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# Ultrastructural Cytology of Human Osteosarcoma Cells*

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**Summary.** The cytology of 6 osteosarcomas was examined by electron microscopy. In keeping with the varied pattern of osteosarcomas seen by light microscopy several types of tumor cells could be differentiated: osteoblast-like, fibroblast-like, chondroblast-like, osteoclast-like and histiocyte-like cells. Moreover, atypical malignant mesenchymal cells and vascular spaces were present. The individual cytoplasmic organelles are not considered to be specific to particular types of cell as seen from the discussion of the significance of rough endoplasmic reticulum, microfilaments and lysosomes. Only examination of the composite pattern of subcellular organelles allows the differentiation of certain cell types. All tumor cells visible in osteosarcomas are considered as modifications of a transformed common progenitor cell. Because of the variegated cytological picture a multipotent mesenchymal cell rather than an osteoblastic cell is assumed to be the ancestor cell.

**Key words:** Osteosarcoma – Ultrastructural cytology – Histogenesis – Cellular modifications.

## Introduction

The electron microscopical features of tumor cells in osteosarcomas have usually been related to those of normal osteoblasts and thus the histogenesis of these tumors has been regarded as a malignant transformation of these cells (Hirohata and Morimoto, 1971; Kay, 1971). Moreover, multinucleated giant cells showing considerable ultrastructural variations were found which may resemble osteoclasts (Hirohata and Morimoto, 1971; Williams et al., 1976) or non-specific malignant giant cells. Although their histogenesis is still under discussion, their morphologic appearances in osteosarcomas favor the conclusion that giant cells develop by fusion of mononuclear osteosarcoma cells (Ghadially and Mehta, 1970).

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However, the histologic and cytologic picture of virus-induced osteosarcomas in animals may vary even more than in human ones. Beside chondrosarcomatous and fibrosarcomatous regions, also seen in human osteosarcomas, areas with fibroxanthosarcomatous, angiosarcomatous and myxoid structures can be observed (Fujinaga et al., 1970). Therefore, Czitrom and coworkers (1976) suggested that a multipotent mesenchymal cell is the origin of malignant bone tumors in animals and that their multipotency is responsible for the formation of different tumor structures.

Contrasting the varying ultrastructural cytology of virally-induced animal osteosarcomas with the more uniform cellular features described in human osteosarcomas, an ultrastructural-cytological review of our own cases of "spontaneous" human osteosarcomas seems to be useful.

## Material and Methods

6 human osteosarcomas were examined. The male:female ratio was 3:3. The ages of the patients ranged between 9 and 54 years, 4 patients were in the second decade of life. 5 tumors were located in the distal end of the femur (3 right, 2 left), and 1 tumor was found in the distal end of the tibia.

Tissue samples for electron microscopic investigation were collected during operation. The material was fixed for 3 h in 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2, postfixed in osmium tetroxide and embedded in Epon. Ultrathin sections were cut and contrasted with lead citrate and uranyl acetate.

For the purpose of comparison the tumor tissue was examined by light microscopy. The following stains were used: H & E, Elastica-Domagk, Goldner's trichrome stain and silver impregnation after Gomori. Additionally for the histochemical demonstration of mucopolysaccharides the alcian blue staining (pH 0.5, 1.0 and 2.5) and PAS reaction were employed.

## Results

*Light Microscopy.* The osteosarcomas showed the varying histological picture familiar from the literature (Uehlinger, 1973; Spjut et al., 1971; Dominok and Knoch, 1977): Generally the tumors consisted of rounded or spindle-shaped malignant mesenchymal cells. The nuclei were pleomorphic and polychromatic. The tumor cells were mostly without clear cellular boundaries, and in some areas the cytoplasm seemed to be vacuolated. Bizarre giant cells and, sometimes, osteoclast-like cells could also be detected. The frequency of mitoses differed. The number of vascular spaces varied, and occasionally abundant ectatic vessels were seen. In addition atypical slit-like spaces lined by tumor cells were observed in some areas. The osteosarcomas always showed areas of varying size in which tumor osteoid was deposited between the malignant cells. This substance was PAS-positive to a varying intensity. Acid mucopolysaccharides were found particularly in close proximity to tumor cells forming a cellular "coat", and in chondroid-like intercellular substances.

*Electron Microscopy.* The cellularity and the production of intercellular substances varied widely both in different parts of the same tumor as well as in different osteosarcomas.

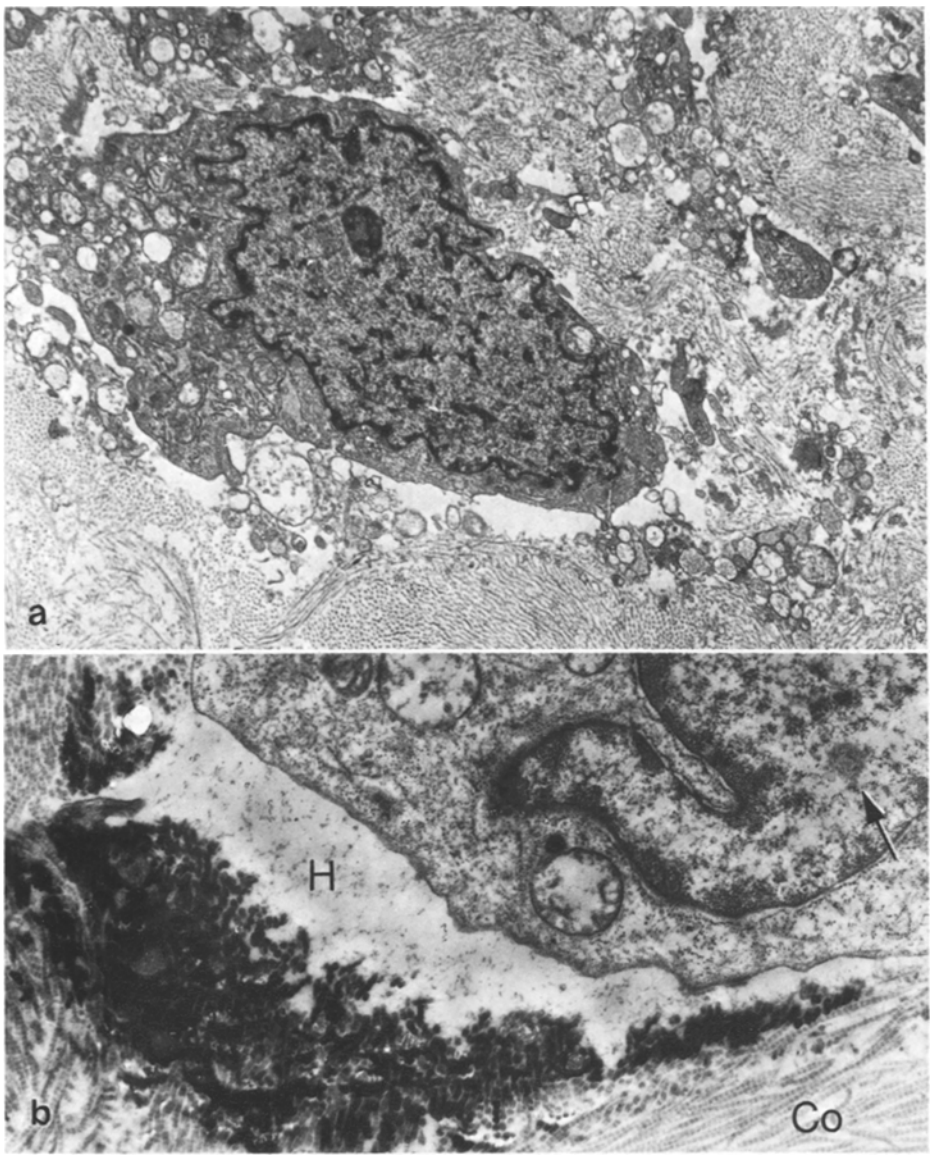



**Fig. 1a and b.** Osteosarcoma cells with abundant intercellular substance. **a** The tumor cell shows an irregularly configurated nucleus and mitochondria as well as endoplasmic reticulum in the cytoplasm. Amidst collagen fibers of the intercellular substance parts of the cytoplasm of other tumor cells are visible (× 9000). **b** In the neighbourhood of an ostoesarcoma cell with an organelle-poor cytoplasm an electron-lucent zone forming a pericellular clear halo (*H*), and cross-banded collagen fibers with deposition of an electron-dense material can be found. The tumor cell contains some hydropic mitochondria, sparse tubes of rough endoplasmic reticulum and free ribosomes in the cytoplasm. Note the nuclear body within the nucleus (→) (× 17,200)

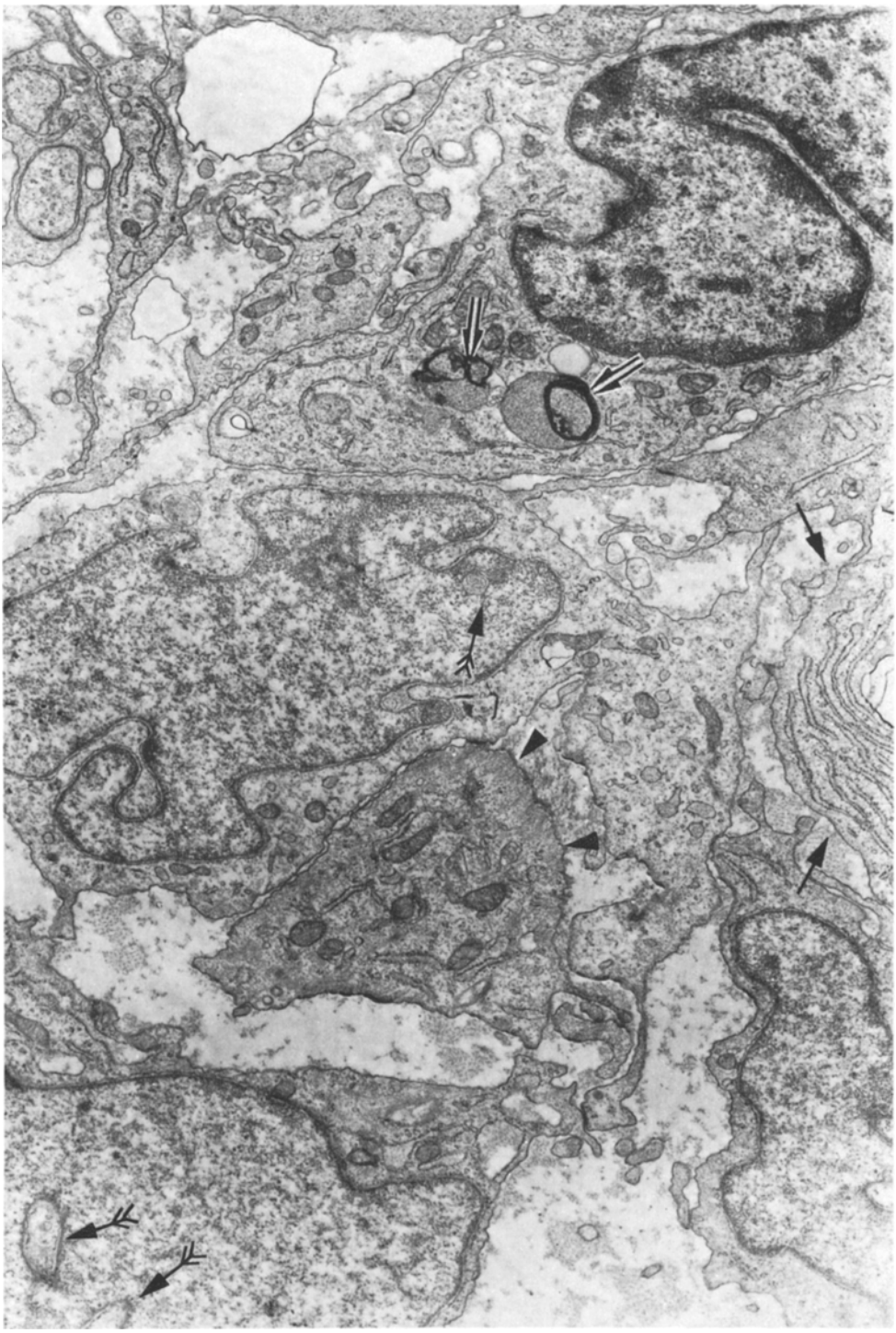


**Fig. 2a–c.** Osteosarcoma cells with different cytoplasmic organelle compositions. Beside rough endoplasmic reticulum (→) lysosomal structures (⇒), mitochondria and microfilaments (▷) may be seen in varying amounts. Generally the nuclei show indentations and invaginations. Note the nuclear pseudoinclusions (↣). The intercellular material consists of a granular and filamentous substance (× 11,600)

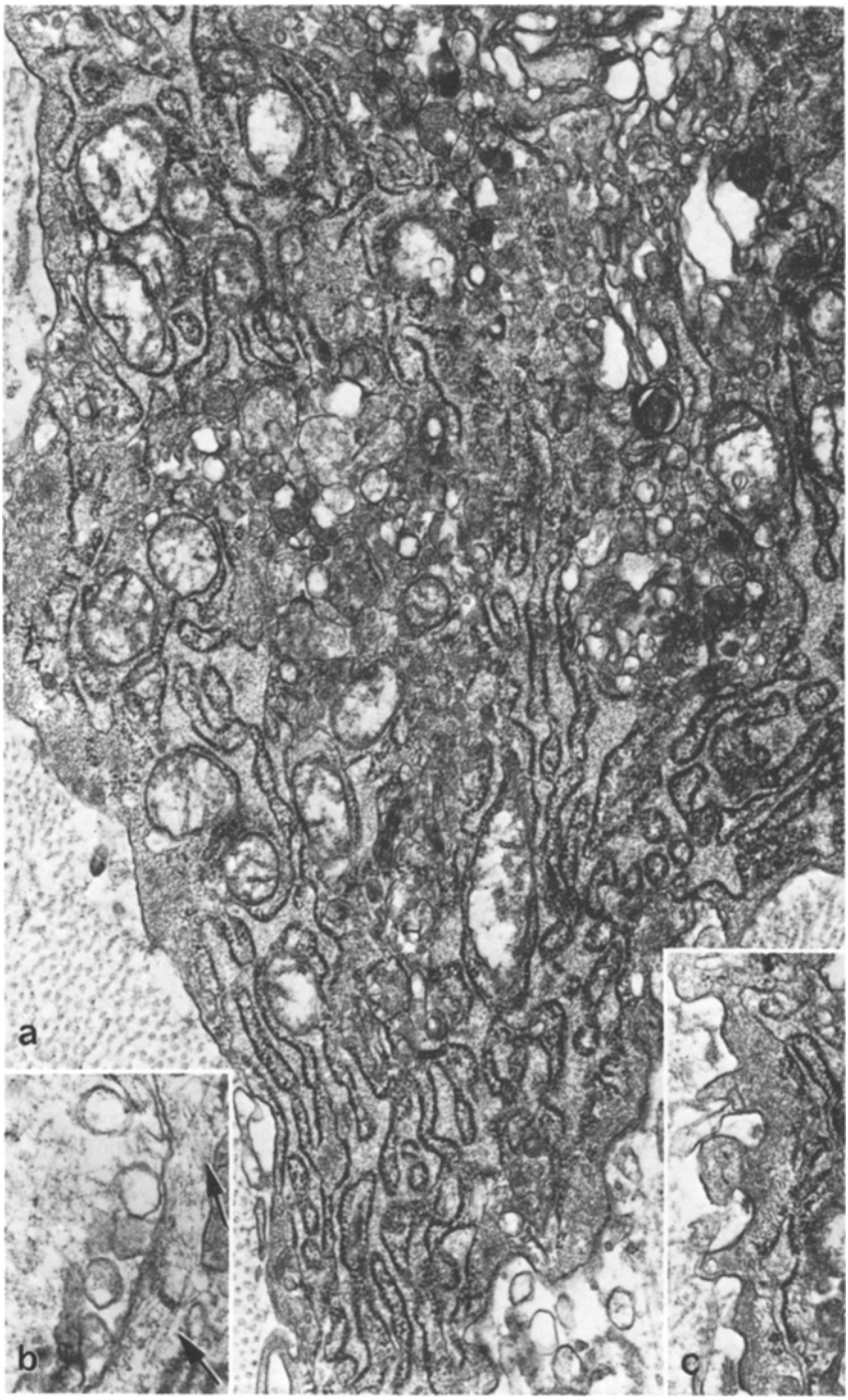



**Fig. 3a–c.** Detail of an osteosarcoma cell. **a** The cytoplasm shows a moderate amount of rough endoplasmic reticulum and mitochondria, a well-developed Golgi apparatus and vesicular structures. Subplasmalemmal microfilaments are also detectable (×24,000). **b** At greater magnification single microfilaments (→) can be demonstrated in subplasmalemmal position. In cellular neighbourhood some vesicular structures are present in the extracellular space (×40,000). **c** Subplasmalemmal network of microfilaments measuring 40–60 Å in diameter (×24,000)

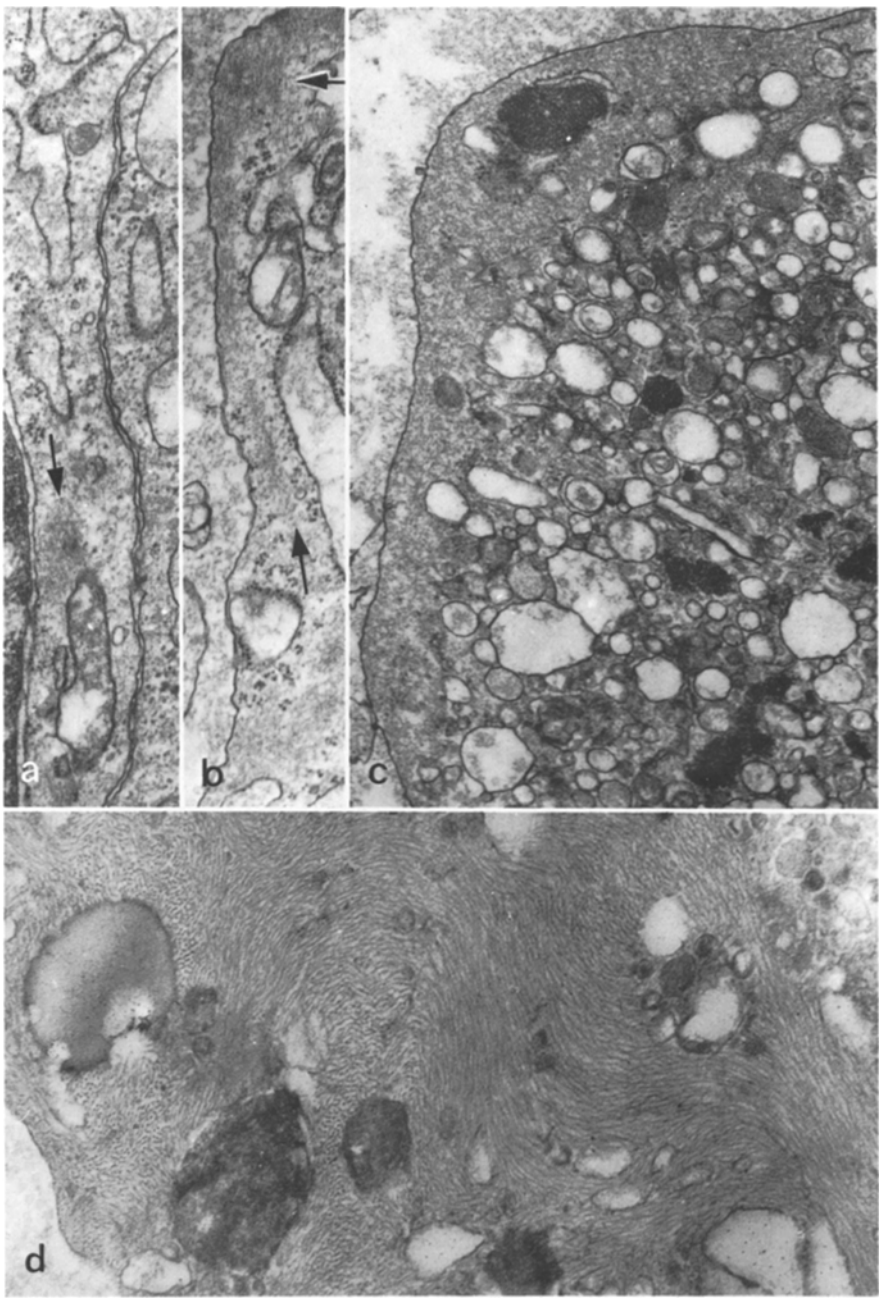



**Fig. 4a–d.** Examples of different cytoplasmic structures in osteosarcoma cells. **a** Details of two tumor cells. Their cytoplasmic membranes are in close proximity to each other. In the left cell microfilaments are seen in juxtanuclear position (→). Moreover, some tubes of rough endoplasmic reticulum and free ribosomes are present (× 24,000). **b** The cytoplasm contains polysomes, mitochondria, dilated tubes of rough endoplasmic reticulum and subplasmalemmal microfilaments (→) (× 24,000). **c** Preponderance of vesicular structures in the cytoplasm. In addition, lysosome-like bodies can be detected. The cell exhibits a clear subplasmalemmal filamentous network (× 24,000). **d** The cytoplasmic picture is dominated by parallel and wavy filamentous structures. Furthermore, lysosomes and a lipid droplet are to be noted (× 24,000)

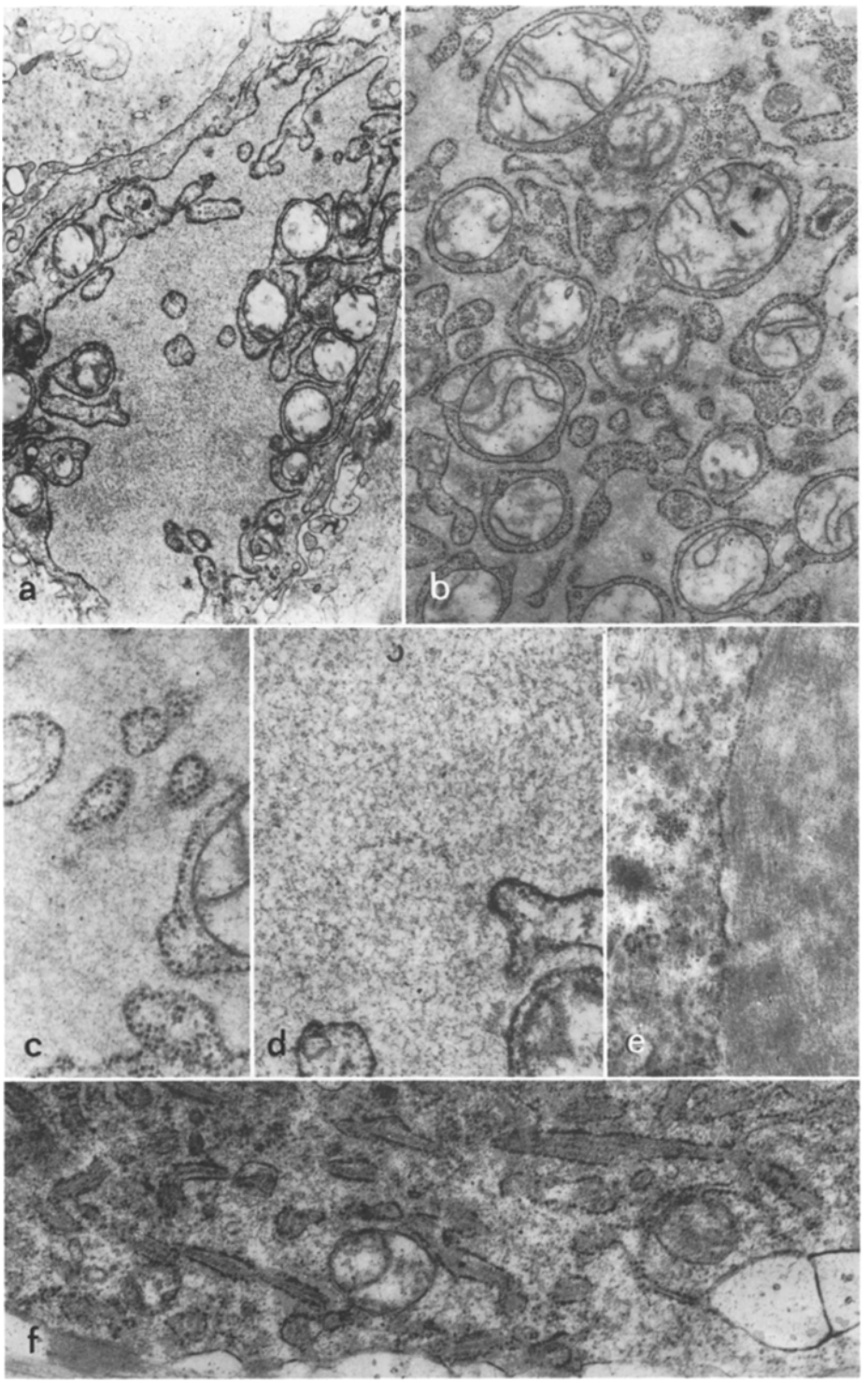



**Fig. 5a–f.** Examples of variations of the rough endoplasmic reticulum in osteosarcoma cells. **a** Extremely distended rough endoplasmic reticulum with a fine-granular content. The mitochondria are hydropic ( × 12,000). **b** Mitochondria are apparently enveloped by membranes of the expanded rough endoplasmic reticulum ( × 16,000). **c** and **d** Fine-granular and fine-filamentous material within the cisternes of the rough endoplasmic reticulum ( × 48,000). **e** Cross-banded filamentous aggregates within the rough endoplasmic reticulum (right side of the picture). They have a periodicity of about 1100 Å ( × 16,200). **f** Fiber-like structures within tubes of rough endoplasmic reticulum. A clear periodicity is not recognizable ( × 24,300).

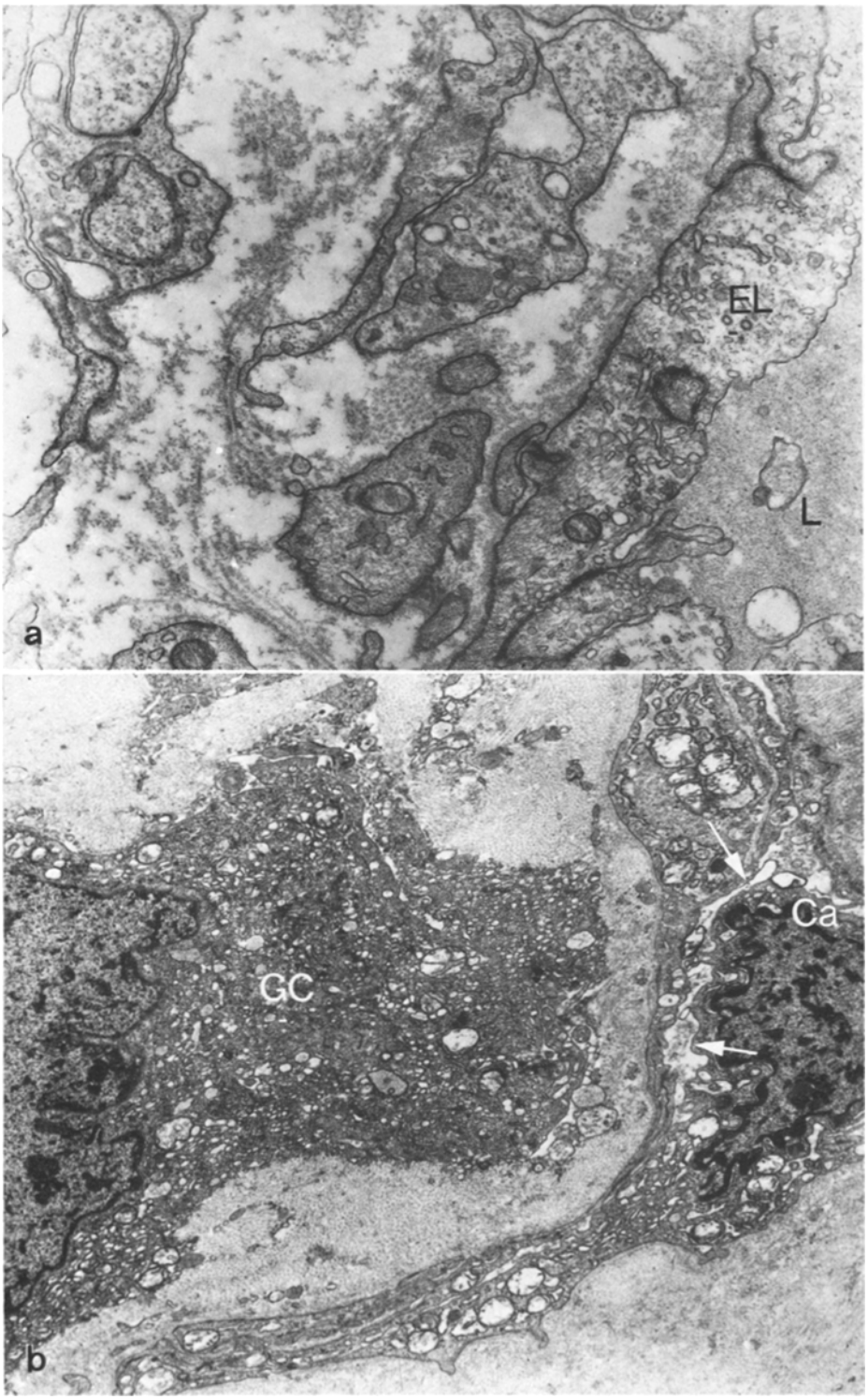
In general the tumor cells possessed a rather bizarre shape (Figs., 1 and 2). The nuclei were irregular, and showed indentations and invaginations of the nuclear membranes. The nuclear configuration sometimes lead to pseudoinclusions of cytoplasmic components. The heterochromatin was finely or coarsely distributed. 1 or 2 nucleoli could be seen, and sometimes nuclear bodies were present (Figs, 1 and 2).

The majority of osteosarcoma cells in each tumor were endowed with a small to moderate amount of rough endoplasmic reticulum, varying numbers of mitochondria of different sizes some exhibiting pleomorphism, and some lysosomes and single lipid droplets without an enveloping membrane (Fig. 2). Mitochondria were well preserved in cells with an electron dense hyaloplasm, but in other cells they showed hydropic change. Lysosomes appeared mostly as so-called secondary lysosomes containing phagocytosed material. Further organelles encounted were free ribosomes, a well-developed Golgi apparatus, free smooth-surfaced vesicles in the cytoplasm and cytoplasmic microfilaments measuring about 40–60 Å and 100 Å in diameter, respectively. The latter filament type was either loosely scattered within the whole cytoplasm or densely packed in larger cytoplasmic areas (Figs. 3 and 4). Some tumor cells were junctioned by specialized cell complexes showing the features of immature desmosomes.

By relating them to the tissue structure and by considering the composition of their cytoplasmic organelles some cell types could be distinguished which resembled cells of normal osteogenesis.

Firstly, there were tumor cells with some ultrastructural characteristics of osteoblasts, chondroblasts and fibroblasts. In these cellular elements the rough endoplasmic reticulum was markedly increased (Fig. 3). The tubes of rough endoplasmic reticulum were multiform: they were slender and arranged in parallel, formed cisterne-like dilatations, or were extremely expanded (Fig. 5). In the latter case the mitochondria were apparently enveloped by membranes of rough endoplasmic reticulum (Fig. 5b). Within the ergastoplasmic tubes a fine-granular or fine-filamentous material could be seen. Sporadically, fiber-like structures of 200–300 Å thickness, but without a clear periodicity, were observed within the rough endoplasmic reticulum (Fig. 5f). In single cells very large sacs of rough endoplasmic reticulum occurred which contained an electron-dense material with a periodic banding of about 1100 Å (Fig. 5e). In osteosarcoma cells with abundant rough endoplasmic reticulum many free ribosomes arranged frequently as polysomes could also be detected. The Golgi apparatus was well developed and sometimes multicentric. In the subplasmalemmal region

**Fig. 6a and b.** Examples of vessels in human osteosarcomas. **a** Atypical vascular spaces with endothel-like cells (*EL*) are junctioned by zonulae occludentes. Pinocytotic vesicles are seen especially on the luminal side (*L* = lumen). A clear endothelial basement membrane is lacking. These cells are cytologically similar to osteosarcoma cells in their proximity (× 17,600). **b** Normally structured capillary vessel (*Ca*) with a slit-like lumen (→). An endothelial basement membrane is present. On the left side of the picture a giant cell (*GC*) with a bizarre nucleus and numerous vesicular structures in the cytoplasm can be seen. Furthermore, there are mitochondria and sparse tubes of rough endoplasmic reticulum in the cytoplasm (× 7500)


EL
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of several cells a felt-like network of microfilaments (single filaments with a thickness of 40–60 Å) was often visible. Moreover, some vesicles and sparse micropinocytotic vesicles were occasionally seen. Some cells showing pseudopodia-like cellular extrusions were localized in lacunar-like cavities. In these cells glycogen granules were encountered in varying amounts.

Secondly, in other tumor cells vesicular structures prevailed within the cytoplasm, as smooth-surfaced and coated vesicles as well as some multivesicular bodies. Coated vesicles were found mostly in the neighbourhood of the Golgi zone. In such cells the presence of several lysosomes and/or laminated bodies was obvious. Thus a histiocyte-like appearance can be discerned in these osteosarcoma cells.

Thirdly, multinuclear giant cells were demonstrable. They possessed a hypertrophied and multicentric Golgi apparatus as well as numerous vesicular structures within the cytoplasm. An additional increase in the number of mitochondria was also noted (Fig. 6b). A typical "ruffled border" or a similar structure was never observed.

Viruses or structures related to a viral infection were not seen.

Finally, atypical vessel-like tubes were encountered. Cytologically, the cells of the lining cellular layer could not be distinguished from the surrounding osteosarcoma cells. Only the pinocytotic vesicles on the luminal side and intercellular junctions like zonulae occludentes were evidence of the endothelial nature of these cells. An endothelial basement membrane was frequently lacking. Beside these atypical structures, normal capillary vessels showing the usual endothelial cells and a basement membrane were seen (Fig. 6).

Intercellular substance was deposited in different quantities. In close proximity to the tumor cells a granular or fine-filamentous material was often found forming a rather electron-lucent halo around single cells (cp. Fig. 1b). Furthermore, in this localization or amidst collagen fibers, thin fibrils with a diameter between 200 and 300 Å could be detected. As a rule they had no periodicity. The collagen fibers showing the typical periodicity of 600–700 Å were arranged either in parallel or in a criss-cross pattern. The accumulation of a very electron-dense material in collagen fiber zones suggested calcification of the osteoid.

## Discussion

Osteosarcoma cells demonstrate well varying cytological appearances which are consistent with a rapidly growing, highly malignant mesenchymal tumor. By subdividing the malignant cells into certain cell groups against the background of comparable normal cell types, tumor cells with similarities to osteoblasts, chondroblasts, fibroblasts, histiocytes and (with certain restrictions) to osteoclasts may be differentiated. Although the ultrastructural similarities with cells of the normal osteogenesis is impressive, the morphological resemblance does not necessarily imply an identical functional significance. Therefore the terms osteoblast-like, chondroblast-like, fibroblast-like, histiocyte-like and osteoclast-like tumor cells should be favored.

Furthermore, atypical malignant mesenchymal cells are present. It is noteworthy that transitional forms between the single tumor cell types as well as between atypical malignant and more differentiated tumor cells are to be seen. Moreover, bizarre giant cells and atypical vascular spaces were observed.

Osteosarcoma cells may show an abundant rough endoplasmic reticulum. However, a large amount of rough endoplasmic reticulum is evidence only of a high rate of synthesis. Although osteoblasts are normally characterized by a well developed rough endoplasmic reticulum (Baud, 1968; Schenk, 1974) other cell types (e.g. fibroblasts and chondroblasts) may also contain numerous tubes of rough endoplasmic reticulum in the cytoplasm as can be seen in fracture repair (Göthlin, 1973). The bizarre configurations of rough endoplasmic reticulum in many tumor cells are only the expression of a raised and disturbed metabolic activity (Steiner, 1977). A special organization of these organelles as branched tubular structures (Jenson et al., 1971) or so-called "Nebenkerne" (Gusek, 1959) was not observed. These variations of the rough endoplasmic reticulum in osteosarcoma cells are the morphological correlate of the differing, in general increased synthesis of mucopolysaccharides and collagen fibers. Furthermore, the occasionally observed fiber-like structures in the tubes of the rough endoplasmic reticulum may be regarded as premature tropocollagen aggregation by analogy with other mesenchymal tumors in which intracytoplasmic collagen fibers are known to occur (Stiller and Katenkamp, 1975). Here and there we detected a cross-banded material in sacs of rough endoplasmic reticulum exhibiting a periodicity like long-spacing collagen. In bone tumors the occurrence of fibrous long-spacing collagen has only been reported in murine osteosarcomas (Marquart et al., 1976).

In several tumor cells many microfilaments were seen. Provided that they have a thickness of about 100 Å they may belong to the so-called cytoskeleton. If they have a diameter of 40–60 Å they are attributed to the actin filament system of cells. Actin filaments are known to occur in osteoblasts (King and Holtrop, 1975; Stanka, 1975) and in osteosarcoma cells (Ghadially and Mehta, 1970; Williams et al., 1976). However, their occurrence is entirely nonspecific (Gabbiani and Montandon, 1977).

Generally, the development of lysosomes, as found in tumor cells of our cases and others reported in the literature (Hirohata and Morimoto, 1971; Williams et al., 1976) is a trait of phagocytosing cells, especially of histiocytes (Gordon and Cohn, 1973). The osteoclasts also belong to the group of cells with resorptive (phagocytosing) tasks (Scott, 1967; Lucht, 1972; Holtrop and King, 1977). These organelles alone are nonspecific and are not sufficient for classifying a cell type because they occur in several varieties of mesenchymal cell.

The giant cells in our tumors cannot simply be equated with osteoclasts in spite of their similar organelle composition, particularly because a "ruffled border" was never present. However, in this connection it is remarkable that in normal osteoclasts the "ruffled border" may disappear under the influence of calcitonin (Kallio et al., 1972). Considering their organelle composition, the giant cells may arise from histiocyte-like tumor cells (amitotic division – fusion

of mononuclear cells etc., for discussion see Aparisi et al., 1977). From our investigations, however, the histogenesis of giant cells cannot be clarified.

Concerning the histogenesis of tumor cells in "spontaneous" human osteosarcomas in general we can consider the following possibilities:

Osteosarcoma cells originate from a multipotent mesenchymal cell. A common progenitor cell can be supposed because continuous transitions between the different cellular modifications exist. Apart from the varied cytology, the atypical vessel-like tubes could favor such an idea (cp. the atypical granulomatous reaction in small virus-induced osteosarcomas – Czitrom et al., 1976).

The other possibility is that tumor cells are constituents of a malignant osteoblast cell line in which the cancerous process causes an abolition of certain gene repressions (cp. Schulz et al., 1977). The inclination of tumor cells for imitating different cell types could be explained by the ontogenetic development of osteoblasts from multipotent mesenchymal cells.

Based on principles of general tumor pathology, and comparison with our previous investigations on mesenchymal tumors and pseudotumorous lesions, we support the first possibility. The production of osteoid would take place under the influence of so-called morphogens (Urist et al., 1977; Hanamura and Urist, 1978). This view would provide a simple explanation of why osteosarcomas as well as chondrosarcomas may occasionally arise in extraosseous sites.

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