior gatric wall



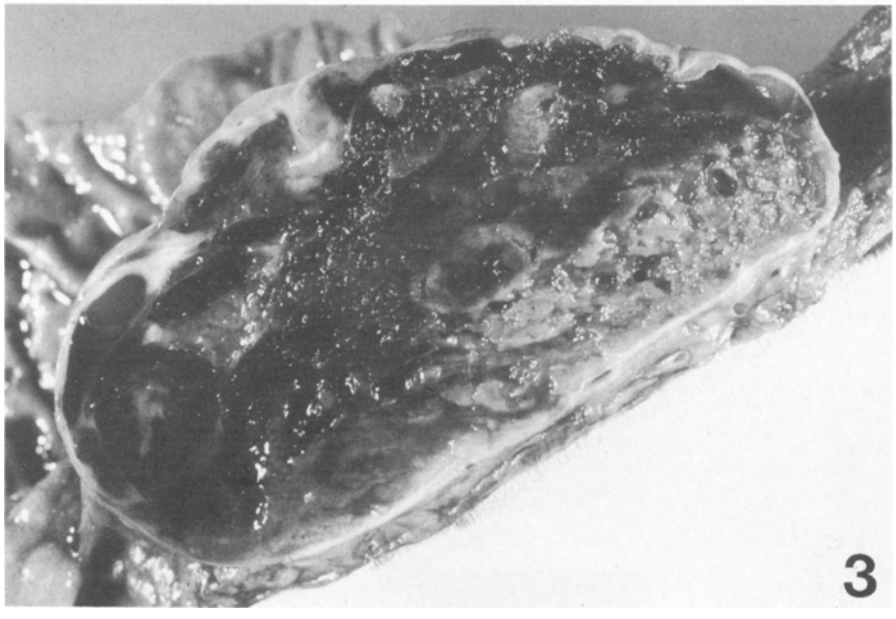


Fig. 2. Nodular tumour bulging into the direction of the gastric cavity (*arrows*) and infiltrating into the gastrocolic ligament (*asterisk*)

Fig. 3. Cut surface of the tumour mass located in the gastrocolic ligament showing haemorrhages and cystic areas

colon. On pancreaticography and choledochography origin from the pancreas could be excluded. At gastroscopy, an impression of the posterior gastric wall at the greater curvature was noted. Biopsy specimens from the overlying gastric mucosa revealed chronic gastritis. Computed tomography of the chest showed a well-circumscribed cystic tumour closely attached to the thoracic aorta. This tumour, 8 cm in diameter, was situated paravertebrally between the lower lobe of the left lung and the diaphragm. An exploratory laparotomy was performed and revealed a bosselated tumour localized in the posterior gastric wall and within the gastrocolic ligament. The mass was removed by partial gastrectomy (Billroth II) and resection of the transverse colon, gastrocolic ligament and the greater omentum. One month later a thoracotomy was performed and a cystic tumour, well-circumscribed but closely attached to the aortic wall, was found. At incision yellowish, necrotic material appeared. A frozen section diagnosis of "necrotic material without any tumour areas" was made and the lesion was removed except where adherent to the aorta. After an uncomplicated postoperative course the patient was discharged in fair general condition. Thirty-one months later tumour masses in the abdomen were detected by computed tomography. In October 1987 a further laparotomy was performed and revealed massive tumour spread to the peritoneum and the minor pelvis with nodules measuring up to 10 cm in diameter. Five weeks later the patient was discharged in poor general condition. Until March 1989 the patient was still alive and was then lost to follow-up.

Results

Macroscopic findings. The surgical specimen consisted of a part of the stomach (Billroth's II gastrectomy), the greater omentum, the gastrocolic ligament and the transverse colon. A nodular tumour originated in the posterior gastric wall and grew into the gastrocolic ligament (Fig. 2). The tumour was situated with its greater part in the gastrocolic ligament and measured 10:7:5 cm. On cross-section the tumour was greyish-white with multiple cystic areas as well as haemorrhages (Fig. 3).

Table 1. List of antibodies used for immunohistochemical cell characterization

Immunogen	Donor	Type	Dilution	Source
Pan-cyto-keratin (CK)	Mouse	M	1:20	Boehringer, Mannheim FRG
Desmin	Mouse	M	1:1000	Dako, Denmark
Neurofilament protein	Mouse	M	1:3	Milab, Sweden
Vimentin	Rabbit	P	1:20	Euro Diagnostic, Netherlands
GFAP[a]	Rabbit	P	1:100	Medac, FRG
S-100 protein	Rabbit	P	1:500	Dako, Denmark
NSE γ-subtype	Mouse	M	1:20	Imogenetics, Belgium
SP1/chromo-granin A	Rabbit	P	1:500	Incstar, USA
Serotonin	Mouse	M	1:500	Dako, Denmark
Leu-enkephalin	Rabbit	P	1:500	Incstar, USA
Met-enkephalin	Rabbit	P	1:500	Incstar, USA
Neuroendo. antigen[b]	Mouse	M	1:500	Ortho, FRG
Somatostatin	Mouse	M	1:50	Novo, Denmark
GRP[c]	Mouse	M	1:500	Hybritech, USA
VIP[d]	Rabbit	P	1:100	Ortho, FRG
ACTH	Rabbit	P	1:500	Dako, Denmark
Gastrin	Rabbit	P	1:1000	Milab, Sweden
Calcitonin	Rabbit	P	1:200	Milab, Sweden
Substance P	Rat	P	1:100	Sera Lab, UK
Neurotensin	Rabbit	P	1:5000	Incstar, USA
Pancreatic polypeptide	Rabbit	P	1:5000	Dako, Denmark
Glucagon	Mouse	M	1:50	Novo, Denmark
Bombesin	Mouse	M	1:100	Hybritech, USA
Insulin	Mouse	M	1:500	Novo, Denmark

P, Polyclonal; M, monoclonal
[a] Glial fibrillary acidic protein; [b] neuroendocrine antigen (clone Phe 5); [c] gastrin-releasing peptide; [d] vasoactive intestinal polypeptide

Methods. The material obtained by surgery was fixed immediately after removal in 10% phosphate-buffered formaldehyde solution (pH 7.4) and paraffin-embedded according to conventional procedures. Consecutive sections, 4 µm thick, were stained with haematoxylin-eosin and Gomori's stain. For immunohistochemical studies the APAAP method (Cordell et al. 1984) was performed. The primary antibodies used are listed in Table 1.

For control purposes, tissues known to contain the respective antigens were included (positive controls). Replacement of the primary antibody by normal serum always revealed negative results (negative controls). For electron microscopy, formalin-fixed tissue was retrieved from paraffin blocks and processed according to standard procedures. Semi-thin and ultra-thin sections were cut with a Reichert OM U4 ultracut microtome. The ultra-thin sections were stained with uranyl-acetate and lead citrate and examined at 80 KV on a Philips EM 400 electron microscope.

Histological findings. In the stomach the tumour was covered only by the mucosa and muscularis mucosae (Fig. 4). Most areas of the tumour appeared characteristic of paraganglioma. They consisted of round or polygonal rather monomorphic cells arranged in a "zellballen" pattern. This was clearly demonstrated by Gomori's stain, where nests of cells were surrounded by reticulin fibres (Fig. 5). However, in those parts of the tumour situated within the muscle layer of the stomach or the gastrocolic ligament the tumour cells were pleomorphic and sometimes vacuolated resembling lipoblasts (Fig. 6). Occasionally, giant cell formation was seen. Only focally was a "zellballen" arrangement observed. By contrast, most cells were arranged in sheets infiltrating the muscle layer. In these areas numerous mitotic figures could also be observed. Paraffin histology of the thoracic lesion revealed fibrous tissue surrounding a pseudocyst filled with necrotic material. Despite careful examination no evidence for structures of a paraganglioma could be detected.

Immunohistochemistry. Diffuse intracytoplasmic staining for glial fibrillary acidic protein (GFAP) (Fig. 7) and neuron specific enolase (NSE) was present in most neoplastic cells arranged in a "zellballen" pattern, whereas in the pleomorphic areas only few scattered tumour cells showed a weak reactivity. Heterogeneity of immunoreactivity was also conspicuous with respect to chromogranin A expression. Diffusely dispersed tumour cells or small tumour cell groups in more differentiated as well as in pleomorphic areas were strongly positive (Fig. 8), whereas other tumour cells showed weak or no reactivity. Cytoplasmic reactivity was shown for neurofilaments (NF) in single tumour cells or small cell groups. Granular cytoplasmic reactivity was observed for ACTH in many tumour cells (Fig. 9). With the antibody to S-100 protein only in some cells suggestive of chief cells weak cytoplasmic and nuclear staining was present. Moderate-to-weak focal cytoplasmic staining for leu-enkephalin (Fig. 10) and vasoactive intestinal poly-peptide (VIP) was noted in few cells in differentiated tumour areas. With the antibody to vimentin only a few single cells were weakly decorated. With antibodies to cytokeratin (CK) strong cytoplasmic reactivity was seen in scattered tumour cells either diffusely distributed or in a global fashion. No immunoreactivity could be observed with the other antibodies used.

Electron microscopy. Ultrastructurally, the tumour was almost exclusively composed of polygonal or elongated cells with often centrally located, oval nuclei with delicate scattered chromocentres and one or two nucleoli (Fig. 11). The cytoplasm contained organelles and few scattered electron dense granules ranging between 100–200 nm in diameter suggestive of neurosecretory nature (Fig. 11 and inset in upper right). The protein cores of the neurosecretory granules lacked enveloping membranes due to formaldehyde fixation and embedding procedures. In the pleomorphic tumour areas within the gastrocolic ligament and the muscle layer of the stomach marked nuclear variability could be observed. Intermediate filaments were present in few cells either dispersed throughout the cytoplasm or as round paranuclear whorls, which resembled the fibrous bodies of neuroendocrine cell neoplasms (fig. 11, inset upper left).

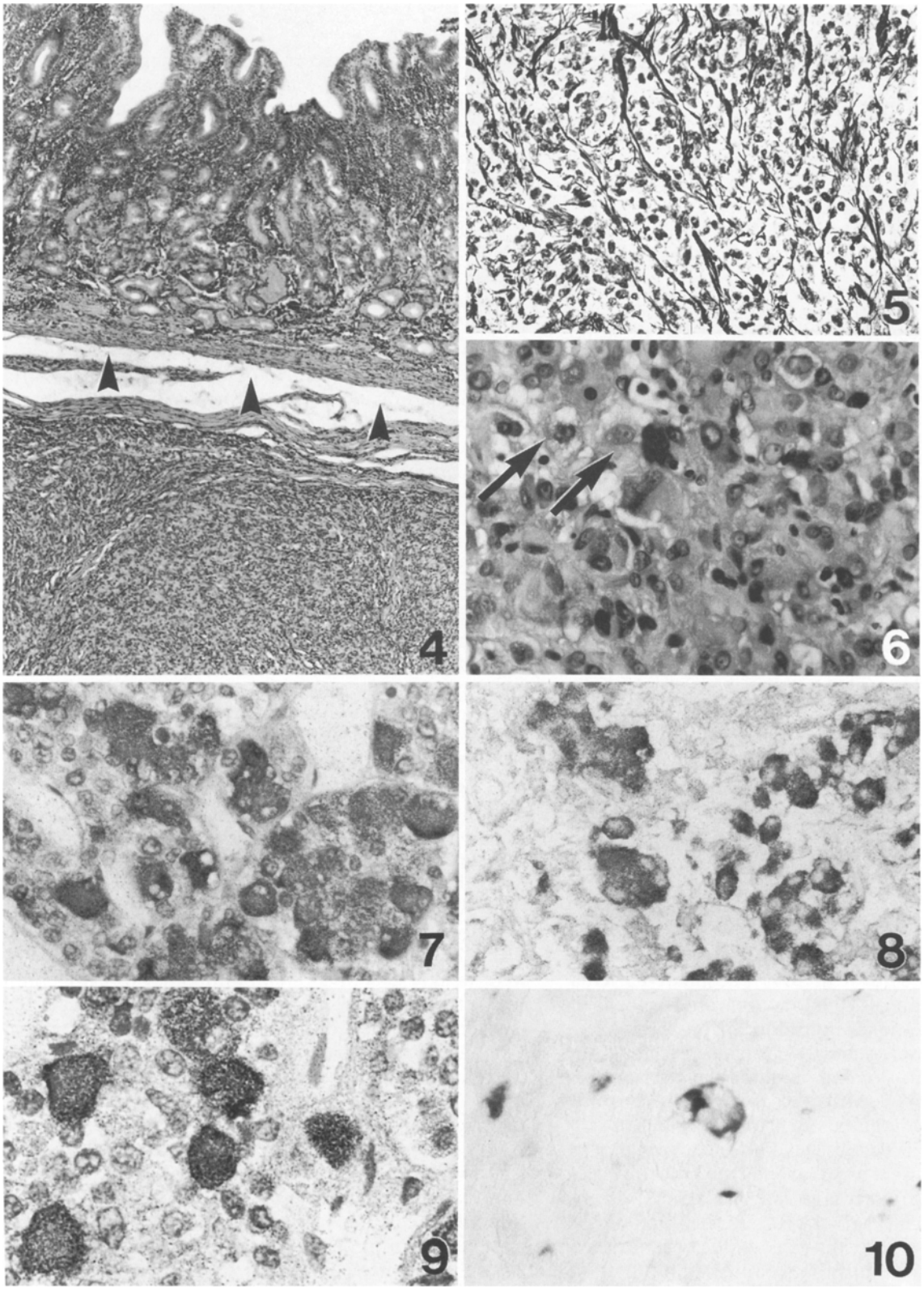


Fig. 4. In the stomach the tumour is only covered by mucosa and muscularis mucosae (*arrow heads*). H&E ×80

Fig. 5. Characteristic "zellballen" pattern of the paraganglioma. Gomori's stain ×280

Fig. 6. Tumour area with pleomorphic cells, some of which resemble lipoblasts (*arrows*). H&E ×360

Fig. 7. Diffuse intracytoplasmic reactivity for GFAP in most of the tumour cells. APAAP ×320

Fig. 8. Many of the tumour cells are strongly positive for chromogranin A. APAAP ×320

Fig. 9. Pronounced granular cytoplasmic immunoreaction for ACTH in many tumour cells. APAAP ×320

Fig. 10. Leu-enkephalin immunoreactivity in few neoplastic cells. APAAP ×320

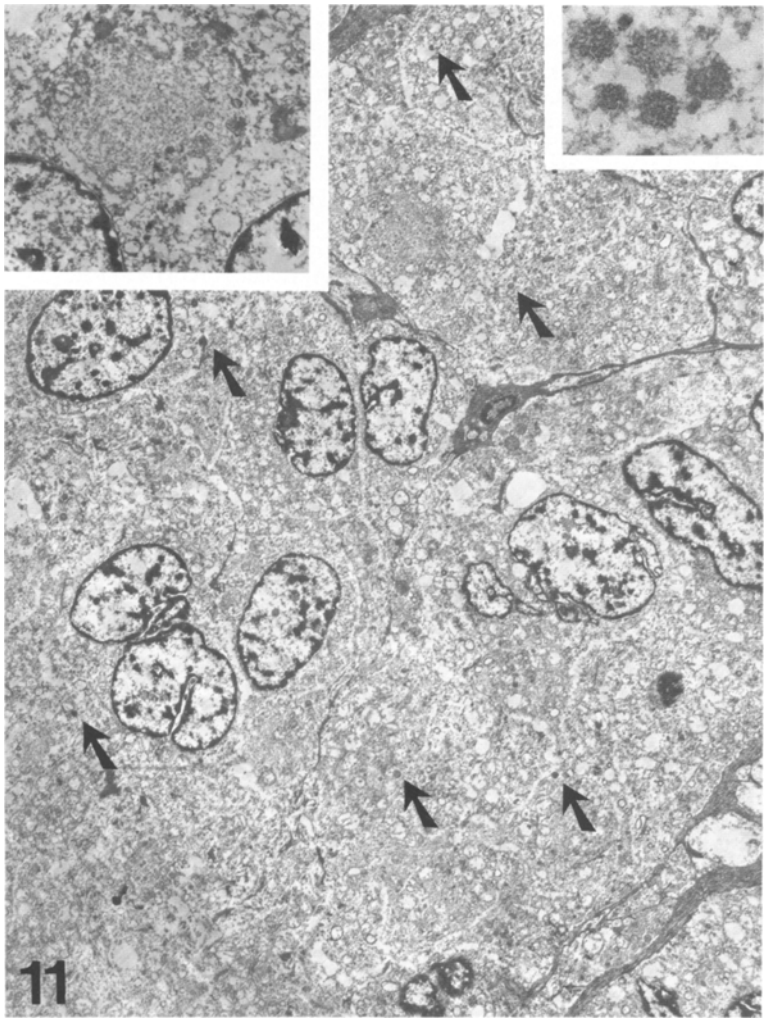


Fig. 11. Polygonal tumour cells revealing oval nuclei with delicate scattered chromocentres and few neurosecretory electron-dense granules (*arrows*). EM ×4800. *Inset, upper right:* higher magnification of electron-dense granules. EM ×48200. *Inset, upper left:* intermediate filaments aggregated as paranuclear whorls. EM ×12960

Discussion

Paragangliomas can rarely be found in visceral organs and, therefore, reports are restricted to single cases. Although they occur relatively often in the small bowel, paraganglioma of the stomach has only once been described (Westbrook et al. 1972). In contrast to our case the aforementioned authors did not confirm the diagnosis by electron microscopy nor could they investigate their case by immunohistochemistry. Since Schwannomas and leiomyogenic lesions with regressive changes are frequently tumours originating from the stomach, Schwannoma, leiomyoma or leiomyosarcoma with cystic areas had to be considered in the differential diagnosis. In contrast to adrenal paragangliomas (phaeochromocytomas) extra-adrenal paragangliomas are usually non-functional (Sonneland et al. 1986), as was our case; therefore, blood and urine chemistry was not diagnostically helpful. By histological examination the tumour could be classified, but behaviour was difficult to evaluate. Malignancy was suggested on the basis of mitoses in the pleomorphic areas as well as of infiltration of the gastric muscle layer and the gastrocolic ligament. According to Lack et al. (1980) and Enzinger and Weiss (1988), cell pleomorphism is not a reliable criterion to predict malignancy, whereas mitotic activity and vascular invasion should be considered as signs of potential malignancy. Sometimes the typical "zellballen" nested cells are pleomorphic and lack the structural organization typical of paraganglioma. They must then be discriminated from carcinoma cells, which was also true for our case where a focally dominant pleomorphic pattern could be seen. Another histological differential diagnosis is carcinoid tumour. However, in general, cells of a carcinoid are more uniform than those of paragangliomas and "lipoblast-like" pleomorphic cells are never seen. Additionally, carcinoid cells have nuclei polarized to the vascular margin of a cell nest, whereas cells of a paraganglioma reveal a centrally located nucleus. A further helpful differential diagnostic feature in our case was the characteristic ultrastructure with the presence of neurosecretory granules and paranuclear intermediate filament whorls (Ironside et al. 1985; Sonneland et al. 1986). Immunohistochemically in some tumour cells we could demonstrate NF, GFAP and S-100 protein, indicating a probable origin from the neural crest (Schroder and Johannsen 1986). In a large series of phaeochromocytomas and paragangliomas Schroder and Johannsen

(1986) demonstrated S-100 protein-positive sustentacular cells in all cases except in two malignant phaeochromocytomas. In our case we could not detect sustentacular cells by electron microscopy or immunohistochemistry. This is in line with other authors who were unable to find such cells in paragangliomas (Toker 1967; Lack et al. 1980; Kliewer et al. 1989). The presence of NSE and chromogranin is characteristic for neuroendocrine tumours, including paragangliomas (Lloyd et al. 1984, 1985; Schroder and Johannsen 1986; Kimura et al. 1988), whereas leu-enkephalin is more specific for paragangliomas (DeLellis et al. 1983). In accordance with other authors (Perrone et al. 1985), we observed the presence of peptide hormones acting as neuroendocrine mediators, like ACTH and VIP, in the tumour. VIP, which is present in many types of neurons, has been reported to be produced by human phaeochromocytoma cells in culture and is considered to be an ectopic product of mammalian chromaffin cells (Tischler et al. 1984). Like Ironside et al. (1985), we were also able to demonstrate CK immunohistochemically in few tumour cells. Some of them revealed a globular immunoreaction, which may be related to the fibrous bodies found by electron microscopy (Ironside et al. 1985).

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