Virchows Arch [Pathol Anat] (1982) 396:91–102

*Virchows Archiv A*
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# Primary Sarcoma of the Gallbladder

## A Light and Electronmicroscopical Study

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**Summary.** Six Swedish cases of primary sarcoma of the gallbladder from the period 1958–1973 and 1 case from 1975 were studied by light and electron microscopy. The literature was reviewed for the period after 1970. Ultrastructural features of sarcoma were investigated in order to exclude poorly differentiated carcinoma. One case was considered to be an embryonal rhabdomyosarcoma, three cases leiomyosarcoma and two were diagnosed as fibrosarcoma. One case, diagnosed as sarcoma of the gallbladder by light microscopy, was omitted because electron microscopical examination revealed a squamous cell carcinoma.

**Key words.** Primary gallblader sarcoma – Electron microscopy – Histopathology

Primary sarcoma of the gallbladder is a very rare disease. The first report of primary sarcoma of the gallbladder appeared in the last century (Schmidt 1893), but the first complete description was made by Landsteiner in 1904. Much dispute has arisen concerning its very existence (Edmondson 1967; Appelman et al. 1970). Evidence has accumulated, however, mostly recorded as single cases. In 1971, Yasama and Yanaka recorded 93 cases in the world literature and added their own three cases. Since then, few other reports have ben published (Tanga 1970; Tamakai et al. 1971; Friedland 1971; Mena et al. 1971; Vaittinen 1972; Carpentier et al. 1973; Montefusco et al. 1973; Richart et al. 1975; Rose 1978).

The aim of the present study is to present the total of registered sarcomas of the gallbladder in Sweden during the period 1958–1973 and to add 1 case of gallbladder sarcoma from our own collection, appearing in 1975 (Table 1). We also want to provide evidence of the existence of primary sarcoma of the gallbladder by an electron microscopical (TEM) investigation, which elimi-

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0340-1227/82/0396/0091/$02.40

**Table 1.** Sarcoma of the gallbladder in Sweden 1958–1973 plus one case from 1975. Summary of age, sex, clinical history, treatment, outcome, macroscopical appearance and histological diagnosis

| Case | Age | Sex | Clinical history | Treatment + outcome | Macroscopy | Histology |
|---|---|---|---|---|---|---|
| 1 | 68 | F | Gallbladder colic for 15 years jaundice 4 years ago, right upper quadrant mass | Laparotomy Died 2 months later Autopsy | Gallstones Gallbladder tumor 24 cm ∅ Secondary growth in liver | Embryonal rhabdomyosarcoma |
| 2 | 91 | F | No history of gallbladder disease | Died of heartfailure Autopsy finding | Gallstones Fundaltumor 2 cm ∅ | Leiomyosarcoma |
| 3 | 77 | M | Biliary symptoms for 20 years Gallstones, radiating right upper quadrant pain | Cholecystectomy Local recurrence No autopsy | Gallstone Gallbladder tumor 8 cm ∅ | Leiomyosarcoma |
| 4 | 72 | M | Abdominal pain, nausea weightloss, jaundice | Autopsy | Exophytic gallbladder tumor, metastases in liver and lungs | Leiomyosarcoma |
| 5 | 75 | F | Right upper quadrant mass | Laparotomy, tumor excision Died 12 months later. No autopsy | Gallstones Gallbladder tumor 15 cm ∅ | Fibrosarcoma |
| 6 | 64 | M | Gallstones, cholecystitis last 10 years cholestatic jaundice | Cholecystectomy Local recurrence Radiotherapy Died 4 months postop. Autopsy | Gallstones Metastases in pancreas, retroperitoneum and brain | Fibrosarcoma |

**Table 2.** Reported histological types of primary gallbladder sarcoma up to 1980. Compiled from ref. [8, 27], Table 2

| | | | |
|---|---|---|---|
| Spindle cell sarcoma | 35 | Angiogenic sarcoma | 9 |
| Round cell sarcoma | 5 | Melanosarcoma | 2 |
| Pleomorphic cell sarcoma | 15 | Neurogenic sarcoma | 1 |
| Myogenic cell sarcoma | 4 | Osteosarcoma | 1 |
| Leiomyosarcoma | 13 | Myxosarcoma | 2 |
| Rhabdomyosarcoma | 6 | Liposarcoma | 1 |
| Fibrosarcoma | 8 | Alveolar sarcoma | 1 |
| Lymphosarcoma | 7 | Fusocellular sarcoma | 1 |
| Giant cell sarcoma | 1 | Chondrosarcoma | ? |
| Reticulum cell sarcoma | 6 | | |
| | | Total | 118 |

nates the possibility of confusion with anaplastic carcinoma. We also summarize the different varieties of reported sarcomas (Table 2).

## Methods

For light microscopy the material was processed in a standard way with formalin fixation, paraffin embedding and staining with hematoxylin and eosin, Werlhoff-van Gieson, and PAS (periodic

acid Schiff), Oil-red-O (ORO), PTAH and Alcian-blue (pH 2.5). For transmission electron microscopy, paraffin-embedded material was used in 5 cases and fresh autopsy material in 1 case. The paraffin-embedded material was cut in 20–30 μ slices and deparaffinized. The tumor area was microdissected under the phase-contrast microscope without staining (Hultquist and Karlsson 1972). In every case 20–30 different areas were studied (Mean 27). The material was then post-fixed in 2% glutaraldehyde in 0.1% cacodylate and 0.1% sucrose, pH 7.2 counterstained in uranyl acetate, embedded in Epon, cut and stained in lead citrate (Brunk and Eriksson 1972). The grids were analyzed in a Jeol-100-C electron microscope. For every case more than 200 fields were studied and more than 50 photographs taken. The fresh material from case 2 was immediately dissected under the dissection microscope, fixed in 2% glutaraldehyde as above and then processed as the rest of the material.

## Clinical Material

Clinical data are given in Table 1.

*Light Microscopy*

*Case No. 1.* In autopsy material an anaplastic tumor with a tremendous variation in cell size was seen (Fig. 1). Most of the cells had a clear vacuolated cytoplasm, while some had a strongly eosinophilic cytoplasm. The nuclei showed coarse irregular chromatin and mitoses, sometimes with abnormal forms. Many cells had more than one nucleus clumped into the center. Some cells were globular with cytoplasmic membranes dividing the cytoplasm into different compartments. The PTAH-staining method revealed cross striation within some of the cells. The interstitial tissue was slightly blue in the Alcian-blue method. Fat staining was negative. The histopathological diagnosis was an embryonal sarcoma, most probably rhabdomyosarcoma.

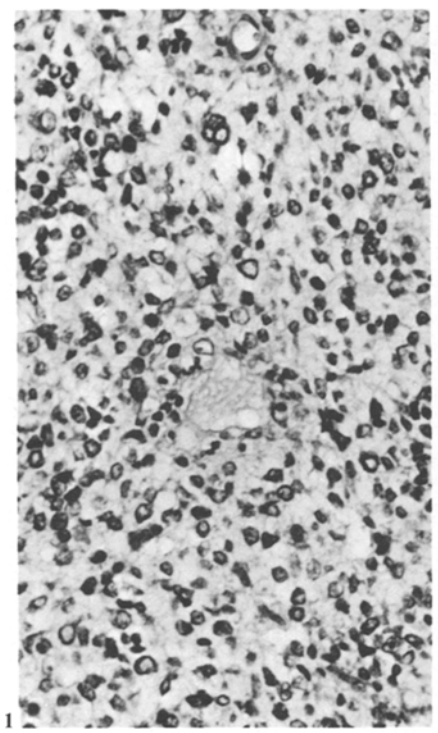
*Case No. 2.* The gallbladder from the autopsy showed features of chronic cholecystitis including foreign body-type giant cells around cholesterol clefts as well as cholesterol granulomas. The tumor (Fig. 3) was built up of elongated spindle-shaped smooth muscle fibers with enlarged hyperchromatic nuclei. Tumor giant cells were numerous. The muscle fibers in most parts of the tumor were arranged haphazardly or in bundles running at right angles to each other. There were 1–3 mitoses per high power field. The tumor mass was formed mainly of fibers which stained yellow with the van Gieson method. Staining with PTAH showed no cross striation. Fat and mucin stains were negative. The histopathological diagnosis was leiomyosarcoma.

*Case No. 3 and 4.* The microscopical picture of the surgically removed tumor was similar to that described in case No. 2. The tumor was rather rich in very thin walled blood capillaries which were arranged haphazardly in the tumor mass. The mitotic activity was very obvious, with many abnormal mitotic figures. The tumor was heavily inflamed (Fig. 4). Autopsy material from case No. 4 showed essentially the same morphological patterns. These cases were considered to be leomyosarcomas.

*Case No. 5 and 6.* Histological sections of the surgically removed gallbladder tumor from case No. 5 (Fig. 5) showed elongated cells with spindle-shaped nuclei. The nuclei were hyperchromatic with very rich mitotic activity. The tumor growth was mostly in whorls with areas of pallisading and with a storiform pattern. The capsule and the liver parenchyma showed tumor infiltrates. Case No. 6 showed a similar picture. Histologically, the tumors were fibrosarcomas.

## Electron Microscopy

At the electron microscopic level in case No. 1, the cells were heterogenous in their ultrastructure, lying singly or in close apposition to one another without junctional attachments. Several types of nuclei could be seen. One (Fig. 6) was large, light, with lobulation, mostly centrally placed in the cytoplasm. Chromatin



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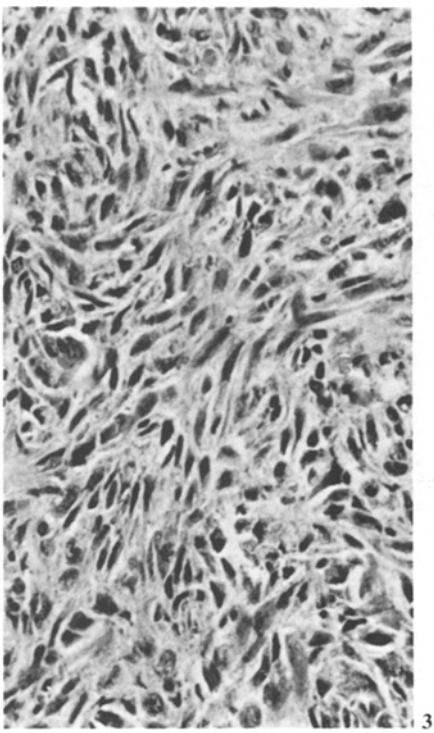

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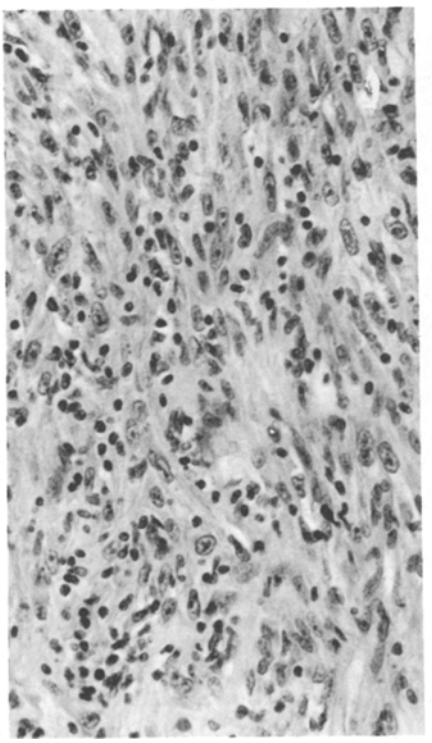

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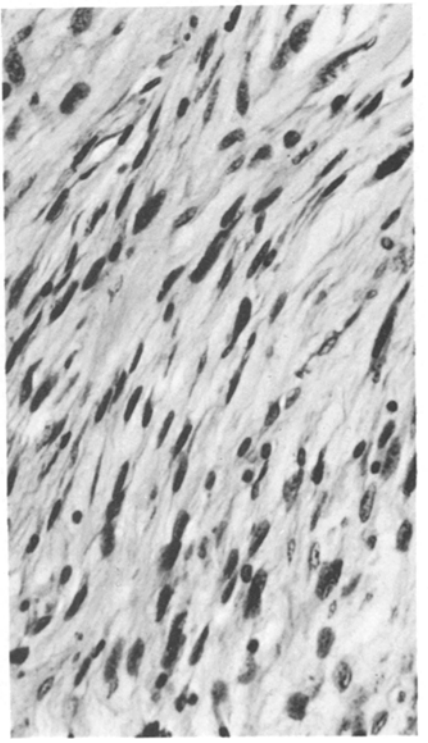

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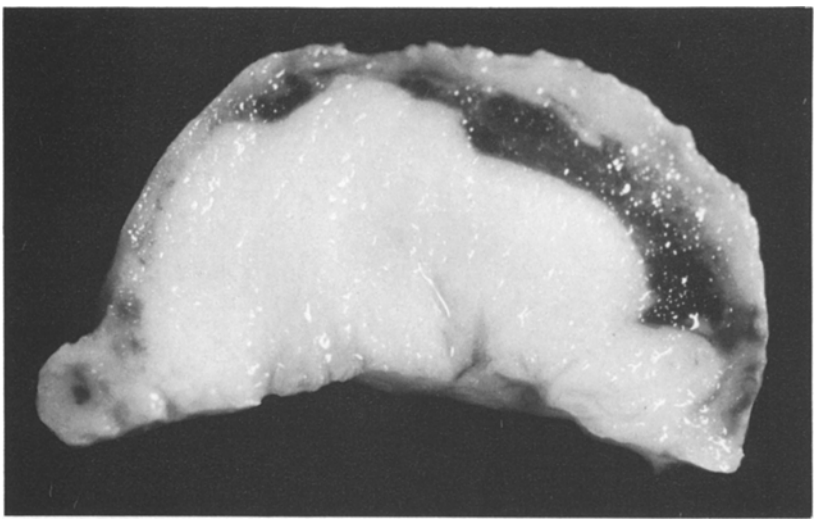

**Fig. 2.** Macrophotograph from fundal tumor of case No. 2 with a greyish-white solid neoplasm and focal necrosis (×6)

was mostly situated at the border of the nuclei. In this cell type numerous filaments could be seen, sometimes haphazardly distributed within the cytoplasm, sometimes orientated in bundles along the longitudinal axis of the cell. It was possible to find both thin (6–8 nm) and thick (10–15 nm) filaments, clear Z-bands and in between something that might be partly remaining or developed I- and A-bands (Fig. 7). Intermingled with these filaments were seen small round particles, probably representing RNA-particles. In other parts of the cytoplasm mitochondria and both free and rough endoplasmic reticulum were encountered. Another cell type (Fig. 8) contained a more ovoid or elongated texture with a round, dense, dark nuclei, containing one or several well outlined nuclei. The cytoplasm contained scanty mitochondria, rough endoplasmic reticulum, glycogen and amorphous substances. A small number of microtubules was also seen but no cross striation or dense filaments as observed in the first type of cell. Cells with a fibroblast like appearance with distended endoplasmic cisterna also appeared. The cytoplasm in this latter cell contained some mitochondria and filaments both thick (10–15 nm) and thin (6–8 nm) type, as well as glycogen particles (Fig. 9). The case was considered to be an embryonal rhabdomyosarcoma.

**Fig. 1.** Photomicrograph of embryonal rhabdomyosarcoma with a polymorphic round cell configuration. From case No. 1 (×250)

**Fig. 3.** Photomicrograph of a leiomyosarcoma with polymorph spindle-shaped smooth muscle fibers and several tumor giant cells. From case No. 2 (×250)

**Fig. 4.** Photomicrograph of leiomyosarcoma with elongated spindle-shaped smooth muscle fibers and heavy lymphocytic inflammation. From case No. 3 (×250)

**Fig. 5.** Photomicrograph of a fibrosarcoma with elongated cells and spindle-shaped nuclei running in parallel bundles. From case No. 5 (×250)

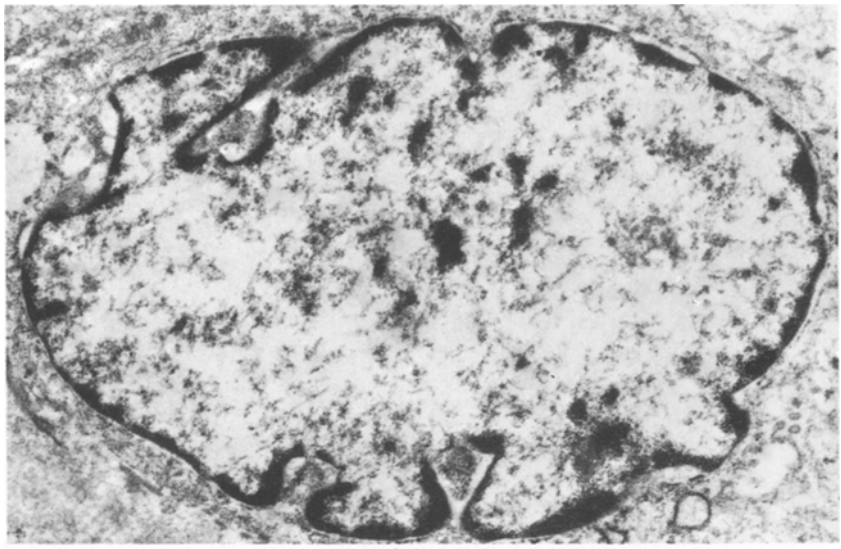

**Fig. 6.** Electron micrograph of large light lobulated nuclei in an embryonal rhabdomyosarcoma. From case No. 1 (orig. mag. ×10,000)

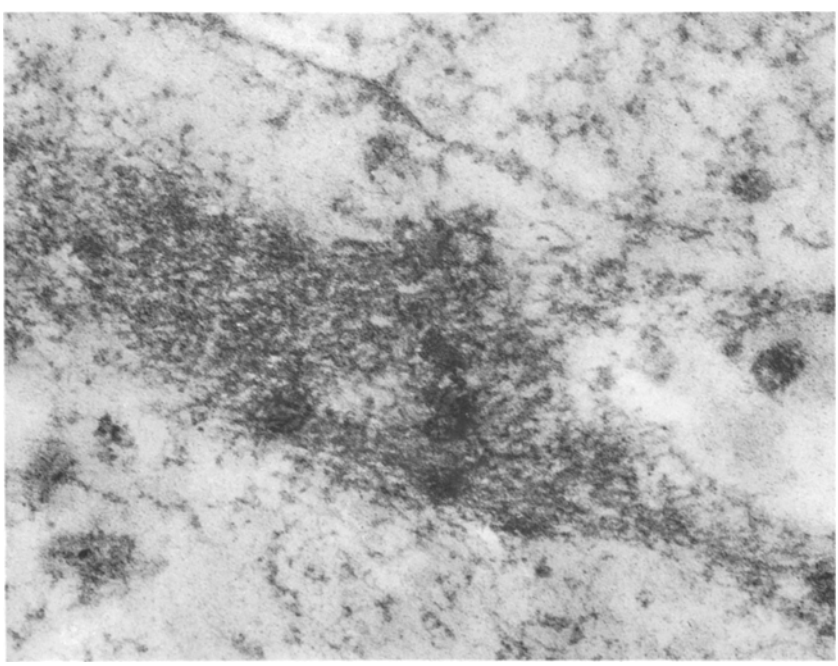

**Fig. 7.** Electron micrograph of embryonal rhabdomyosarcoma with thin and thick myofilaments with focal periodicity (Z-bands). From case No. 1 (orig. mag. ×33,000)

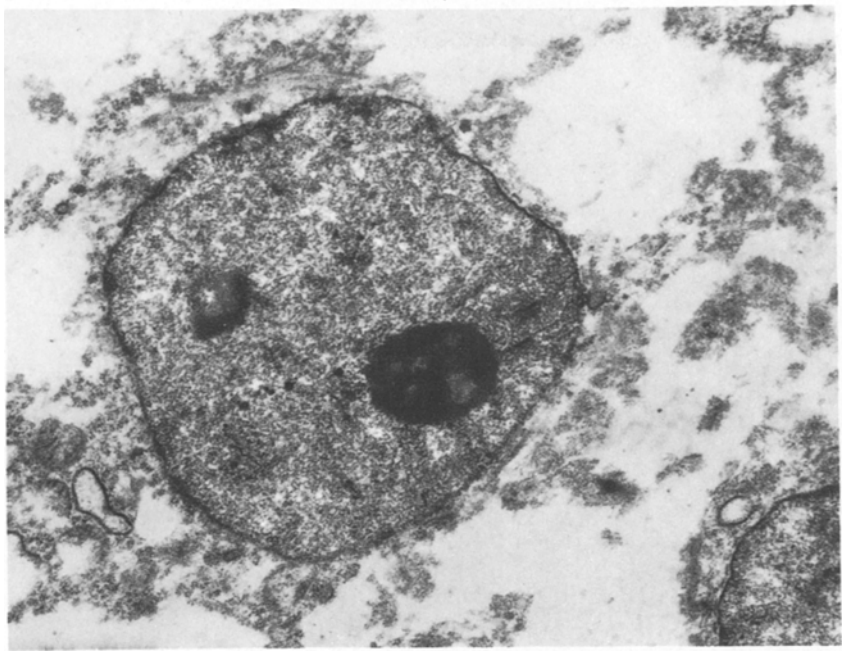

**Fig. 8.** Electron micrograph from embryonal rhabdomyosarcoma with rounded dark cell containing several dense nucleoli. From case No. 1 (orig. mag. ×10,000)

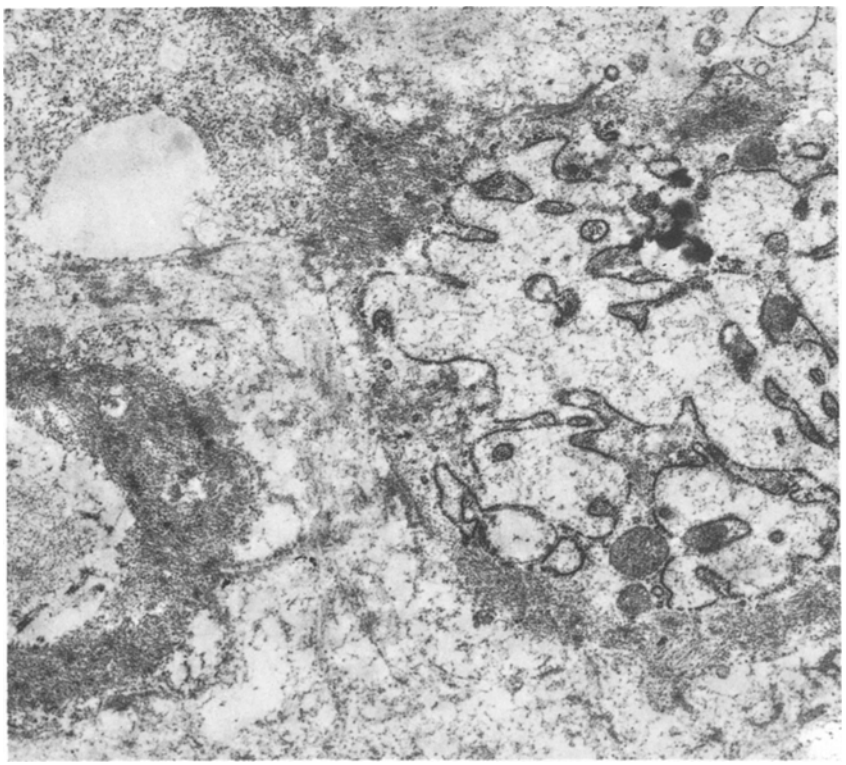

**Fig. 9.** Electron micrograph from embryonal rhabdomyosarcoma with a fibroblastic cell and a dark cell. In between, within the cytoplasm, thick and thin filaments with periodicity of Z-bands. From case No. 1 (orig. mag. ×10,000)

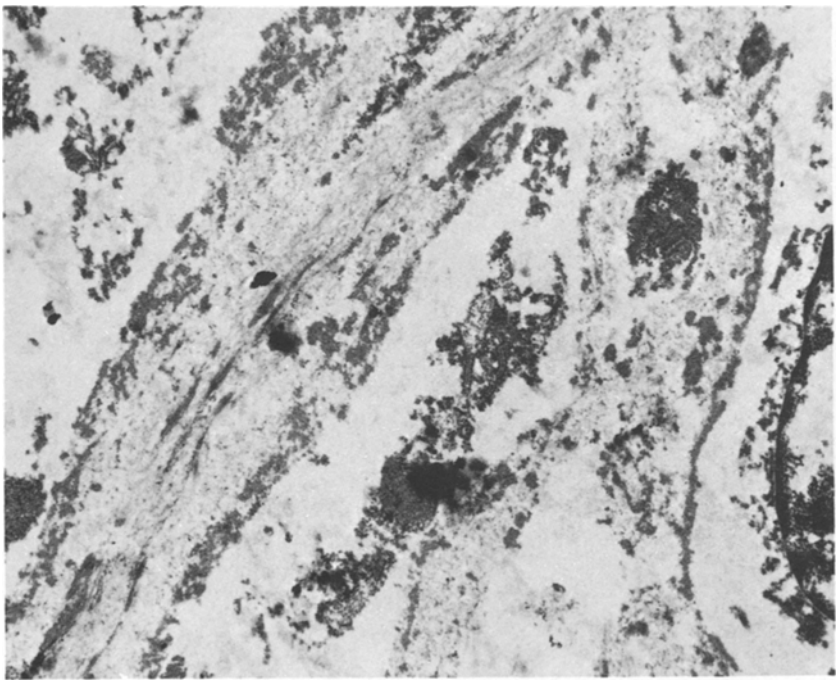

**Fig. 10.** Electron micrograph from leiomyosarcoma containing elongated or ovoid nuclei. Along the cell border are bundles of myofilaments showing focal periodicity. From case No. 2 (orig. mag. × 5,000)

In the cases Nos. 2, 3 and 4 the cells were elongated or ovoid with indentation and folds of the nuclear membrane. The chromatin mainly outlined the nuclear border or was randomly dispersed within the nuclei. The cytoplasm contained an abundance of free ribosomes and a smaller amount of mitochondria and rough endoplasmic reticulum. Along the cell border, bundles of myofilaments with focal periodicity were seen (Fig. 10). No clear cut desmosomes were observed. The interstitial matrix was composed of fine amorphous material, scanty collagen bundles or other thin fibrils. Pinocytosis could be traced only occasionally. These cases were considered to be leiomyosarcomas.

Cases Nos. 5 and 6 showed spindle-shaped elongated cells with cigar formed nuclei, mostly diffusely distributed chromatin, but also a quite substantial amount of heterochromatin along the nuclear border (Fig. 11). One or several nucleoli were observed, often quite compact in outline. The cytoplasm was dominated by a proliferation of rough surface endoplasmic reticulum, mostly situated at the polar edge of the nuclei. The cytoplasm often contained dilatated cisternae with ribosomes and polysomes. In the interstitium, collagen fibers were rich in number and often in close apposition to the fibrocytic cell. Sometimes more polymorphic fibroblastic cells were seen with large cell processes, distended cisterna and evident Golgi-complexes. No outer membrane was observed and no clear cut desmosomes (Fig. 12). These two cases were diagnosed as fibrosarcomas.

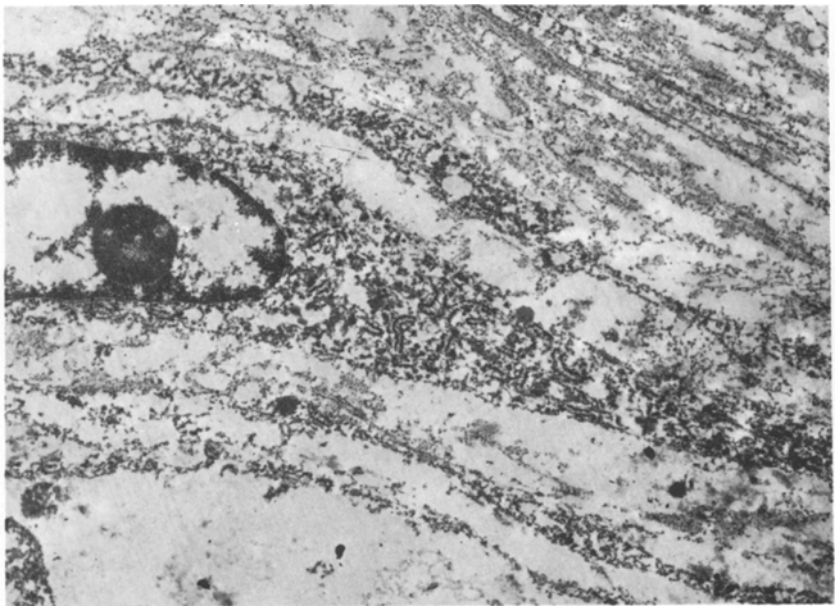

**Fig. 11.** Electron micrograph from fibrosarcoma with spindle-shaped elongated cells containing cigar-formed nuclei and a substantial amount of rough surface endoplasmic reticulum, mostly situated at the polar edge of the nuclei. From case No. 5 (orig. mag. ×5,000)

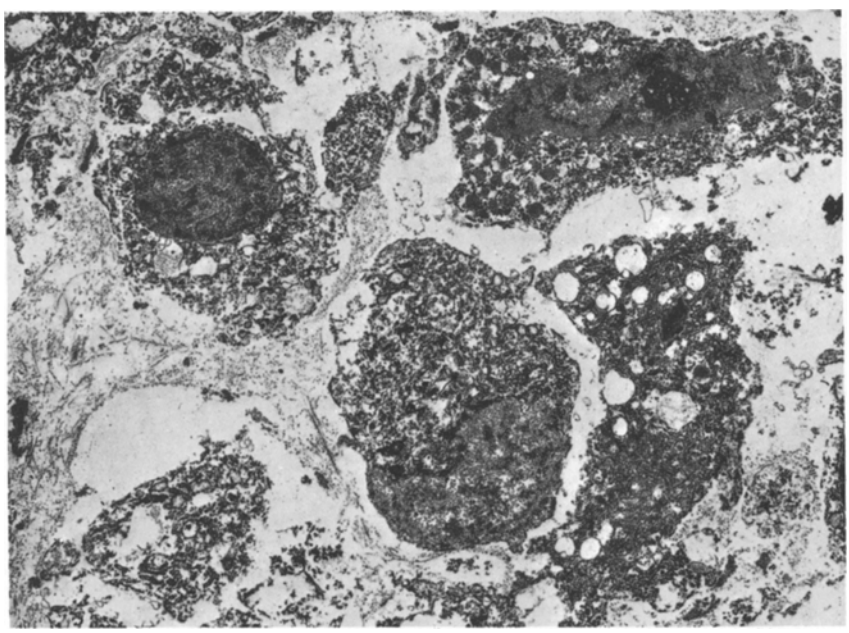

**Fig. 12.** Electron micrograph of fibrosarcoma with polymorphic fibroblastic cells exhibiting cytoplasmic processes, distended cisterna and Golgi-complexes. From case No. 5 (orig. mag. ×2,600)

## Discussion

Sarcoma of the gallbladder is an uncommon entity. The total number of documented primary sarcomas of the gallbladder exceeds 100 cases in the world literature which has been reviewed twice within the last ten years (Yasuma et al. 1971; Vaittinen 1972). The youngest patient was 17 (Richart et al. 1975) and the oldest was 86 years old (Vaittinen 1972). The disease was more frequent in women (Yasuma et al. 1971; Vaittinen 1972). Gallstones were present in 79% of all cases (Vaittinen 1972). We report 4 females and 2 males, the oldest patient was 91 and the youngest was 64 years old (average age 74,5 years). In our study, gallstones occured in 5 of 6 cases (83%).

The Swedish Cancer Registry has been operating since 1958, but it was not until 1970 that special sites in the Registry were available enabling subdivision of the extrahepatic bile duct system. During the period 1970–1973 two cases out of 1,411 malignancies were sarcomas, thus comprising a frequency of 1.4 per one thousand (The cancer registry: Cancer incidence in Sweden 1959–1973).

The histogenesis of sarcoma in the gallbladder is not clear. Sarcomas may arise from an aberrant tissue component, normally absent, such as striated muscle (Yasuma et al. 1971).

Gallstones and chronic inflammatory changes have been considered to be promoting factors in the pathogenesis of gallbladder sarcoma (Yasuma et al. 1971; Vaittinen 1972; Carpentier et al. 1973). It is conceivable that fibrosarcoma may develop as a result of chronic inflammation of the gallbladder because of prolonged fibroblastic proliferation.

The *diagnosis of sarcoma of the gallbladder* is usually impossible at admission and is obtained from patients either at the time of operation or at necropsy.

*Histologically,* different types of sarcoma of the gallbladder were reported in the literature (Table 2). To the best of our knowledge, the diagnosis of sarcoma of the gallbladder has never been supported by transmission electron microscopy (TEM) investigation in published cases. The spindle-cell sarcoma is most often reported together with pleomorphic cell sarcoma and leiomyosarcoma (Yasuma et al. 1971). We believe that a number of fibrosarcomas and leiomyosarcomas were described as spindle-cell sarcomas. It seems likely that a number of rhabdomyosarcomas have been diagnosed as pleomorphic cell sarcoma (Yasuma et al. 1971). Edmonson (1967) added one unpublished case of rhabdomyosarcoma and also put forward the possible existence of a chondrosarcoma of the gallbladder. We have not, however, been able to trace such a case in the world literature. Appelman et al. (1970) reported two cases of an unusual pleomorphic spindle-cell form of carcinoma of the gallbladder and discussed the possible relation to sarcoma of the gallbladder. Microscopic features of this tumor included extensive round and spindle-cell proliferation without stromal differentiation.

Before the diagnosis of a primary sarcoma of the gallbladder can be made, a pleomorphic spindle-cell carcinoma or poorly differentiated adenocarcinoma must be excluded. This is only possible with the TEM technique (Appleman et al. 1970). We found one case of malignant tumor of the gallbladder from the Swedish Cancer Registry, diagnosed as sarcoma, which had to be omitted

because autopsy examination and an electron microscopic investigation revealed a squamous cell carcinoma.

Electron microscopic studies from tissue fixed in ordinary 10% formalin and/or paraffin embedded material has been an established technique for several decades (Lehner et al. 1966; Zeitoun et al. 1970; Hultquist et al. 1972; Johannessen 1977; Ghadially 1980; Sobel et al. 1980). In spite of formalin fixation and paraffin embedding, it was surprising how much the overall preservation permitted an ultrastructural analysis. Nuclei and subcellular structures such as fibrillar components were often well preservered.

The case of embryonal rhabdomyosarcoma showed a very plemorphic picture in TEM with different types of nuclei and filaments of both thin (6–8 nm) and thick (10–15 nm) type. The bundles were oriented along the long axis, with clear Z-band and also slightly outlined I- and A-bands. No outer membrane was seen, nor were tight junctions observed. In light microscopy, the tumor had a round cell structure with light and dark cells, thus giving a pleomorphic picture. It was also possible to find cross striation at that level. In electron microscopy, the number of filaments was, however, quite substantial and it was possible to further subdivide the elements involved in the tumor. The picture is in keeping with early reports of an embryonal rhabdomyosarcoma (Kroll 1967; Morales et al. 1972; Sarkar et al. 1973; Amemiya et al. 1975; Ghadially 1980).

The cellular configuration in cases Nos. 2, 3 and 4 with myofibrillar densities along the periphery of the cell, running along the long axis of the cell, made us believe that these cases were leiomyosarcomas. The cellular outline is consistent with what has been previously described (Ferenczy et al. 1971; Tobon et al. 1973; Böcker et al. 1975; Morales et al. 1975; Ghadially 1980).

Two cases, Nos. 5 and 6, showed the evident characteristics of fibrosarcomas (Ross 1968; Crocker et al. 1969; Stiller et al. 1975; Churg et al. 1977; Ghadially 1980) with both fibrocytelike and fibroblastic cells. They contained abundant rough endoplasmic reticulum, widened cisterna and a rich amount of collagen fibres.

Sarcomas of the gallbladder, like other malignant tumors of mesenchymal origin, are often very aggressive with a poor prognosis, in spite of extensive surgical treatment, radiotheraphy or chemotherapy (Yasuma et al. 1971; Vaittinen 1972; Carpentier et al. 1973). The tumor is, however, not always incurable. Vaittinen (1972) reported one case with 5 years postoperative survival.

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Accepted March 3, 1982