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Osteosarcoma with a clear-cell component

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Summary. The reports concerns the light microscopical and ultrastructural findings obtained in three conventional osteosarcomas with an unusually high admixture of clear cells, whose presence appeared to be responsible for the marked change in the histological pattern of these tumours. In the tumours with a prevailing fibroblastic component the clear cells were either irregularly scattered throughout the tumour in the form of small groups, or they formed large groups sharply demarcated against the fusicellular areas of the tumours. In two cases it was shown that their cytoplasm contained exaggerated glycogen deposits accompanied by the formation of glycogen-containing phagolysosomes and occasional empty vacuoles. In the third case the clear cells showed vacuolar degeneration with numerous single-membrane-bound, empty vacuoles. In contrast to the clear-cell chondrosarcoma we did not find S-100 protein in clear cells of our osteosarcomas. Such findings could be particularly significant in the differential diagnosis of bone tumours.

Key words: Osteosarcoma – Clear cell – Histopathology – Electron microscopy

Introduction

The histological structure of conventional osteosarcoma is characteristic and generally well-known, so that the morphological recognition of this tumour is quite easy in the majority of cases. Recently, however, we have encountered osteosarcomas with an unusually high admixture of clear cells, whose presence altered the basic pattern of these tumours to such degree that their diagnosis was difficult. The character of the clear tumour cells, which occasionally contained exaggerated glycogen deposits or numerous intracytoplasmic vacuoles, was elucidated only after an electron microscopical examination. In view of the fact that the problem has not received appropriate attention (Dahlin and Unni 1986; Huvos 1979; Mirra 1980; Roessner 1985; Schajowicz 1981; Schulz 1980; Spjut et al. 1971), the presentation of the following report concerning three osteosarcomas with a clear-cell component seems to be justified.

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Case reports

Case 1. F.K., a male of 17 years of age, was admitted to hospital because of swelling of and pain in the right knee joint for three months. X-ray films revealed a partially osteolytic and partially osteoplastic focus affecting the distal metadiaphysis of the femur and extending into the adjacent tissues. An exploratory excision was carried out on September 17, 1986 and after the diagnosis had been established preoperative treatment with Methotrexate and folic acid was started. Due to the small effects of this treatment and the accompanying toxic reaction, an amputation was performed at the upper third of the femur on November 4, following which adjuvant chemotherapy was commenced. In January 1987 multiple lung metastases developed.

Case 2. K.Z., a male of 54 years of age was admitted to hospital because of pain in and reduced mobility of the knee joint of 3 months duration. X-ray examinations revealed the presence of an extensive osteolytic lesion expanding the lateral epimetaphysis of the distal femur and invading the adjacent soft tissues. On May 21, 1986, a provisional excision, enucleation of the focus and cement plombage were carried out. After osteosarcoma had been diagnosed, amputation of the limb at the femoral level was performed on June 2, 1986. The patient is currently receiving adjuvant chemotherapy and no signs of tumour recurrence and/or metastasis have been reported.

Case 3. R.M., a girl of 13 years of age, was admitted to hospital because of pain in her left knee joint for 5 months. Her history revealed allergy towards Penicillinum and bronchial asthma associated with eczema treated with Aminophylline, Ketotifenum and autovaccination. X-ray examinations showed a regular lo-

bulated area of destruction affecting the proximal metadiaphysis of the tibia, containing scattered sclerotic foci. A provisional excision was carried out on November 3, 1986 and one week later preoperative chemotherapy based on a combination of high-dosage Methotrexate, Doxorubicin hydrochloride, Bleomycin chloride, Actinomycin D, Cyclophosphamide, and Vincristin sulfate (The T12 schedule according to Rosen) was started. The initial effects of such treatment were quite good, but later the tumour appeared to have invaded the surrounding soft tissues and therefore amputation of the limb at the femoral level was carried out on May 14, 1986. The patient is currently undergoing chemotherapy. So far no recurrence or tumour metastasis has been recorded.

Material and methods

The tissues obtained by biopsy excision or amputation were fixed in a neutral formalin solution for light microscopical examination. All the tissue samples were embedded in paraffin without previous decalcification and the sections obtained from the paraffin tissue blocks were stained with haematoxylin and eosin, Masson's blue trichrome stain, the P.A.S. method with and without previous amylase digestion, alcian blue at pH 1.0 and 2.5, or Gomori's silver impregnation method for reticulin fibres. Frozen sections were stained with Sudan III and Sudan Black B prior to and after extraction with acetone and chloroform respectively. Some tissue samples removed from the bones of amputated limbs for the purpose of histological examinations were decalcified with Kristensen's solution and further processed as described above.

The formalin-fixed, paraffin-embedded sections from our three osteosarcomas were also examined for S-100 protein by the immunoperoxidase technique (Weiss and Dorfman 1986). Positive control slides were cut and prepared in the same way from a clear-cell chondrosarcoma and chondroma to ensure a working staining system. Rabbit anti-S-100 protein antibody (Dako, Kopenhagen, Denmark) was used for this purpose.

The tissues for electron microscopy were removed during the operation and immediately fixed by immersion in 3.25% glutaraldehyde mixed with a pH 7.2 phosphate buffer (1:7) for 24 h at 4° C. The material has been further processed in the same way as described previously (Povýšil et al. 1977). Ultrathin sections were examined with a Tesla BS 500 electron microscope.

Results

Case 1

Light microscopy of the tissue sample obtained by the exploratory excision revealed a widely necrotic fibroblastic osteosarcoma with focal osteoplasia and chondroplasia. The fibroblastic tumour component was composed of spindle-shaped cells some of which had a water-clear cytoplasm (Fig. 1) usually containing demonstrable glycogen deposits. The clear tumour cells were present in the vicinity of the necrotic areas. After chemotherapy the tumour was mostly necrotic but contained areas of viable fusiform tumour cells, some of which showed a clear cytoplasm.

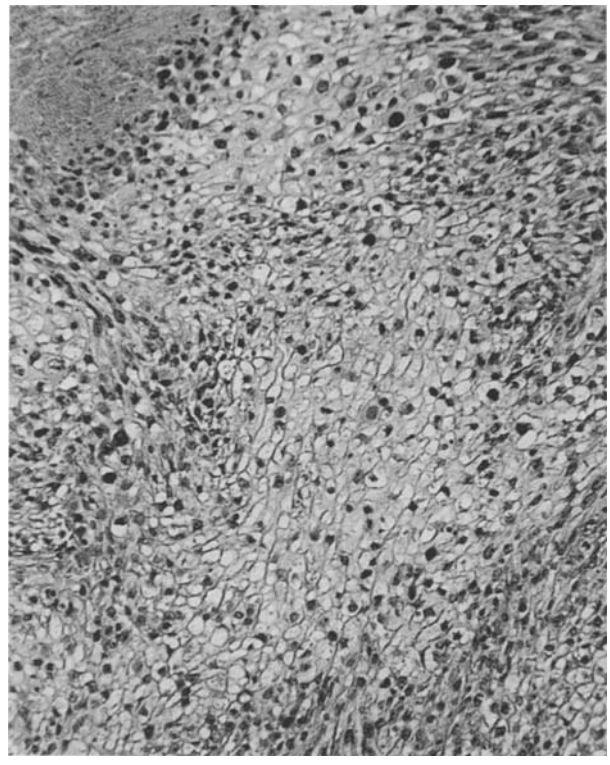

Fig. 1. *Case 1.* Clear tumour cells containing glycogen as observed in the neighbourhood of a necrotic focus in the osteosarcoma. H. and E., ×275

Case 2

The tissue sample from the first biopsy revealed osteoblastic osteosarcoma containing areas composed of poorly differentiated spheroid or fusiform cells with an admixture of giant multinucleate cells (Fig. 2). The tumour was partly teleangiectatic. It also contained large foci composed of clear cells only (Fig. 3) which showed a small amount of glycogen after formalin fixation. Such tumour cells were found in the close vicinity of the osteoid islets or trabeculae and the vascular spaces. In other instances the clear-cell areas merged with areas of osteoplasia in which the clear tumour cells appeared to be smaller. The tumour tissue also contained focal round-cell inflammatory infiltrates.

Light microscopical examination of tumour tissue obtained during the amputation revealed structures similar to those observed in the biopsy sample. The major part of the tumour was necrotic, however, so that it was not possible to assess the presence of clear cells with certainty.

Case 3

In tissue obtained from the first excision the tumour appeared to be a characteristic, mostly fibro-

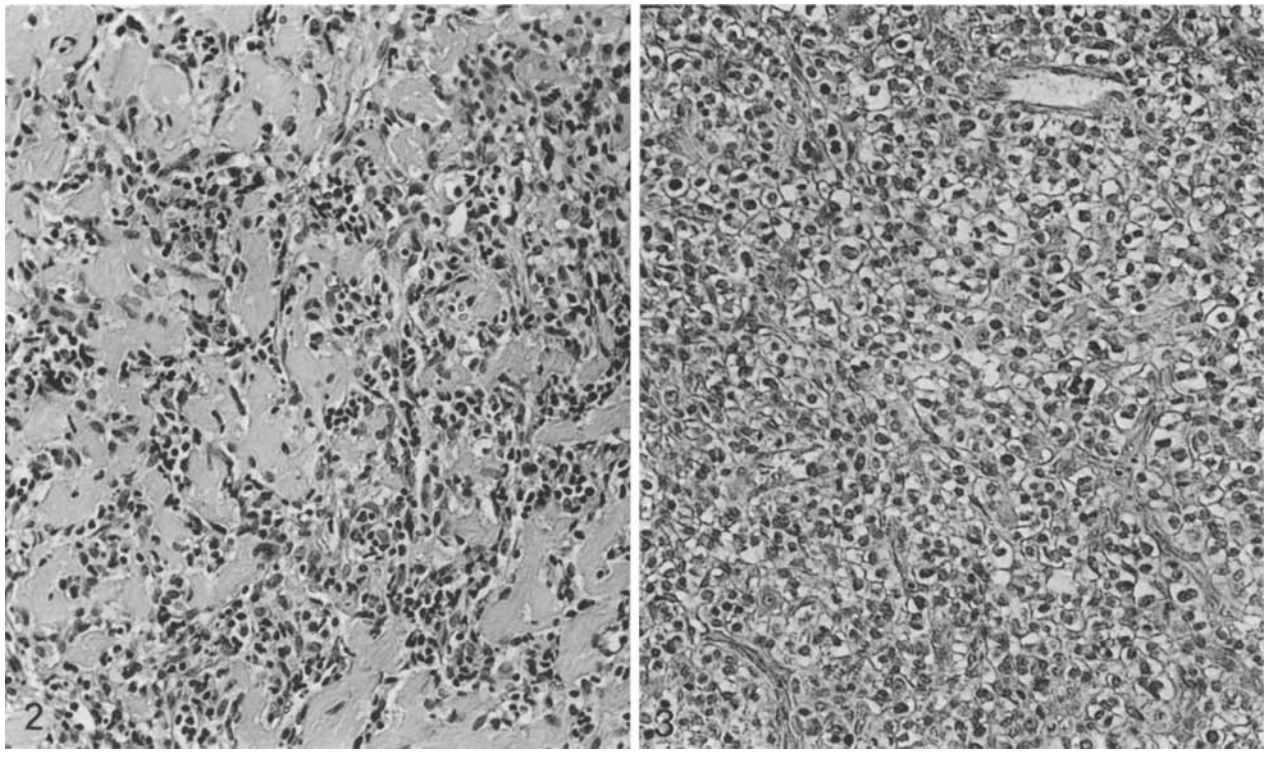

Fig. 2. *Case 2.* Area of the osteosarcoma showing neoplastic osteoplasia. Poorly differentiated small malignant osteoblasts can be seen between the osteoid trabeculae. H. and E., ×275

Fig. 3. *Case 2.* Clear-cell area of the osteosarcoma. H. and E., ×275

blastic osteosarcoma with occasional small areas of neoplastic chondroplasia. Neoplastic osteoplasia was found in a number of places in the form of islets or trabeculae of osteoid (Fig. 4) with slight indication of mineralization.

The major part of the tumour was occupied by large areas of neoplastic cells with atypical nuclei and a markedly vacuolated or water-clear cytoplasm (Fig. 5). Such areas were usually sharply demarcated against the surrounding tumour tissue. In places, however, clear cells were present in the form of small groups only. They were situated both between the spindle-shape cells of the fibroblastic component and the trabeculae of the malignant osteoid. We were unable to demonstrate the presence of glycogen, lipids or other substances in their cytoplasm. Individual clear cells were embraced by reticulin fibres, clearly visible in silver-impregnated sections. After chemotherapy the tumour was largely necrotic, but small foci of clear cells similar to those identified in the first excision were found.

We did not find S-100 protein in the clear cells of our tumours contrary to the positive immunoreactivity of the control cartilagineous lesions.

In electron microscopy the findings were as follows. In Case 1, the tissue samples contained tumour cells showing the characteristics of fibroblasts with several irregular profiles of rough endoplasmic reticulum. In clear-cell areas there were cells showing an electron lucent cytoplasm studded with glycogen granules (Fig. 6). Irregularly scattered cytoplasmic organelles similar to those found in changed neoplastic cells occurred between the glycogen granules. Some of these cells contained numerous autophagosomes containing glycogen and occasional cytoplasmic organelles such as mitochondria and rough endoplasmic reticulum.

In Case 2, poorly differentiated mesenchymal cells we seen together with larger fibroblasts and osteoblasts with marked cytoplasmic glycogen accumulation (Fig. 7). Such glycogen-rich cells showed well-developed rough endoplasmic reticulum, preserved mitochondria and occasional phagolysosomes containing glycogen and rests of mitochondria. The clear cell tumour areas contained electron lucent cells with scarce intra-cytoplasmic organelles and scattered glycogen granules (Fig. 8). Some of such cells contained single membrane-bound empty vacuoles (Fig. 8). Gradual transi-

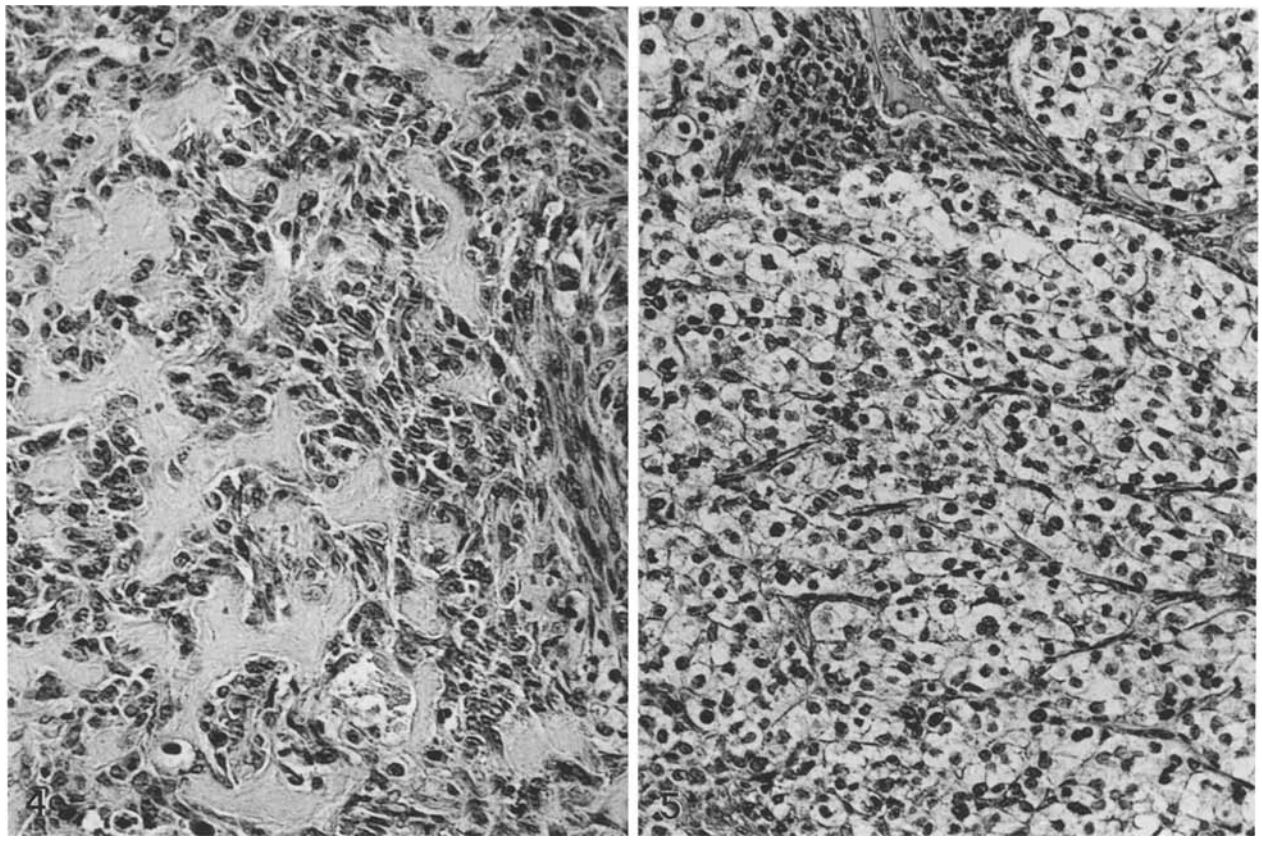

Fig. 4. *Case 3.* Area of the osteosarcoma showing neoplastic osteoplasia. H. and E., × 300

Fig. 5. *Case 3.* Clear-cell area of the osteosarcoma. No glycogen was demonstrated in these cells. H. and E., × 275

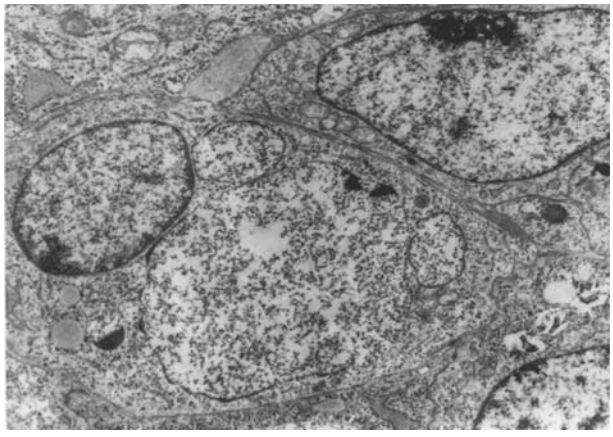

Fig. 6. *Case 1.* Tumour cells from a clear-cell area of the osteosarcoma. Their cytoplasm contains a large amount of glycogen granules, occasional lipid droplets, mitochondria, rough endoplasmic reticulum and glycogen containing phagolysosomes. × 5250

tions were observed between the neoplastic osteoblasts, the glycogen-rich cells and the electron lucent cells. Electron lucent cells were also observed in areas of osteoplasia with macrophages and lymphocytes of inflammatory infiltrates often situated in their vicinity.

In Case 3, the fusicellular areas were composed of elongated cells with an irregularly shaped, rough endoplasmic reticulum (Fig. 9). Such areas did not differ essentially from similar fibroblastic areas observed in conventional osteosarcomas examined in our laboratory (Povýšil 1986).

The cytoplasm of clear cells contained numerous single-membrane-bound vacuoles (Fig. 10). Some of them contained an amorphous substance of intermediate density. Dilated rough endoplasmic reticulum, small inclusions most probably representing regressive mitochondria and occasional dense bodies of the lysosomal apparatus were found between the vacuoles. The vacuolated cells were usually closely packed, leant against one another with their cell membranes and were interconnected with primitive desmosomes (Fig. 10). The nuclei of such cells were usually ovoid with small indentations of the nuclear surface and the heterochromatin was accumulated at the nuclear membrane and scattered irregularly in the form of tiny clumps throughout the whole nucleus. In compari-

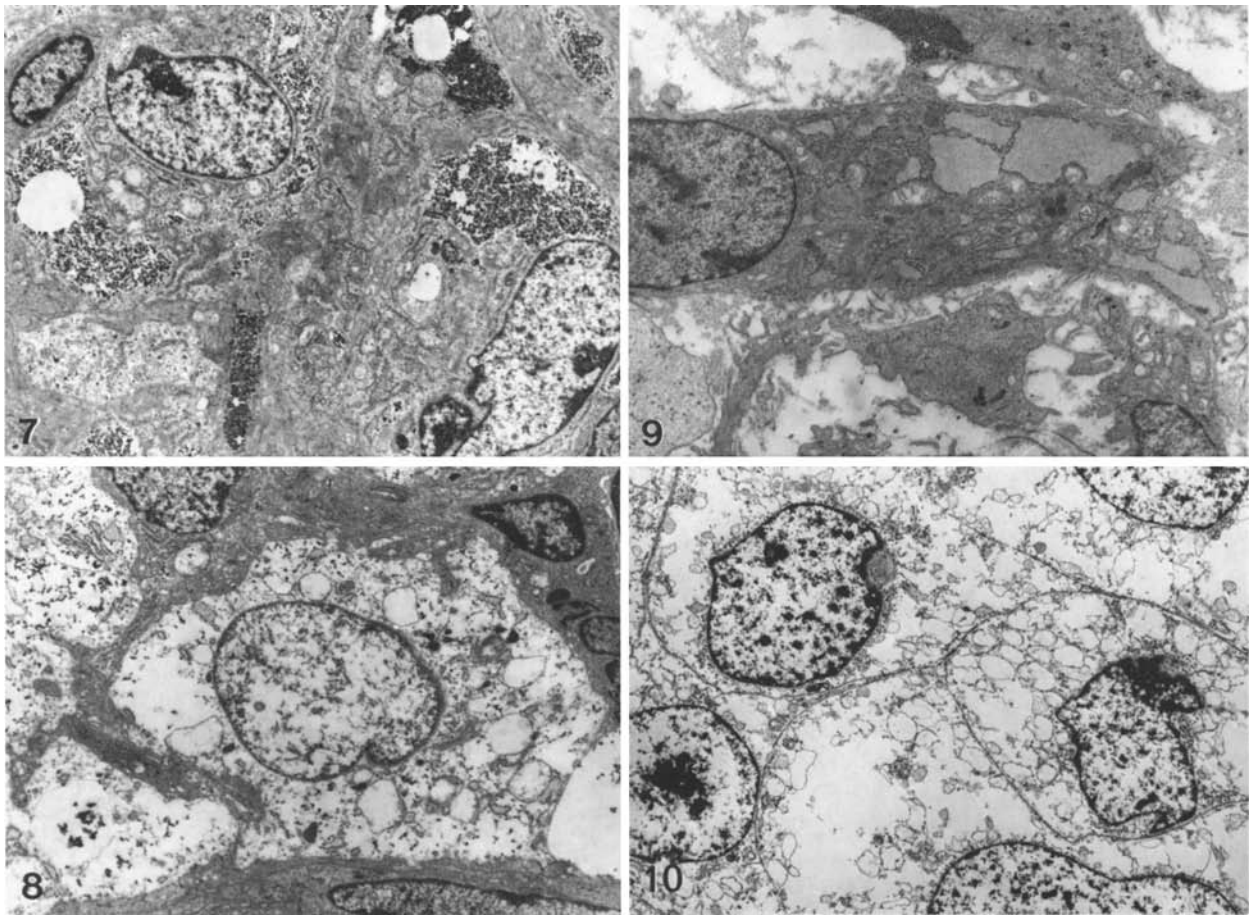

Fig. 7. *Case 2.* Tumour osteoblasts whose cytoplasm contains a large amount of glycogen granules, mitochondria and rough endoplasmic reticulum. × 3850

Fig. 8. *Case 2.* An electron lucent tumour cell showing regressive changes. Its cytoplasm contains a few glycogen granules, dense bodies and single membrane-bound empty vacuoles. × 4000

Fig. 9. *Case 3.* Tumour fibroblast as found in the fusicellular area of the osteosarcoma. Its cytoplasm contains irregular rough endoplasmic reticulum. × 6100

Fig. 10. *Case 3.* Tumour cells from the clear-cell tumour component showing numerous single membrane-bound empty cytoplasmic vacuoles. The cytoplasmic organelles show severe regressive changes and are therefore difficult to identify. × 3800

son with the nuclei of the spindle-shaped cells of the fusiform cellular areas, the euchromatin appeared rarefied. Single cells, the majority of whose vacuoles contained an amorphous substance of intermediate density, and cells with somewhat numerous granules of the lysosomal apparatus were found in the clear-cell areas.

Discussion

Clear cells have been recorded in connection with bone tumours with different storage materials. Glycogen accumulation has been seen in clear cell chondrosarcoma (Angervall and Kindblom 1980; Bjornsson et al. 1984; Faraggiana et al. 1981; Povýšil and Matějovský 1985; Salzer-Kuntschik 1981; Unni et al. 1976), chordoma (Dahlin and Unni 1986; Erlandson et al. 1968; Povýšil and Matějovský 1985; Povýšil 1986; Schajowicz 1981) and Ewing's sarcoma (Povýšil 1986). Lipid storage occurs in liposarcoma (Schajowicz 1981) and increased formation of glycogen-containing phagolysosomes or intracytoplasmic inclusions of intercellular mucoid substances occurs in chordoma (Erlandson et al. 1968; Povýšil and Matějovský 1985). In osteosarcoma, the finding does not seem to have received sufficient attention in the literature. In our cases the underlying condition was either glycogen accumulation or vacuolar degeneration of the cytoplasm of the tumour cells. These clear cells did

not stain for S-100 protein and so a chondrogenic origin is improbable because chondrogenic tumours are positive by this method (Weiss and Dorfman 1986). Glycogen was found in increased quantity in the spindle-shaped fibroblasts and undifferentiated mesenchymal cells, usually unassociated with the production of intercellular substance. Osteoblasts of the clear cell type embedded in osteoid mass or lying between trabeculae or neoplastic bone appeared to be a rare finding. There were transitional forms between the osteoblasts and clear cells.

In the third osteosarcoma discussed here the clear cells had clearly regressive nuclei and contained single-membrane-bound intracytoplasmic empty vacuoles. In our opinion such cells represent regressive cells showing vacuolar degeneration of the cytoplasm. It is somewhat difficult to explain the development of vacuoles, because the material available permitted the disclosure of only single cells with initial vacuolar transformation. An interesting finding recorded in Case 2 was the occurrence of electron lucent cells with vacuolated cytoplasm simultaneously containing an increased amount of glycogen and scattered glycogen-containing phagolysosomes. The question of whether vacuolated cells represent advanced stages of changes preceded by cytoplasmic glycogen accumulation and the formation of phagolysosomes should be considered. The possibility of the development of some of the vacuoles from swollen mitochondria can not be ruled out.

The aetiopathogenesis and the significance of increased glycogen accumulation and the vacuolar transformation of the cytoplasm retain obscure despite the possibility existing, in our opinion, that these changes are of a regressive character. Neither arteriography nor the surgical intervention proper were connected with an untoward clinical event in any of the cases presented here. None of the patients was subjected to cytostatic treatment before biopsy. Patient No. 3, however was regularly treated with Aminophylline and Ketotifenum because of an allergic condition. The general pathological data indicate the possible significance of hypoxia in the pathogenesis of the changes discussed. This interpretation can possibly be supported by the finding of extensive necrosis in Case 1 and the presence of occasional mural thrombi in the dilated vascular spaces observed in the tumour of Case 2. Conspicuous focal inflammatory infiltrates were present in the otherwise intact tumour tissue of Case 2 and 3.

Despite the fact that we feel unable to elucidate with certainty the causes of clear cell transformation in our osteosarcomas, the practical significance of this finding appears to be beyond all doubt. The unusual findings obtained in Case 3 can be used as evidence of the possibility that the clear cell transformation of some tumour cells in mesenchymal tumours may develop without any accumulation of glycogen, or lipid or mucoid substances. In a preoperative or exploratory excision the finding of such areas might be a source of differential diagnostic doubt; elucidation appears to be impossible without the aid of electron microscopy. The conditions to be ruled out include the metastasis from a clear cell carcinoma and possibly also clear cell sarcoma of the tendon sheaths. Clear cell chondrosarcoma mostly occurs in elderly individuals presenting a different X-ray picture. The tumour cells contain S-100 protein and ultrastructurally retain the features of chondrocytes showing a distinctly scalloped surface (Angervall and Kindblom 1980; Weiss and Dorfman 1986; Povýšil and Matějovský 1985). The so-called osteosarcoma with an endocrine pattern (Meister et al. 1980; Scranton et al. 1975) contains malignant epithelioid cells whose character has not been specified and it therefore appears impossible to assess the relationship to the clear cells found in our cases. Malignant mesenchymomas contain a rhabdomyoblastic component and liposarcomatous structures (Schajowicz 1981).

In our opinion, the clear cell component of osteosarcoma does not represent a histogenetically separate component of the tumours although such an impression might easily arise from the light microscopic study of Case 3. It seems probable that similar but smaller areas of clear cells have hitherto escaped attention. In reviewing our collection of 100 osteosarcomas another 7 cases were shown to contain single clear cells or small groups of them, but their character could not be determined by electron microscopy.

The possible relationship of clear-cell transformation to the biological nature of osteosarcoma remains unexplained. Clear-cell chondrosarcomas have been acknowledged to represent tumours of low grade malignancy in relation to conventional chondrosarcomas (Bjornsson et al. 1984). The prognosis of chordoma, another skeletal tumour with a high glycogen content, is also quite favourable (Dahlin and Unni 1986; Huvos 1979; Schajowicz 1981). The problem has not been studied hitherto in the case of Ewing's sarcoma because of the small number of a cases with clear-cell transformation. Similar reasons have also prevented us from evaluating the problem in the case of osteosarcoma with a clear-cell component.

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