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Case report

Malignant paraganglioma of the uterus

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Summary. We report a malignant uterine paraganglioma in a 40-year-old female, who died 7 months after the initial diagnosis. On light microscopy the tumour showed a typical *zellballen* pattern as well as a pronounced cellular pleomorphism. In many tumour cells hyaline globules were demonstrated within the cytoplasm. Immunohistochemically the lesion was characterized by the presence of neuron-specific enolase, protein gene product 9.5 and synaptophysin, and electron microscopically by the occurrence of neurosecretory granules.

Key words: Uterus – Paraganglioma – Intracytoplasmic hyaline globules – Immunohistochemistry – Electron microscopy

Introduction

Paragangliomas of visceral organs are rare lesions which have been reported to occur at a variety of sites (Schmid et al. 1990). Among them is the uterus in which, to date, a conventional paraganglioma (Young and Thrasher 1982) and two melanocytic paragangliomas (Tavassoli 1986) have been described, and a further paraganglioma has been briefly mentioned in an abstract (Tenti et al. 1991). The first three cases showed an apparently benign course as evidenced by a follow-up period without metastases and recurrences between 2 and 3.3 years.

Therefore, to our knowledge, we are the first to present a clinically malignant paraganglioma of the uterus, the diagnosis of which was confirmed by both immunohistochemistry and electron microscopy.

Case report

In February 1990 a 40-year-old women, para 1, abortus 1, underwent hysterectomy because of a fast-growing uterine tumour thought to be a leiomyoma. The histopathological diagnosis of malignant paraganglioma was made and the patient was carefully checked 1 month later, when neither recurrence nor metastases were found. In June 1990, however, the patient had to undergo two operations because of a recurrent tumour affecting the whole minor pelvis with resulting obstruction of the small bowel. Thereafter no further treatment was given and she was discharged to home, where she died from massive metastatic tumour spread in September 1990. An autopsy was not performed.

The majority of the material obtained surgically was fixed in 10% formaldehyde solution, paraffin embedded and processed conventionally. Consecutive sections of 4 μm were stained with haematoxylin and eosin and Gomori's stain. A smaller part of the tumour was frozen in liquid nitrogen and stored at −70° C for further immunohistochemical studies using the alkaline phosphatase – antialkaline phosphatase method. The primary antibodies used are listed in Table 1. For electron microscopy, tissue was also fixed in 2.5% glutaraldehyde and processed according to standard procedures.

Pathological findings

Macroscopically the initial surgical specimen consisted of the uterus without adnexae and showed a nodular tumour within the myometrial layer of the corpus. On cross-sections, the tumour, 4.3 cm in diameter, was greyish-white to yellow and showed multiple foci of haemorrhage and necrosis. It was sharply demarcated from the surrounding tissue (Fig. 1). By light microscopy, most areas of the tumor were composed of rather monomorphic polygonal cells with a slightly enlarged round or ovoid nucleus. The latter were centrally located and exhibited one or two distinct nucleoli. Occasional giant tumour cells were evident. The cells were arranged in a characteristic *zellballen* pattern (Fig. 2), which was impressively outlined by Gomori's stain, showing nests of cells surrounded by reticulin fibres (Fig. 3). In other areas the tumour cells became progressively more pleomorphic, manifest at worst as cells with one or often multiple hyperchromatic nuclei with eosinophilic nucleoli (Figs. 4, 5). Here, the tumour lost its typical *zellballen* pattern and showed large foci of necrosis (Fig. 6) as well as vascular invasion. A large number of atypical mitoses were distributed unevenly all over the tumour. Interestingly, in monomorphic as well as in pleomorphic cells, eosinophilic hyaline globules were found within the cytoplasm, sometimes closely attached to the nucleus (Fig. 7). In contrast to its macroscopic appearance, the tumour microscopically showed irregular infiltration of adjacent myometrium. The recurrences were identical in histomorphology to the primary tumour, thus confirming our diagnosis of malignant paraganglioma.

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Table 1. Primary antibodies used for immunohistochemical studies

Antibody to	Clonality	Dilution	Source
ACTH	P	1:500	Dako, Copenhagen, Denmark
Bombesin	M	1:100	Hybritech, San Diego, Calif., USA
Calcitonin	P	1:200	Milab, Malmö, Sweden
Chromogranin A	P	1:500	Incstar, Stillwater, Minn., USA
Desmin	M	1:1000	Dako
Gastrin	P	1:1000	Milab
GFAP	P	1:100	Medac, Hamburg, FRG
Glucagon	M	1:50	Novo, Bagsvaerd, Denmark
GRP	M	1:500	Hybritech
Insulin	M	1:500	Novo
Leu-encephalin	P	1:500	Incstar
Leu 7	M	1:100	Becton Dickinson, Mountain View, Calif., USA
Met-encephalin	P	1:500	Incstar
NSE	M	1:20	Innogenetics, Antwerpen, Belgium
Neurofilaments	M	1:3	Milab
Neurotensin	P	1:5000	Incstar
Pancreatic polypeptide	P	1:5000	Dako
Pan-cytokeratin	M	1:20	Boehringer, Mannheim, FRG
PGP 9.5	P	1:500	Ultraclone, Cambridge, UK
S-100 protein	P	1:500	Dako
Serotonin	M	1:500	Dako
Somatostatin	M	1:50	Novo
Substance P	P	1:100	Sera Lab, Vienna, Austria
Synaptophysin	P	1:5000	Max Planck Institute, Munich, FRG
Ubiquitin	P	1:2	Cambridge Res. Bioch., UK
Vimentin	P	1:20	Eurodiagnostic, Apeldoorn, The Netherlands
VIP	P	1:100	Ortho, Neckargemünd, FRG

ACTH, Adrenocorticotropic hormone; GFAP, glial fibrillary acidic protein; GRP, Gastrin releasing peptide (= Bombesin); NSE, neuron-specific enolase; PGP, protein gene product; VIP, vasoactive intestinal polypeptide

Immunohistochemically many tumour cells showed diffuse intracytoplasmic staining for neuron-specific enolase (NSE) (Fig. 8) and protein gene product 9.5 (PGP 9.5). Synaptophysin could be demonstrated only in single cells (Fig. 9). However, pleomorphic cells were never decorated. No immunoreactivity could be observed with the other antibodies used. In particular, the intracytoplasmic hyaline globules did not react with any antibody.

By electron microscopy the tumour was found to be composed exclusively of chief cells, whereas sustentacular cells could not be identified. The cell membranes were straight and closely opposed to each other by a few small desmosome-like junctions. The periphery of tumour cell clusters was separated from the stroma by a continuous basal lamina (Fig. 10, inset lower left). According to the electron density of the cytoplasm, which reflected a varying amount of rough endoplasmic reticulum, the chief cells were divided into "light" and "dark" types. A minority of these cells contained occasional granules 180 nm in diameter, which were composed of an electron-dense core surrounded by a distinct halo beneath an enveloping membrane (Fig. 10, inset upper right). Most interesting was the presence of intracytoplasmic large spherical, non-membrane-bound "filamentous" bodies (Fig. 10). Most filaments were 6–8 nm (some 25 nm) in diameter and lacked periodicity in the longitudinal dimension. For the most part the filaments formed rosette-like aggregates of larger size with a diameter of 50 nm on average (Fig. 10, inset lower right). Moreover, very rarely, cilia could be detected, which revealed an unusual 9 + 0 configuration (Fig. 10, inset upper left).

Discussion

Paragangliomas of the uterus are extremely rare tumours; only three have been described previously (Young and Thrasher 1982; Tavassoli 1986) and one other has been reported very briefly (Tenti et al. 1991). These lesions revealed a characteristic and diagnostically helpful *zellballen* pattern on light microscopy, which was also true for most areas of our case, which also showed enlarged round nuclei with distinct nucleoli. A differential diagnosis of alveolar soft part sarcoma (Guillou et al. 1991) and carcinoid tumour of the uterus (Post et al. 1966; Falchetti et al. 1989) was considered. Moreover, other areas of the present tumour exhibited pro-

Fig. 1. Nodular tumour within the muscle layer of the uterine corpus

Fig. 2. Typical nesting of tumour cells. H&E, ×250

Fig. 3. Characteristic *zellballen* pattern of the tumour. Gomori's stain, ×250

Fig. 4. Atypical tumour cells with enlarged nuclei and prominent nucleoli. H&E, ×420

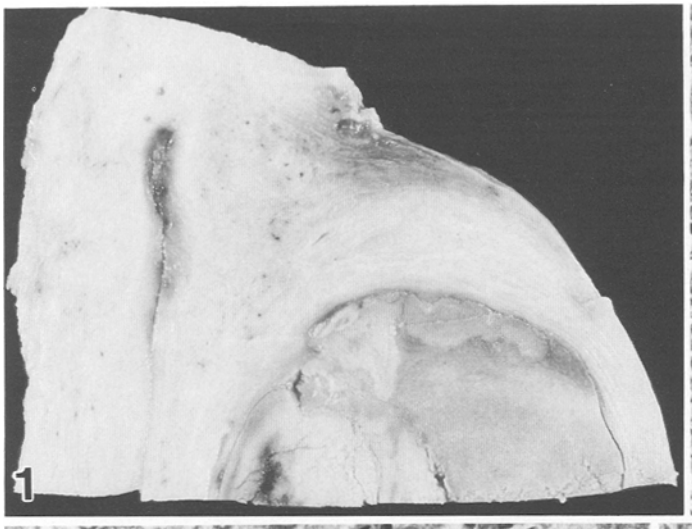
Fig. 5. Pleomorphic, multinucleated tumour cells amongst monomorphic ones. H&E, ×250

Fig. 6. Tumour necrosis surrounded by pleomorphic tumour cells. H&E, ×250

Fig. 7. Many tumour cells reveal intracytoplasmically eosinophilic hyaline globules, sometimes closely attached to the nucleus. H&E, ×480

Fig. 8. Positive immunostaining for neuron-specific enolase in many tumour cells. APAAP, ×300

Fig. 9. Synaptophysin immunoreactivity in a few neoplastic cells. APAAP, ×300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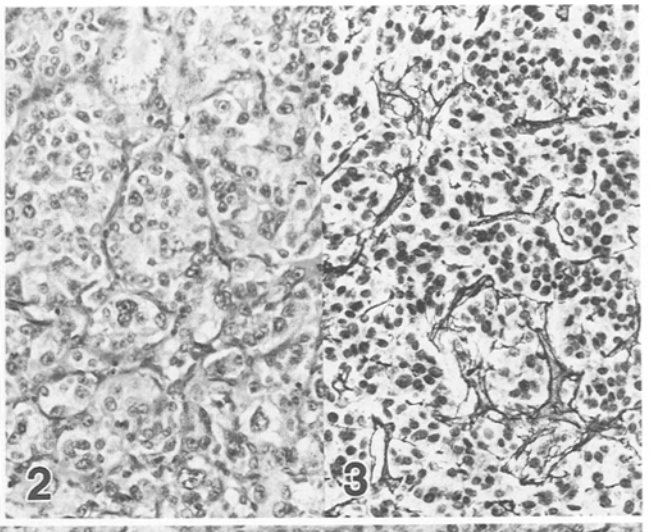
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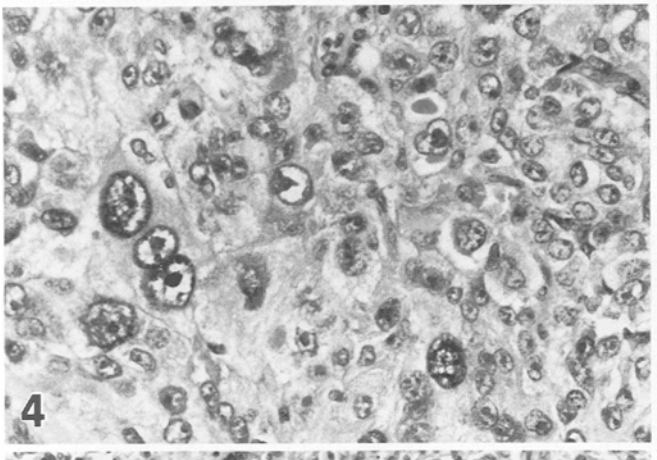
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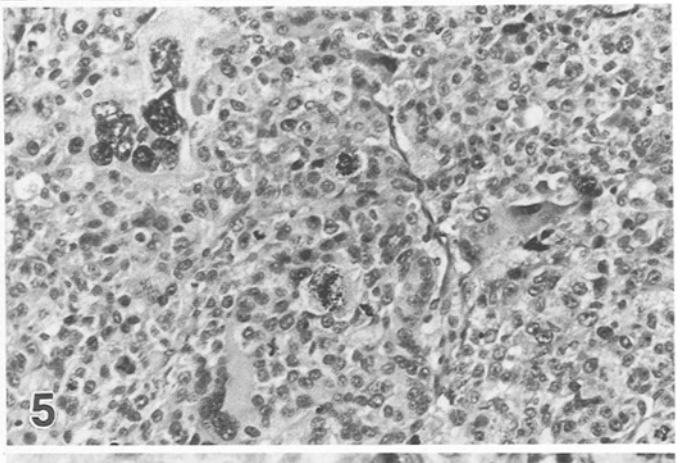
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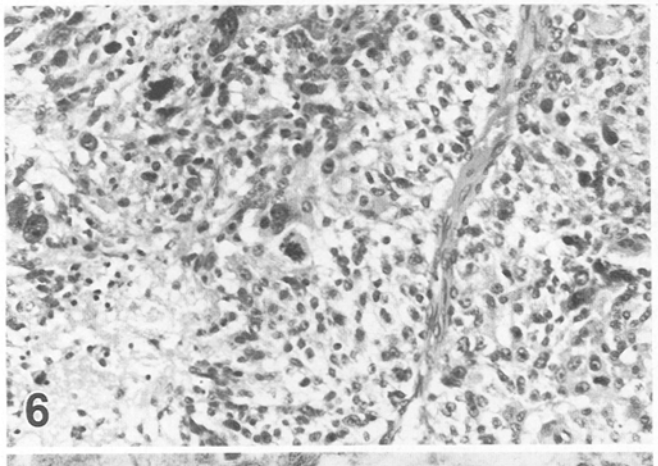
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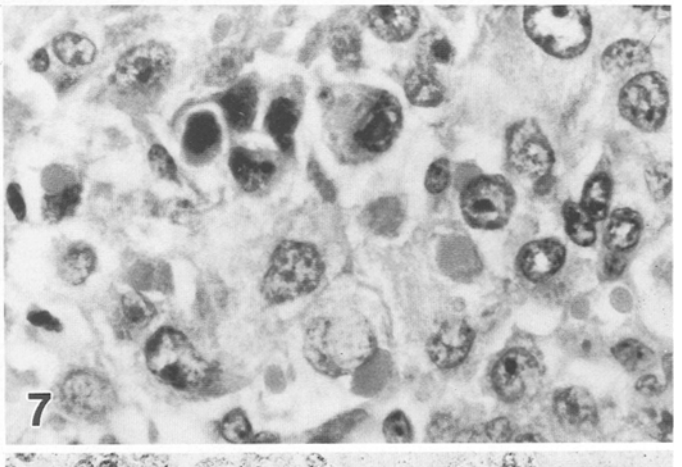
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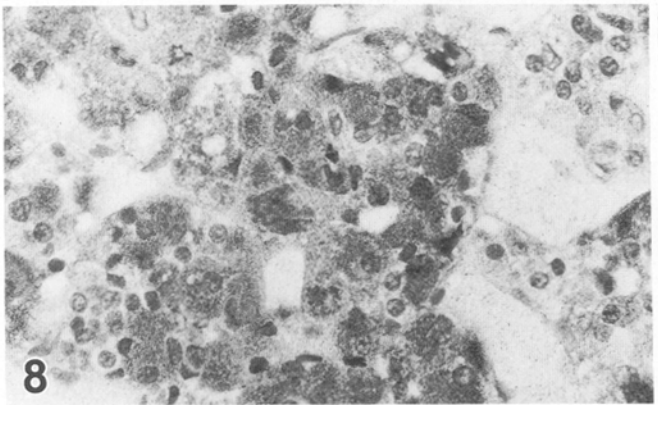
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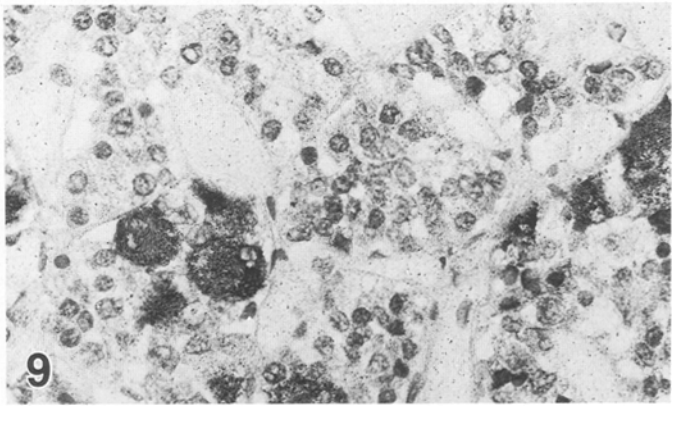
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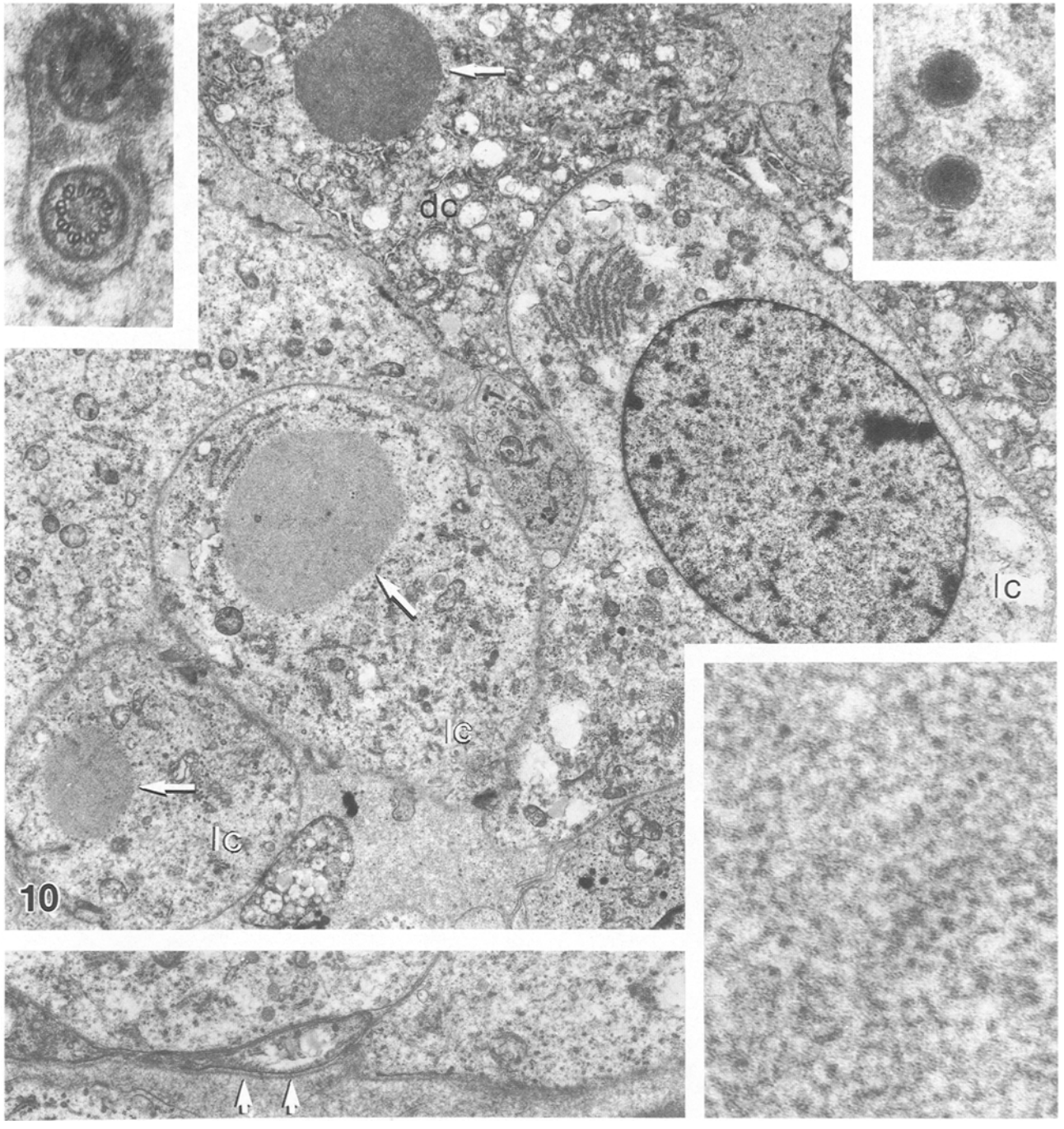

Fig. 10. Ultrastructural demonstration of "light" and "dark" chief cells (*lc* and *dc*) with intracytoplasmic hyaline globules (*arrows*), ×10200. These globules are composed of filamentous structures forming irregular rosette-like aggregates (*inset*, lower right, ×48000). Intracytoplasmically, neurosecretory granules can only rarely be found, exhibiting a characteristic electron-dense core and an enveloping membrane (*inset*, upper right, ×54000). In transverse sections of some tumour cells, cilia with a 9+0 structure surrounded by an invaginating vacuolar membrane can be seen (*inset*, upper left, ×62000). The periphery of tumour nests is separated from the stroma by a continuous basal lamina (*arrows, inset*, lower left, ×21500)

nounced pleomorphism, numerous atypical mitoses and necrosis, and had, in consequence, to be distinguished from symplasmic leiomyoma (Dworak and Meybehm 1988), high-grade leiomyosarcoma (Hart and Billman 1978) and metastasis from an extrauterine primary (Kumar and Hart 1982; Mazur et al. 1984). All these differential diagnoses except carcinoid were ruled out by immunohistochemical demonstration of NSE, PGP 9.5, chromogranin and neuropeptides (Hamid et al. 1987); Kliewer and Cochran 1989), negative immunoreaction for actin, desmin and cytokeratin, and ultrastructurally by the finding of neurosecretory granules (Hamid et al. 1987) in paragangliomas. Carcinoids are usually composed of uniform cells with nuclei rather polarized to the vascular margin of a cell nest and lack the pronounced cell pleomorphism and mitotic activity present

in our tumour. Moreover, in contrast to paragangliomas, carcinoids reveal expression of cytokeratin (Höfler and Denk 1984). The negative immunoreaction for chromogranin was in line with the very rare detection of neurosecretory granules even by electron microscopy. These granules are known to be the location of chromogranin (Varndell et al. 1985). We were able to demonstrate intracytoplasmic hyaline globules in many tumour cells, an original finding in uterine paragangliomas. Interestingly, Linnoila et al. (1990) described hyaline globules in a series of 98 paragangliomas of the sympathetic chain. These occurred more frequently in benign (59%) than in malignant (32%) tumours. At present the exact nature of hyaline globules, which can also be found in pathologically altered tissue of many other organs, is unclear. For lesions of the liver, central nervous system and skeletal muscle ubiquitin has been found to play a major role in the formation of hyaline globules (Lowe et al. 1988).

In general, the likely behaviour of paragangliomas is difficult to assess, but, in the case under discussion, malignancy was indicated by numerous atypical mitoses, necrosis and vascular invasion. Pleomorphism on its own, however, has been found not to be a criterion of malignancy (Linnoila et al. 1990). Additionally, decreased expression of neuropeptides (Linnoila et al. 1988) and a significantly reduced number of glial fibrillary acidic protein and S-100-protein-positive sustentacular cells (Kliewer et al. 1989) have been thought to correlate with a poor prognosis, which corresponds to our findings.

Judging from the published cases, including the present one and two unpublished cases from H.J. Norris (cited by Young and Thrasher 1982), paragangliomas of the uterus seem to arise in females of reproductive age with an average age of 40.8 years (range 13–51 years). Hormonal activity may have some influence on tumour development and/or growth. However, further studies on a larger number of such rare lesions are required before firm conclusions can be drawn.

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