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# Electron Microscopy of Uterine Leiomyosarcomas

W. Böcker and H. Strecker

Abteilung für Gynäkologische Histopathologie und Elektronenmikroskopie der Universitäts-frauenklinik (Abt.-Vorst.: Prof. Dr. H.-E. Stegner) und Pathologisches Institut der Universität Hamburg (Direktor: Prof. Dr. G. Seifert)

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*Summary.* Electron microscopic studies of uterine leiomyosarcomas disclose a wide range of differentiation in the neoplastic cells. According to the cytoplasmic appearance undifferentiated, myoblastic, and fibroblast-like cells can be distinguished. Derivation of these cells from mesenchymal cells or poorly developed smooth muscle cells of the myometrium is suggested by the finding of numerous variously differentiated intermediate cell types. The cytoplasm of more differentiated tumour cells usually contains myofilament bundles. Three types of filaments are detected: small, intermediate and thick ones. While the thin ones probably are actin filaments, the intermediate and thick filaments are suggested to represent nonspecific filaments described in a variety of other cell types. Fibroblastic cell types are more often encountered and are usually better differentiated in the premenopausal woman. The possible role of endogeneous hormonal factors on the differentiation process of tumour cells is discussed.

## Introduction

The light microscopic appearance and the diagnostic criteria of uterine leiomyosarcomas have been extensively discussed in previous publications (Hall, 1971; Kempson and Bari, 1969; Montague *et al.*, 1965; Silverberg, 1971; Taylor and Norris, 1966). According to these authors the presence of invasive growth, the number of mitoses, and the degree of cellular abnormalities are the most important criteria on which to base the diagnosis of sarcoma.

Up to this time little is known about the ultrastructure and about the cytoplasmic differentiation of leiomyosarcoma cells. In the present study two leiomyosarcomas were analysed with the aim to provide informations to the following aspects:

1. general ultrastructure of leiomyosarcoma cells;
2. cellular differentiation and filament system;
3. ultrastructural differences in dependence on the hormonal status of the patient.

## Material and Methods

### *Clinical Material*

Biopsy samples of tumour tissue were obtained from two patients referred to the gynecologic department of the university of Hamburg.

*Case 1.* This 37 year old female had a laparotomy and total hysterectomy because of an uterine leiomyosarcoma. Five months later she was admitted to our hospital because of multiple pelvic tumour nodules and infiltration of the right parametrium. Physical examination revealed additional cutaneous metastases in the abdominal wall and a lung metastasis, about 4 cm in diameter. One of the cutaneous metastases was excised. Half of the tumour was used for light and electron microscopic investigation. Additionally the resistance of

tumour cells to several cytostatic agents was tested in cell cultures. According to these results the patient was treated by intraperitoneal injections of Trenimon® (total 1 mg) and Endoxan® (total 1 g). The patient continued to deteriorate and expired two months later with generalized peritoneal carcinosis and widespread metastasis of the right lung. No autopsy was performed.

*Case 2.* This patient, a 68 year old postmenopausal woman, presented a large pelvic tumour, which had developed six months after supracervical hysterectomy and bilateral salpingo-oophorectomy because of multiple "uterine leiomyomas" at another hospital. A second laparotomy was performed with excision of the tumour including a small lamella of the upper vagina. At this time no lymph node metastases were found. Following the operation the patient received 6000 R of external radiation to the pelvis. No follow up.

### *Histologic and Electron Microscopic Investigation*

Tumour tissue for histologic examination was fixed in formalin and embedded in paraffin. Sections were stained with hematoylin-eosin, periodic acid-Schiff, Masson trichrome and van Gieson stains. *Mitotic counts* were made on a binocular Leitz microscope using a 40 X high objective and 12.5-X wide eypieces. Mitoses were counted in the most active area by determining the number of 4 sets of 10 high power fields (HPF). For electron microscopy small pieces of tissue were fixed at 4° C by immersion for 2–3 hours in 2.5% phosphate buffered glutaraldehyde, ph 7.4. The pieces were postfixed in 1% osmium tetroxide, dehydrated in alcohol and embedded in Westopal W. Semithin sections were cut with diamond knifes, mounted on noncoated grids, stained with uranyl acetate and lead citrate and examined with a Zeiss EM 9A electron microscope.

## Results

### *Histologic Examination*

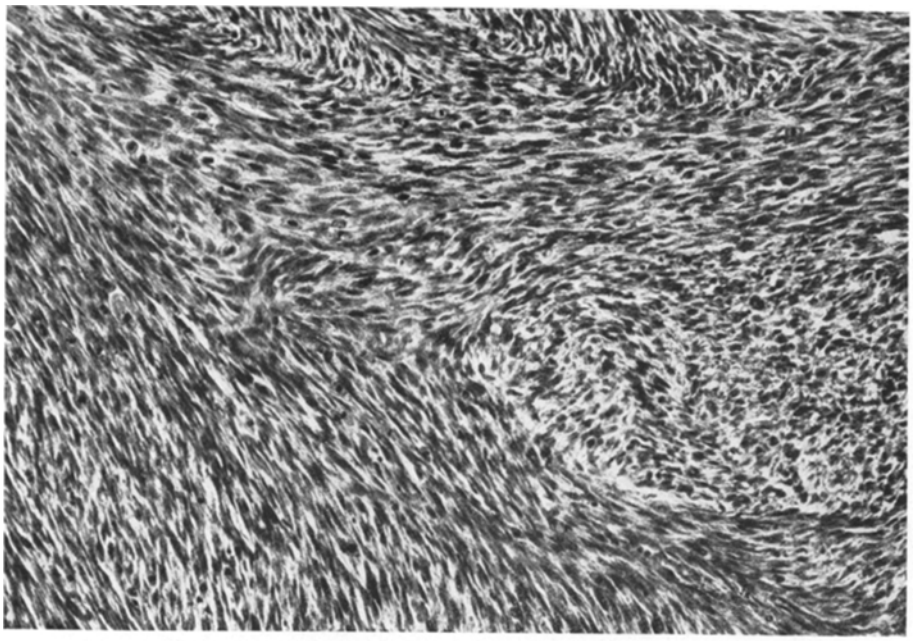
In microscopic appearance, both tumours varied considerably on the basis of cellular atypia, pleomorphism, and fascicular differentiation (see Table 1).

Table 1. Comparison of light microscopic features and menstrual status of two cases of leiomyosarcomas

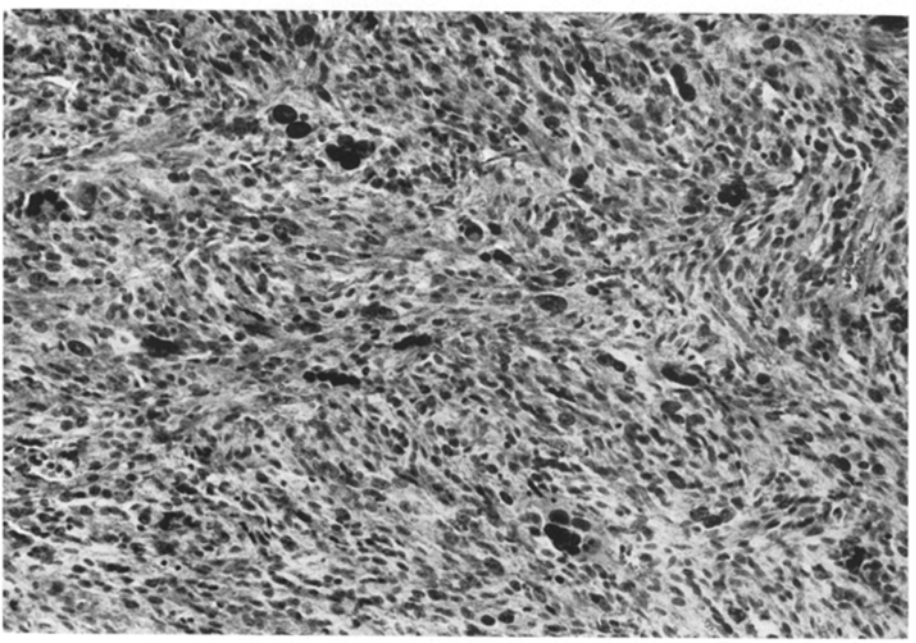
| Case | Menstrual status | Fascicular architect. | Mitotic rate[a] | Cytologic atypia | Tumour giant cells |
|---|---|---|---|---|---|
| 1 | premeno-pausal | moderately preserved | 112 | little | none |
| 2 | postme-nopausal | destroyed | 67 | moderate | many |

[a] Mitoses/10 HPF.

In case 1 the low power architecture (Fig. 1a) was that of a cellular muscular neoplasm with moderately preserved fascicular arrangement of tumour cells. The outstanding feature of this tumour was its extremely high mitotic activity combined with a clearly invasive growth. In case 2 the fascicular architecture was generally lost (Fig. 1b). Numerous tumour giant cells with partly hyperchromatic nuclei were seen. The number of mitoses was, compared with the reports in the literature, still very high but only half of that seen in case 1.



a



b

Fig. 1 a and b. Two cases of leiomyosarcomas. (a) Case 1. Leiomyosarcoma with well preserved fascicular architecture and a great number of mitoses. ×250. (b) Case 2. Leiomyosarcoma with numerous tumour giant cells and nuclear pleomorphism. Fascicular architecture destroyed. ×250

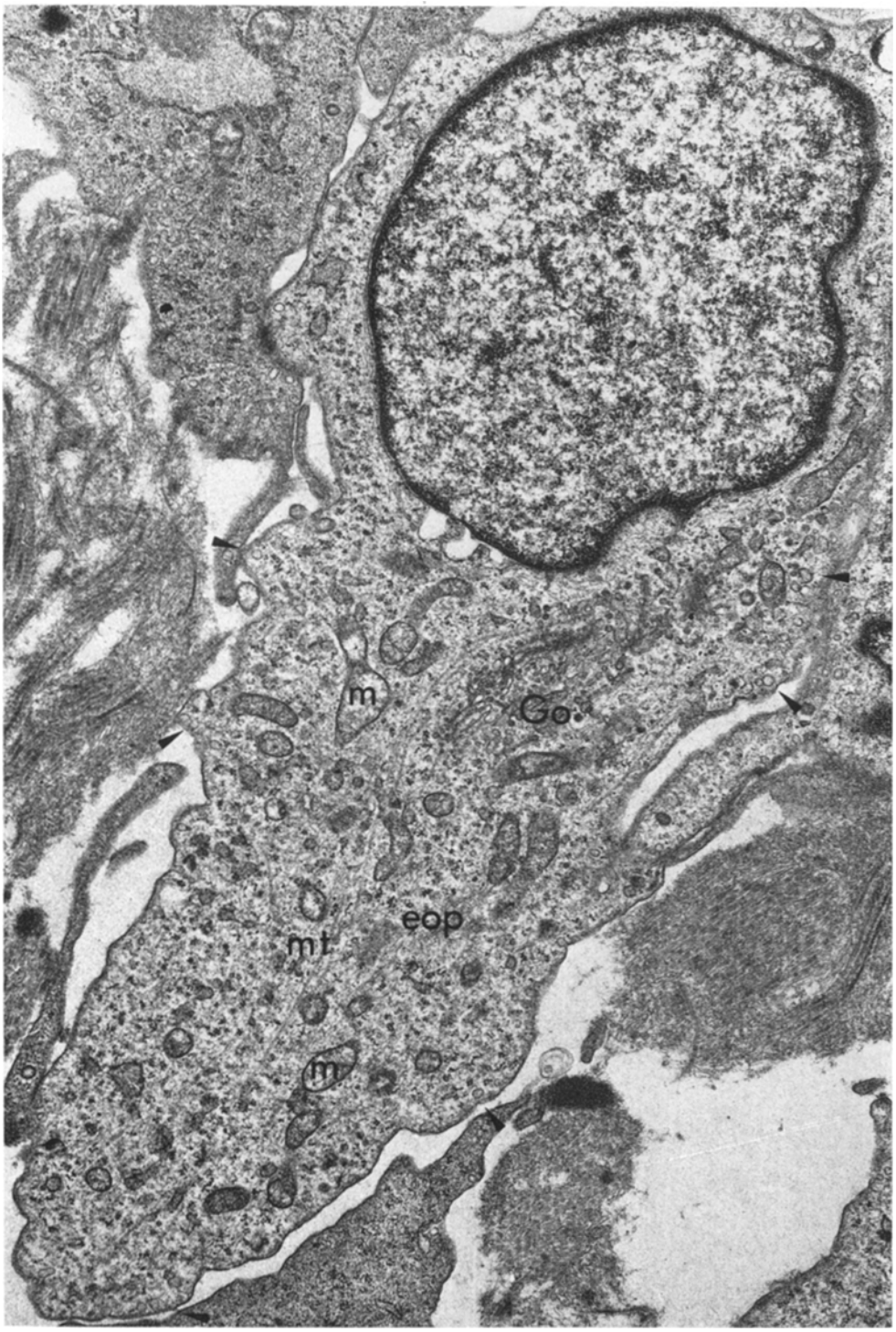



Fig. 2. Immature tumour cell with numerous free ribosomes, a moderately developed Golgi complex (*Go*), microtubules (*mt*), and scattered mitochondriae (*m*). Note numerous vesicles at the surface of the cell (arrows) and a few electron opaque areas (*eop*). ×14770

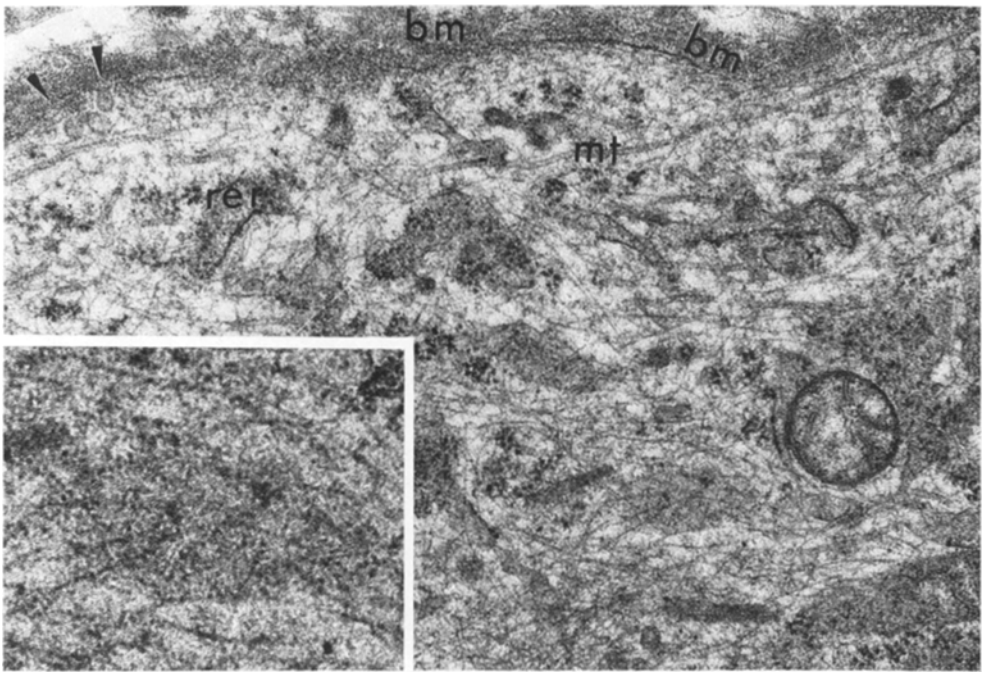



Fig. 3. Cytoplasm of an immature tumour cell. Numerous intermediate filaments, some microtubules (*mt*), and small lamellae of rough endoplasmic reticulum (*rer*) are present. Note basal membrane (*bm*) and small inpocketings at the surface (arrows). ×35500. Inset: High magnification of an electron opaque area with intermediate filaments. ×77200

*Electron Microscopic Examination*

Ultrastructurally three basic cell types could be distinguished in both cases herein to referred as immature cell, as myoblastic, and as fibroblastic cell type. In case 2 predominantly cells intermediate in cytoplasmic appearance between myoblastic and fibroblastic cell types were encountered. Tumour giant cells, which were only present in case 2, were oval to spindle shaped and measured 25 to 30 u in their greatest diameter. They contained nuclei with a smooth contour. The cytoplasmic organelle content of these cells did not differ from that of the mononuclear counterparts.

The intercellular space varied considerably between neighbouring cells. It contained bundles of collagen and some elastic fibers. A glycocalyx was developed in only a small number of tumour cells.

*Immature Cell.* These cells usually showed an excentrically located euchromatic nucleus with smooth contours (Fig. 2). Randomly oriented throughout the cytoplasm ribosomes could be found, often arranged in small clusters. Few lamellae of rough endoplasmic reticulum were only occasionally encountered. A moderate number of mitochondria was present, sometimes irregular in shape. The juxtanuclear cytoplasm often contained a well developed Golgi apparatus with many irregular anastomosing tubules and saccules. The most conspicuous feature of immature cells was its abundance of filaments (Figs. 2 and 3). These

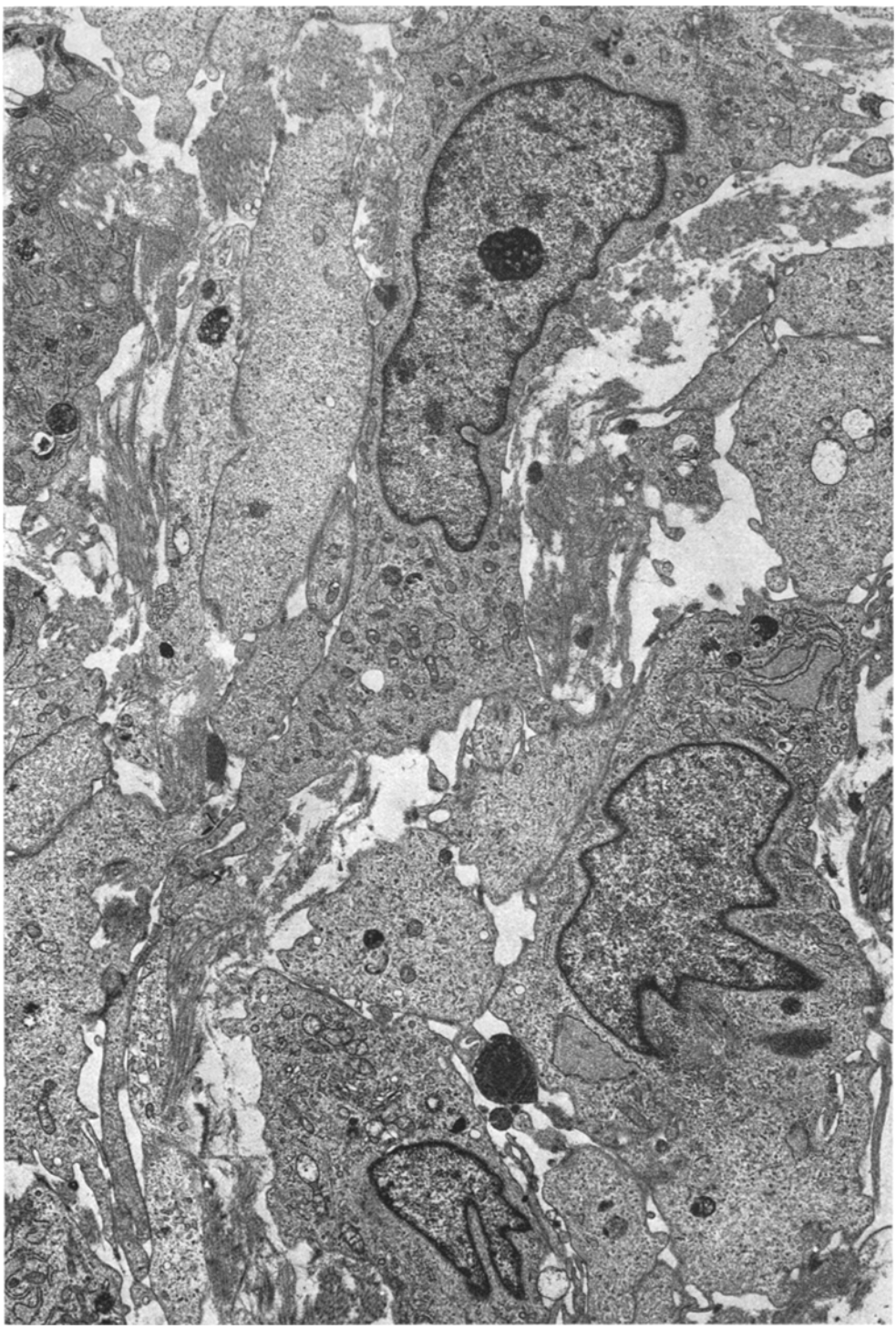


Fig. 4. Low power electron micrograph with predominantly myoblastic cells. The interstitial space contains collagen fibrils. Note deep indentations in some nuclei. ×6950

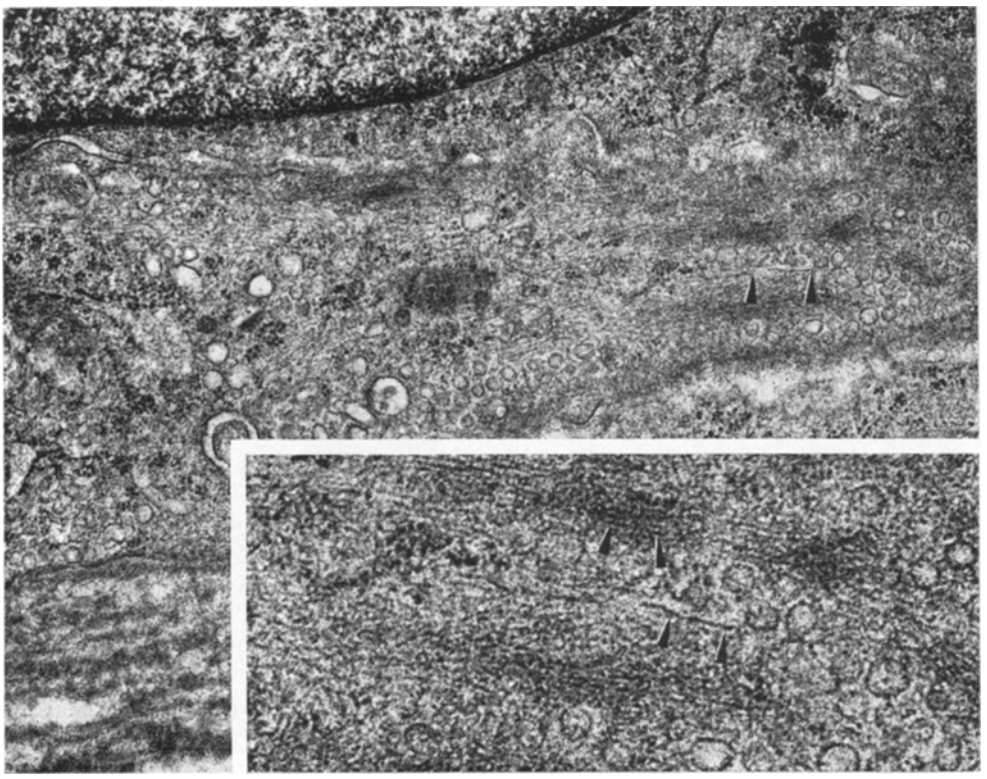


Fig. 5. Process of myoblastic cell with numerous myofilament bundles. Note small vesicles between the filaments (arrows). ×13300. Inset: High magnification of myofilaments with three dark bodies. The thick filaments (arrows) have a diameter of about 150 A. ×50100

filaments were 80–100 A in diameter. Only occasionally the filaments were arranged in networks some of which contained an electron opaque material (Fig. 3, inset). These areas measured 210 nm in width and about 530 nm in length. In addition to "100" A filaments often microtubuli could be visualized traversing the cytoplasm of the cell in the long axis. These microtubuli were 28 nm in diameter and could be traced over a maximal length of 2.1 u (Fig. 2). To the following cells a number of stages with increasing cytoplasmic differentiation was seen.

*Myoblastic Cell Type.* In electron micrographs of low magnification the cytoplasm of these cells was of even somewhat varying electron density (Fig. 4). The elongated euchromatic nucleus often contained a prominent nucleulus which was very dense with distinctly compact thread- or ball-like pattern. Some nuclei had deep infoldings in their nuclear membrane. The most differentiated tumour cells resembled smooth muscle cells. However, in contrast to smooth muscle, where myofilaments extend throughout the cytoplasm, the myofilaments of leiomyosarcoma cells were often confined to limited areas. Usually these areas had no or only a few other cell organelles (Fig. 5). Contrary to 100 A filaments the thin myofilaments seemed to be less uniformly electron opaque and occasion-

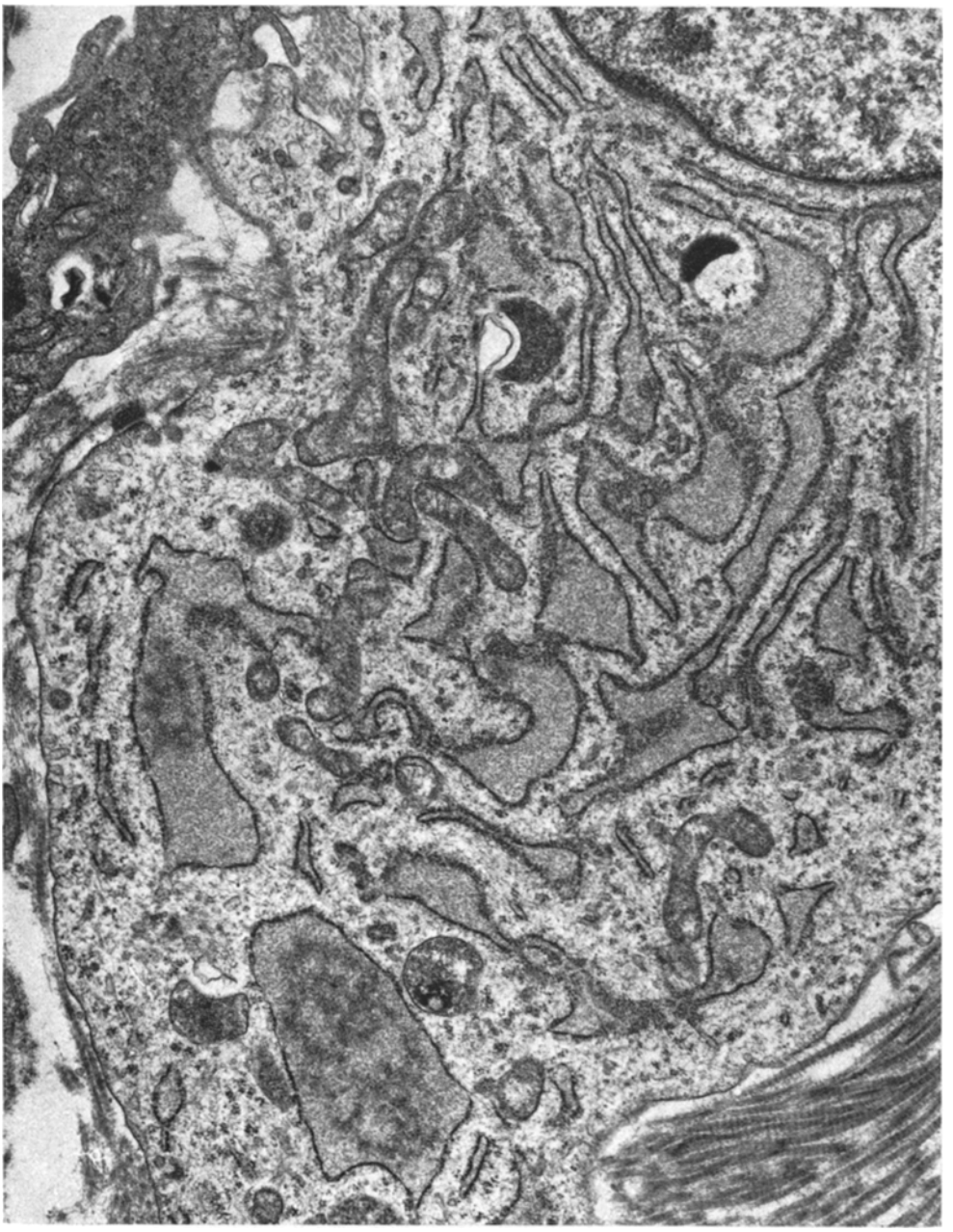


Fig. 6. Fibroblastic cell type with dilated cisterns of rough endoplasmic reticulum. Note small vesicles at the surface of the cell and collageneous fibrils in the interstitium. ×16350

ally formed microfilamentous webs. Dark bodies were found to be closely associated with myofilaments. They measured about 330 nm in length and 150 nm in width and under high resolution they contained thick filaments of 150 A in

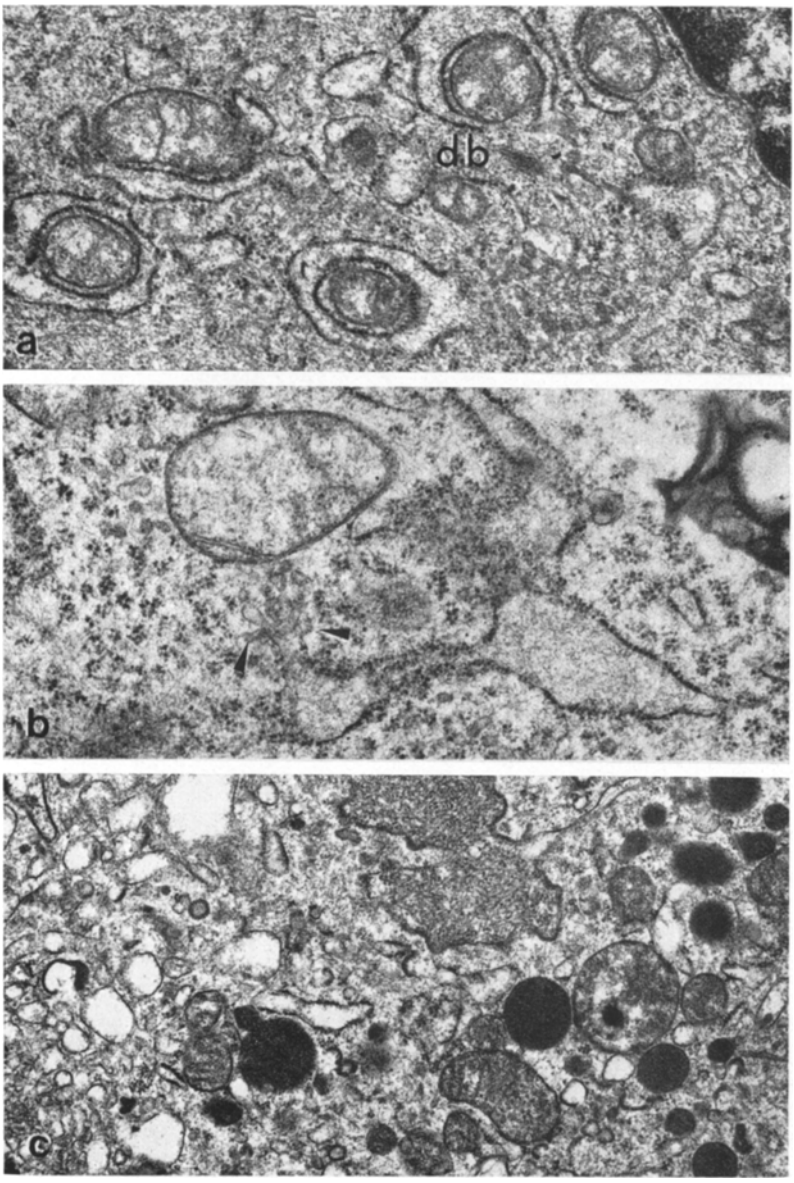



Fig. 7. Cytoplasmic details of a cell with fibroblastic appearance. (a) Cisterns of rough endoplasmic reticulum concentrically located around mitochondriae. Note dense bodies (*db*) in the cytoplasm. ×25790. (b) Small cytoplasmic sacs protruding from cisterns of the rough endoplasmic reticulum, possibly forming transitional vesicles (arrows). ×26000. (c) Numerous small vesicles and vacuoles containing osmiophilic fine floccular material, suggested to be primary lysosomes. ×16000

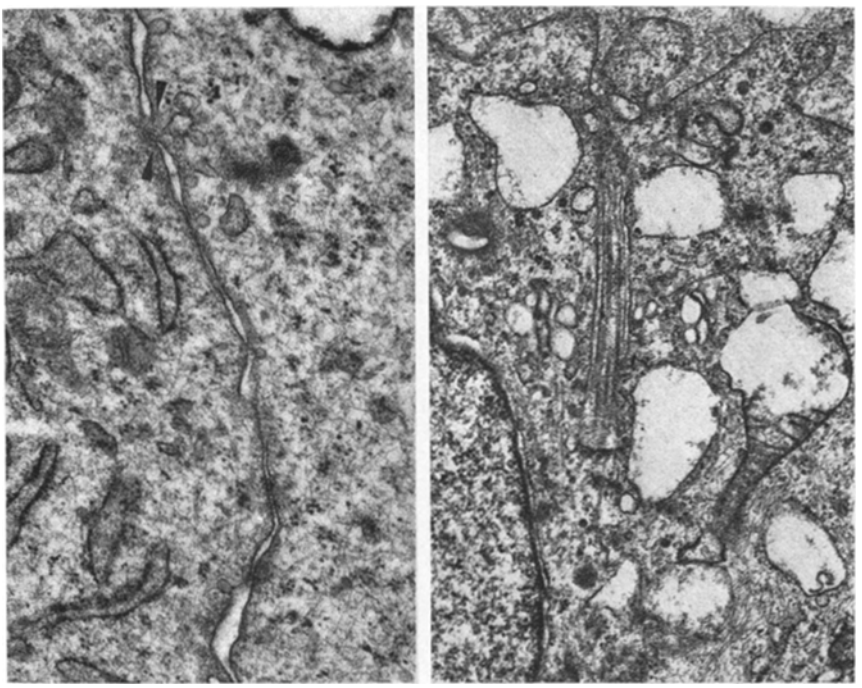


Fig. 8. Surface specializations of tumour cells. Left: Junction of zonula adhaerens type. Note cytoplasmic continuity between two tumour cells (arrows) and occasional surface vesicles. ×24700. Right: Part of myoblastic cell with well developed cilium. ×17500

diameter. In a small number of cells numerous small vesicles, measuring from 86 nm to 130 nm, could be observed between the myofilament bundles. Some dense areas were developed along the plasma membrane of the tumour cells. Micropinocytotic vesicles on the surface of the tumour cells varied widely in number but were found in each tumour cell. Their diameter was from 56 to 81 nm.

At various points adjacent tumour cells came into close contact, forming specializations of nexus and zonula adhaerens type (Fig. 8). These junctions were developed between smooth muscle cells as well as between myoblastic and fibroblastic cells.

*Fibroblastic Cell Type.* This cell is characterized by its abundance of rough endoplasmic reticulum in its cytoplasm (Fig. 6). The cisternal spaces were commonly dilated and contained a finefloccular material. Sometimes the cisterne were concentrically located around mitochondriae (Fig. 7a). In some areas smooth cytoplasmic sacs could be observed protruding from the rough endoplasmic reticulum (Fig. 7b). In regard to surface specializations, filaments, mitochondriae, and free ribosomes these cells partly correspond to myoblastic cells. Although a quantitative study on the cell organelles was not performed, the size of the Golgi fields and the number of lysosome-like vesicles (Fig. 7c) seemed to be increased in these cell when compared with the myoblastic cell type. Some of the tumour giant cells showed hyperplastic Golgi fields (Fig. 9).

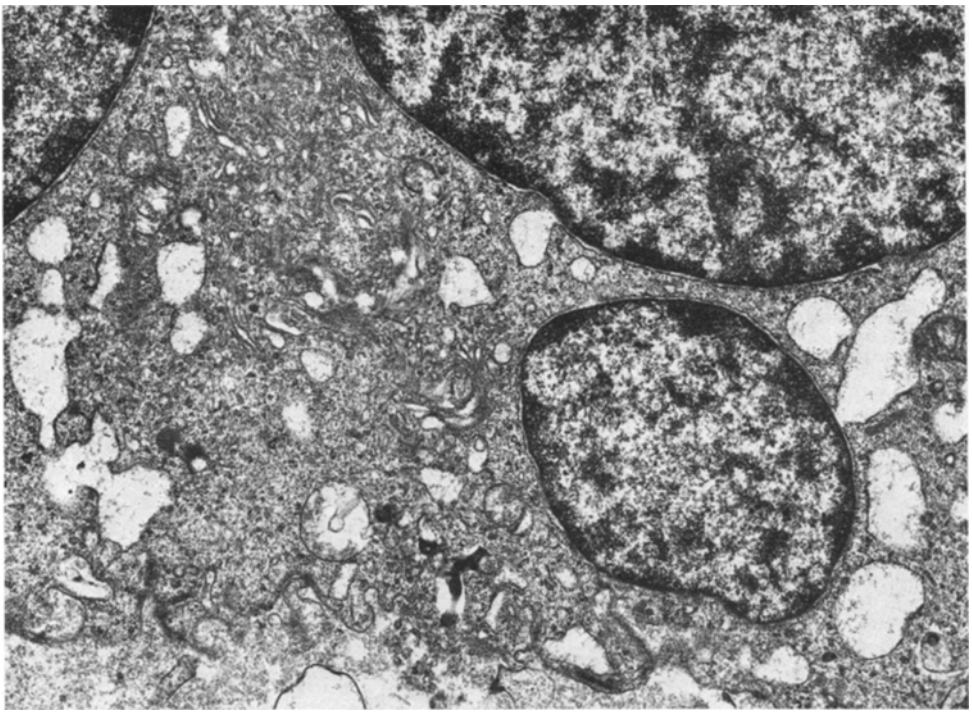


Fig. 9. Part of a tumour giant cell with hyperplastic Golgi complex. ×15300

## Discussion

In the present study it is shown that leiomyosarcomas, which are of a homogeneous cell population by light microscopy, reveal a series of cells with different cytoplasmic differentiation at electron microscopic level. At the one extreme there are more or less undifferentiated cells characterized by the perdominance of 100 Å filaments, free ribosomes and a moderately developed Golgi complex. There are no or only a few features in this cell indicating a differentiation toward smooth muscle. At the other extreme there are cells that appear differentiated containing myofilaments in their cytoplasm. However the cytoplasmic features characteristic for smooth muscle are usually incompletely developed: e.g. myofilament bundles, dense bodies, tiny invaginations and vesicles, a polysacharide coat, and occasional junctions. The third cell type with a fibroblastic appearance, nearly exclusively found in the tumour of the premenopausal woman, is probably involved in the formation of collagen fibers.

Although recent investigations have shown that smooth muscle, including the myometrium, contain actin and myosin (Needham and Schoenberg, 1968) there is still much discussion about the ultrastructure of this cell. Thus thin filaments with diameters from 30–80 A and thick filaments with diameters from 100 to 350 Å have been described (Campbell *et al.*, 1971; Cooke and Fay, 1972; Gwynn *et al.*, 1974; Kelly and Rice, 1969; Rosenbluth, 1971; Yamauchi and

Burnstock, 1969). The three filament systems, seen in the two cases of leiomyosarcomas, strikingly resemble those found in cultured smooth muscle cell (Campbell *et al.*, 1971). Thus in addition to thin filaments intermediate (about 100 A) and thick (150 A) filaments are observed. The thick filaments of leiomyosarcoma cells, when compared with myosin filaments of rhabdomyoblasts, have a more uneven beaded appearance. This suggests: (a) that thick filaments in our tumour cells merely represent a subclass of intermediate filaments and this possibility seems to us more likely, as Rash *et al.* (1970) described two classes of intermediate filaments with either diameters of 80–95 or 115–130 A in differentiating heart cells, (b) that the thick filaments represent a special aggregation of smooth muscle myosin. Evidence, speaking in favour of the latter hypothesis is seen in the description of similar arrangements of dense bodies—thick filaments in smooth muscle of guinea pig taenia coli (Cooke and Fay, 1972) as well as in pictures of isotonically contracted gizzard smooth muscle (Kelly and Rice, 1969) which look like Fig. 5 in our work.

Randomly oriented networks of 100 A filaments are found to be a feature of immature tumour cells. Similar arrangements have been described in cultured smooth muscle (Campbell *et al.*, 1969) and ten days chick embryo gizzard (Bennett and Cobb, 1969). Recently we were also able to demonstrate these filaments in developing young rhabdomyoblasts of mixed mesodermal tumour of the uterus (Böcker and Stegner, 1975) and in analogy to the function in other cells we suggested a cytoskeletal role (see also Ferrans and Robberts, 1973). This may also apply to undifferentiated leiomyosarcoma cells.

As already mentioned there is a wide spectrum of cells with varying degree of cytoplasmic differentiation and one is tempted to assume a development of more advanced stages from immature tumour stem cells. The striking similarity between different cells of the presented tumours and corresponding cells during the development of smooth muscle (Yamauchi and Burnstock, 1967) support this conclusion. This would however include that leiomyosarcomas of the uterus have their origin in either mesenchymal cells with the potential of mitosis and specific differentiation (stem cells responsible for the renewal of the myometrium ?) or poorly developed smooth muscle cells.

Recent studies have shown that the *age of the patient* with leiomyosarcoma is of *great prognostic significance*. Thus the premenopausal patient has a better prognosis than the postmenopausal one without recognizable histological or macroscopical difference. Ultrastructurally the most obvious difference between the two cases in this study is the occurrence of cells with a well differentiated fibroblastic appearance in the premenopausal woman. This finding is interesting because Ross and Klebanoff (1971) have observed a highly developed rough endoplasmic reticulum and Golgi complex in the smooth muscle of the rat uterus of mature animals in estrus or in prepubertal animals primed with estradiol. Thus may be, that endogeneous hormones may have an effect on the differentiation process of leiomyosarcoma cells and by this influence the biologic behaviour of the tumour. But this is far from conclusive and much further study in this field of pathology is needed.

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Dr. med Werner Böcker
Pathologisches Institut der Universität
D-2000 Hamburg 20
Martinistraße 52
Federal Republic of Germany