Virchows Arch A Path. Anat. and Histol. 389, 27–41 (1980)

Virchows Archiv A

Clear-Cell Chondrosarcoma

A Light- and Electron-Microscopic and Histochemical Study of Two Cases

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Summary. Two cases of clear-cell chondrosarcoma located in the upper end of the right femur of men aged 30 and 40 years are reported. The roentgenologic appearances suggested a chondroblastoma. Both patients are alive, one and four years after surgical removal of the tumor.

Glucosaminoglycans were studied with cationic dyes at different pH, with and without pretreatment with testicular hyaluronidase, and with the Scott technique at the light-microscopic level. Ultrastructurally, the glucosaminoglycans were studied with the high iron diamine and dialyzed iron techniques and glycogen with the PATCH-method. Light-microscopically, the tumors were characterized by clear vacuolated cells with distinct cytoplasm boundaries and scattered multinucleated giant cells of osteoclast type. Histochemical studies at the light-microscopic level indicate the presence of chondroitin 4- and 6-sulphate but no keratosulphate. Ultrastructurally, the predominant clear-cells showed features characteristic for chondroblasts. The cytoplasm showed areas lacking organelles and containing a low-density, finely granular matrix. These areas are considered to correspond to the clear cytoplasmic vacuoles seen under the light microscope. Most of the organelles were seen in the perinuclear region. The irregular tumor cells formed delicate protruding cytoplasmic extensions, which delineated intercellular spaces appearing as vacuoles under the light microscope. The benign multinucleated giant cells had an ultrastructural appearance typical of osteoclasts. Histochemical analysis at the electron-microscopic level showed the presence of sulphated glucosaminoglycans in the intercellular matrix and in association with the cytoplasmic membrane. Glycogen and non-sulphated acid glucosaminoglycans were found within the cytoplasm of the clear-cells.

Key words: Chondrosarcoma – Clear-cell chondrosarcoma – Glucosaminoglycans – Histochemistry – Ultrastructure.

Introduction

Sixteen cases of a rare cartilaginous tumor of low grade malignancy, called clear-cell chondrosarcoma, which originally had been considered benign, were

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0340-1227/80/0389/0027/$03.00

recently described from the Mayo Clinic (Unni et al. 1976). In several instances the chondroid matrix and foci of bone formation had led to an erroneous initial diagnosis of atypical chondroblastoma or osteoblastoma. Roentgenographically, the lesion was usually well defined and indistinguishable from chondroblastoma. However, the clear-cell chondrosarcomas occur in an older age-group than chondroblastomas. The designation clear-cell chondrosarcoma refers to the peculiar histological appearance, that is clear, vacuolated tumor cells with distinct cytoplasmic boundaries. A constant finding in these tumors was scattered, small, osteoclast-like giant cells. Very recently, Le Charpentier et al. (1979) reported five cases of clear-cell chondrosarcoma (one of which was included in the Mayo Clinic series) and briefly described the electron-microscopic appearance in three of them.

This paper describes two cases of clear-cell chondrosarcoma of the upper end of the femur, which were studied histochemically with respect to glucosaminoglycans and carbohydrates: One case was studied electron-microscopically and the histochemical analysis was in this case extended to the ultrastructural level.

Case Reports

Case 1. A thirty-year-old man, who suffered from poliomyelitis as a child, from which he recovered without any sequelae, consulted in April 1979 for pain in his right hip. The pain started one and a half years earlier after trauma to the right hip and gradually increased, so that he limped and had to use a cane. Finally the pain became so severe that he was unable to walk at all. At examination the mobility of the hip joint was normal. Plain radiograms disclosed a well delineated osteolytic area with partly sclerosed distinct borders within the head and neck of the right femur (Fig. 1). Angiography showed some delicate vessels within the osteolytic area and diffuse opacification. There were no signs of arteriovenous shunting.

In June 1979 the area was explored and the lesion curetted. The defect was filled with transplanted bone from the iliac bone. Since then no further surgery has been performed. The patient has been followed with repeated roentgenographic examinations and in September 1979 there is no sign of recurrence, and the transplanted bone has homogenized.

Case 2. A forty-year-old man, who for many years was active as a speedway driver, consulted in 1976 because of severe pain in his left hip. During the time he was active as a speedway driver he had sustained numerous traumata, including a tibial fracture and a hepatic rupture. He had also been operated upon for duodenal ulcer and for cholelithiasis. Before he sought medical care the pain had gradually increased over a period of several months, and he finally limped severely and had constant pain when walking or standing. Plain roentgenograms showed a large osteolytic area within the head and neck of the left femur (Fig. 2). This was well delineated, with partly sclerosed margins and a few delicate bony septa inside the lesion. The roentgenographic appearance was interpreted as a benign bone cyst. After one year, in March 1977, the osteolytic area was explored and the lesion was curetted. The histopathologic anatomic diagnosis was chondrosarcoma and the patient was referred to the Department of Orthopedic Surgery, Sahlgren's Hospital, Göteborg, for radical surgery. In May 1977, a left hemipelvectomy was performed. Since then the patient has been seen at regular intervals, without any signs of recurrence or metastases. At examination in April 1979 the patient is well and walks with a prosthesis without using a cane.

Pathology

Histological, Histochemical and Electron-Microscopic Methods

The surgical specimens were fixed in 4% formaldehyde solution and embedded in paraffin. Five-micron sections were stained according to the van Gieson method and with hematoxylin

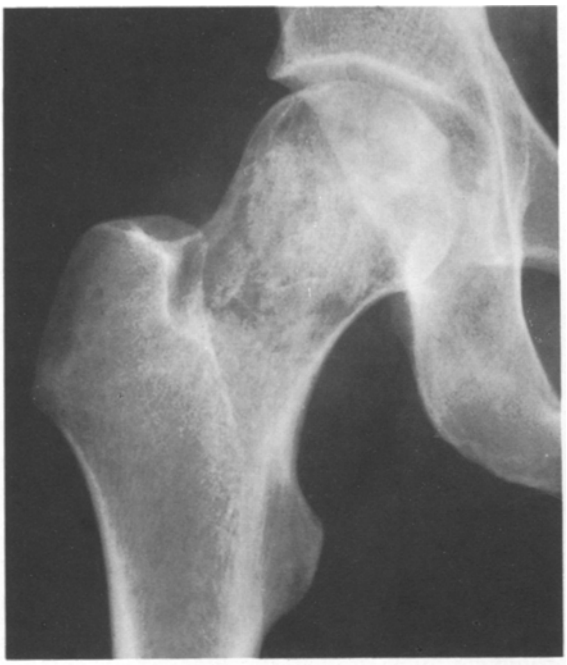


Fig. 1. Case 1. Plain radiogram of the right hip showing an osteolytic area within the head and neck of the femur

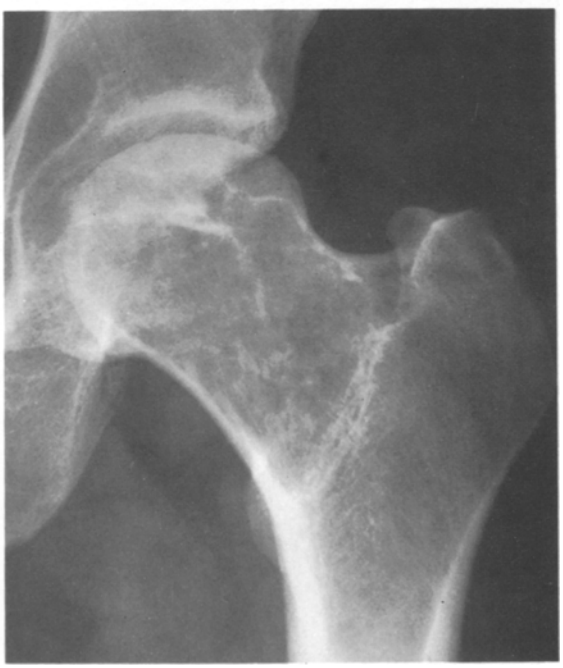


Fig. 2. Case 2. Plain radiogram of the left hip showing a sharply delineated osteolytic area with partly sclerotic margins within the neck and head of the femur

and eosin. Staining with Alcian blue at pH 2.5 and 1.0 and with toluidine blue at pH 4.0 and 1.0 was used for the demonstration of glucosaminoglycans, and the PAS method was used for studying vic-glycols. Pretreatment of sections with testicular hyaluronidase (hyaluronidase from bovine testes, type IV, Sigma) and staining with Alcian blue and toluidine blue at the above-mentioned pHs was used for further characterization of glucosaminoglycans. Staining with Alcian blue at controlled electrolyte concentrations for determination of the 'critical electrolyte concentration', according to Scott and Dorling (1965), was performed as previously described (Kindblom and Angervall 1975). Pretreatment of sections with diastase (2,800 u/g, E. Merck, Darmstadt, Germany) and staining with the PAS method was used for the identification of glycogen.

The concanavalin A – horseradish peroxidase – diaminobenzidine method (ConA-HRP-DAB) was performed with and without diastase digestion and with and without prior oxidation with 1% periodic acid (10 min) according to Katsuyama and Spicer (1978).

For electron microscopy, small pieces from one of the tumors (case 2) were immersed in 2,5% glutaraldehyde, washed in cold buffer, postfixed with 1% OsO_4 for 1 h, and thereafter dehydrated in ethanol, embedded in Epon 812 and cut in an LKB Ultrotome III. One micron thick sections were stained with toluidine blue, and thin sections were stained with uranyl acetate and lead citrate prior to examination in a Philips 400 electron microscope.

For ultrastructural cytochemistry cryostat sections of the specimen were stained with dialyzed iron (DI) (Wetzel et al. 1966) or high iron diamine (HID) (Spicer et al. 1978).

Some pieces of tumor were embedded without postfixation with osmium tetroxide and thin sections of these blocks were stained with the PATCH-SP-method (Thiery et al. 1967).

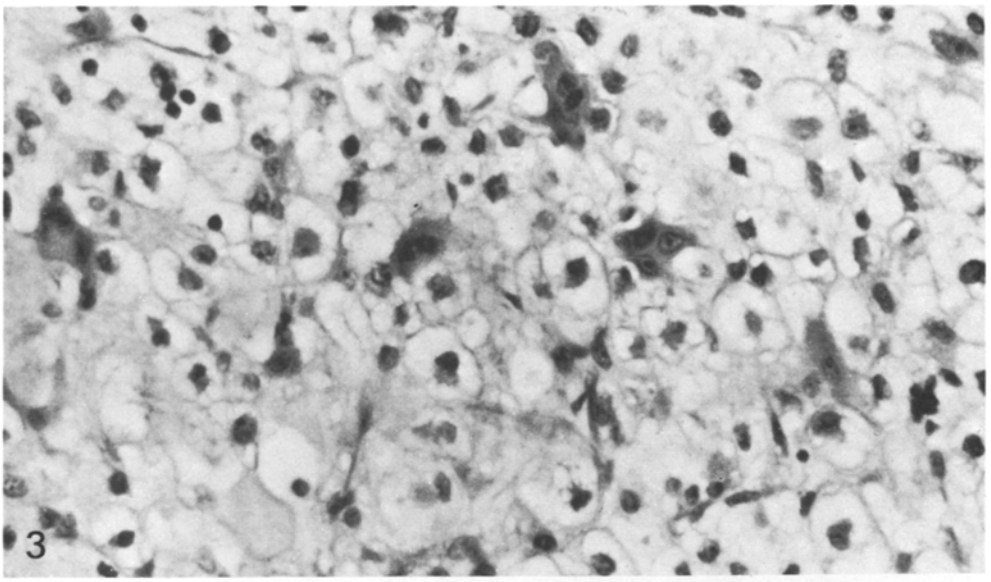
Light-Microscopic Appearance

Case 1. The tumor tissue had a prominent finely lobular appearance. The tumor cells had a centrally or eccentrically placed nucleus surrounded by empty vacuoles separated by cytoplasmic septa (Figs. 3 and 4). In some areas the tumor cells had a foamy cytoplasm without distinguishable separated vacuoles. Cellular and nucelar pleomorphism was relatively slight; very few mitotic figures could be found. Multinucleated osteoclast-like giant cells were seen alone or in small groups (Figs. 3 and 5). Most of these cells were relatively small with, usually, 5–15 small, uniform nuclei. Occasionally these osteoclast-like giant cells showed signs of phagocytic activity and contained up to 40 nuclei. Some areas showed a transition towards the appearance of myxoid chondrosarcoma of bone. There were few foci containing chondro-osteoid tissue, which was at least partly calcified. Between the small tumor lobules there was a sparse vascular stroma containing mainly delicate capillary-like vessels and occasional wide, thin-walled, angulated vessels.

Fig. 3. Case 1. Solid tumor area with clear cells and scattered small multinucleated giant cells of osteoclast-type. H&E, ×440

Fig. 4. Case 1. Toluidine blue-stained 1 micron thick Epon-section of clear-cell chondrosarcoma showing multivacuolated tumor cells, somewhat resembling lipoblasts, and showing distinct nuclei and nucleoli. The dark staining of the intercellular matrix is due to metachromasia. The giant cells are deeply orthochromatically stained. ×900

Fig. 5. Case 1. Clear-cell chondrosarcoma with an area showing a hyaline tumor matrix and osteoclast-like giant cells in the border zone giving a somewhat chondroblastoma-like appearance. H&E, ×110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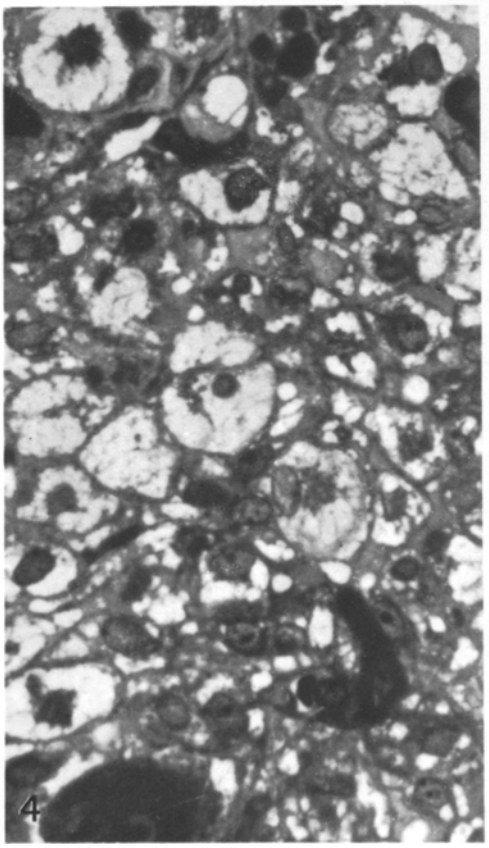
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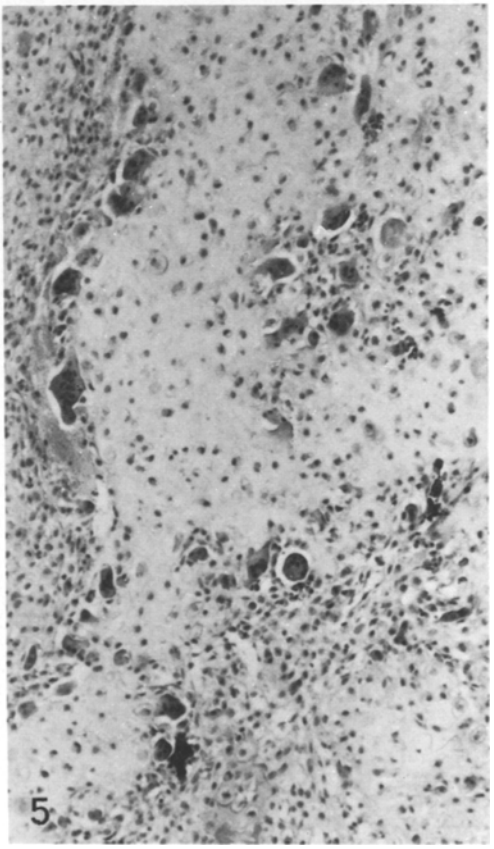
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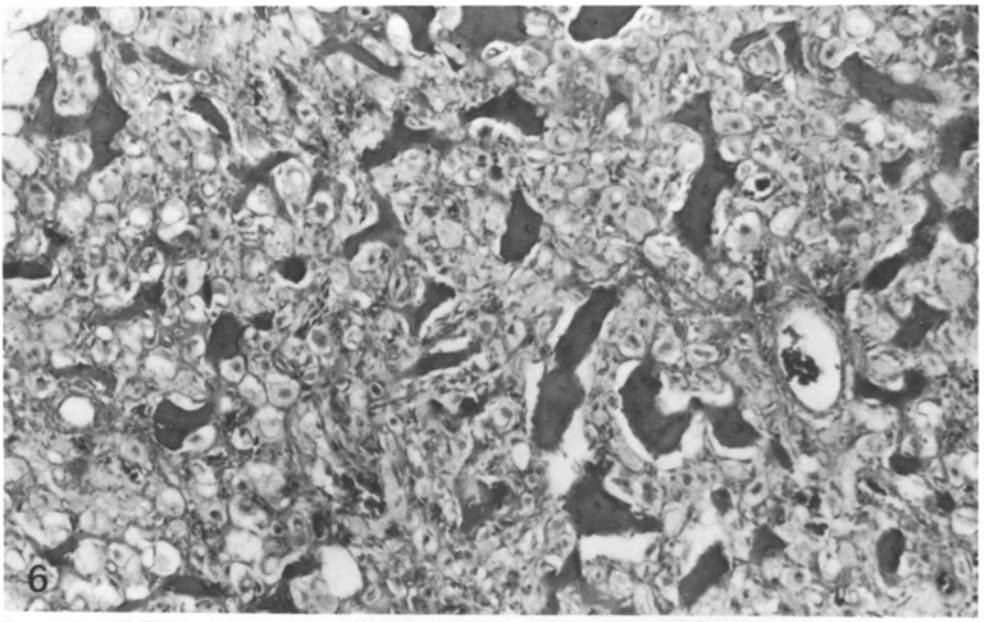

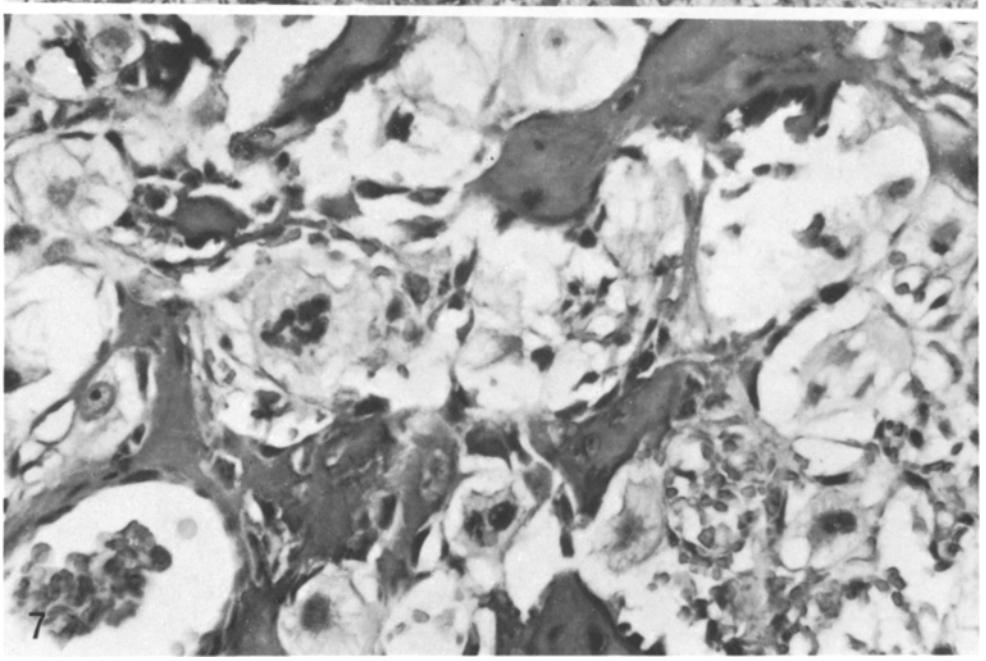

Fig. 6. Case 2. Area showing clear-cell chondrosarcoma growing within bone supposed to be host bone. H&E, ×150

Fig. 7. Case 2. Neoplastic large clear cell within a highly vascular stroma between osteoid trabeculae, lined by osteoblasts – somewhat osteoblastoma-like appearance. A few tumor cells have two or more nuclei. H&E, ×440

Case 2. The curetted tissue had the appearance of regressively changed chondro-osteoid tissue with uneven calcification in some areas. The chondrocytes had a pale, multivacuolar cytoplasm and a centrally or excentrically located nucleus. In some areas there were large, vacuolated "spider-web" cells with a distinct, finely granular cytoplasm around the nucleus, resembling those of rhabdomyoma of adult type.

In small areas a transition towards an osteoblastoma-like appearance was seen, with highly vascular stroma containing multivacuolated tumor cells, as in the hyaline chondro-osteoid areas (Fig. 6). Especially in these areas, finely multivacuolated cells were seen which often had an appearance similar to atypical lipoblasts (Fig. 7). The osteoid in these areas was outlined by small osteoblasts and bone trabeculae contained mature osteocytes and signs of calcification. Throughout the tumor tissue there were scattered, small, multinucleated osteoclast-like giant cells.

The operative specimen obtained at hemipelvectomy showed multiple residues of tumor tissue of the same appearance as in the curetted material. The tumor tissue grew diffusely in the medullary bone. Apart from the typical multivacuolated mononuclear tumor cells, there were occasional bi – or multinucleated cells with a similar cytoplasm. The nuclei often had one distinct nucleolus. In some areas the tumor cells were more chondroblast-like, without distinct cytoplasmic vacuoles. Scattered, small, osteoclast-like cells were also seen in this specimen. The pleomorphism was slight or moderate, only occasional cells showing large, hyperchromatic nuclei with prominent nucleoli, and mitotic figures were very sparse.

Electron-Microscopic Appearance

Ultrastructurally, the clear cells predominated. The nucleus was mostly centrally situated and showed a single nucleolus and heterochromatin condensed at the periphery. The nuclei had a rounded, oval and often cleft appearance (Fig. 8). Occasional cells of this type contained two nuclei. A constant finding in the cytoplasm were areas lacking organelles and containing a low-density, finely granular matrix (Figs. 8 and 9). At low magnification these areas gave the impression of multiple cytoplasmic vacuoles. However, there were no delineating membranes separating these clear areas. A rim of denser cytoplasm containing short segments of rough endoplasmic reticulum was often seen at the periphery. Most of the rough endoplasmic reticulum was seen in the perinuclear region and formed parallel, narrow tubular structures or dilated cisternae. The mitochondria were rounded or filamentous in type and often closely associated with the rough endoplasmic reticulum (Figs. 8 and 9). Distinct Golgi zones were seen in the perinuclear areas.

The tumor cells had an irregular shape with microvillous projections and showed delicate cytoplasmic extensions into the wide intercellular spaces (Figs. 9 and 10). These extensions from neighbouring cells delimited spaces which, at low magnification, had the appearance of extracellular vacuoles. The content

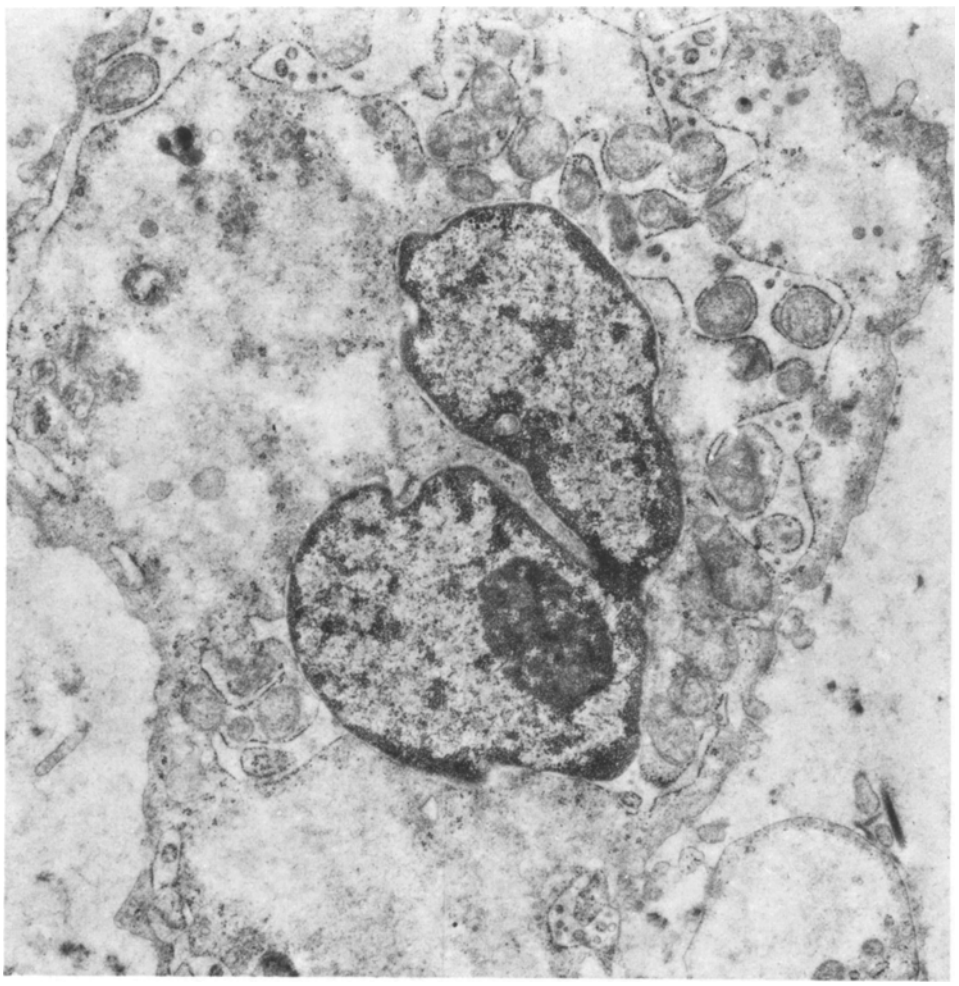

Fig. 8. Case 1. Clear-cell with pale-staining "clear" cytoplasmic areas without organelles and rounded mitochondria protruding into wide cisternae of RER. The cleft nucleus contains a prominent nucleolus. × 10,000

of these extracellular spaces had the same ultrastructural characteristics as the clear areas within the cells.

The benign multinucleated osteoclast-like cells were easily recognized and showed characteristically a ruffled border (Figs. 10 and 11). The cytoplasm contained an abundance of mitochondria, which were ovoid or elongated and showed a dense matrix. The rough endoplasmic reticulum was sparse and appeared mostly as parallel membranes. There were numerous small vacuoles and vesicles within the cytoplasm. These giant cells usually showed two to four nuclei, which were small and uniform and had a distinct small nucleolus and evenly distributed chromatin.

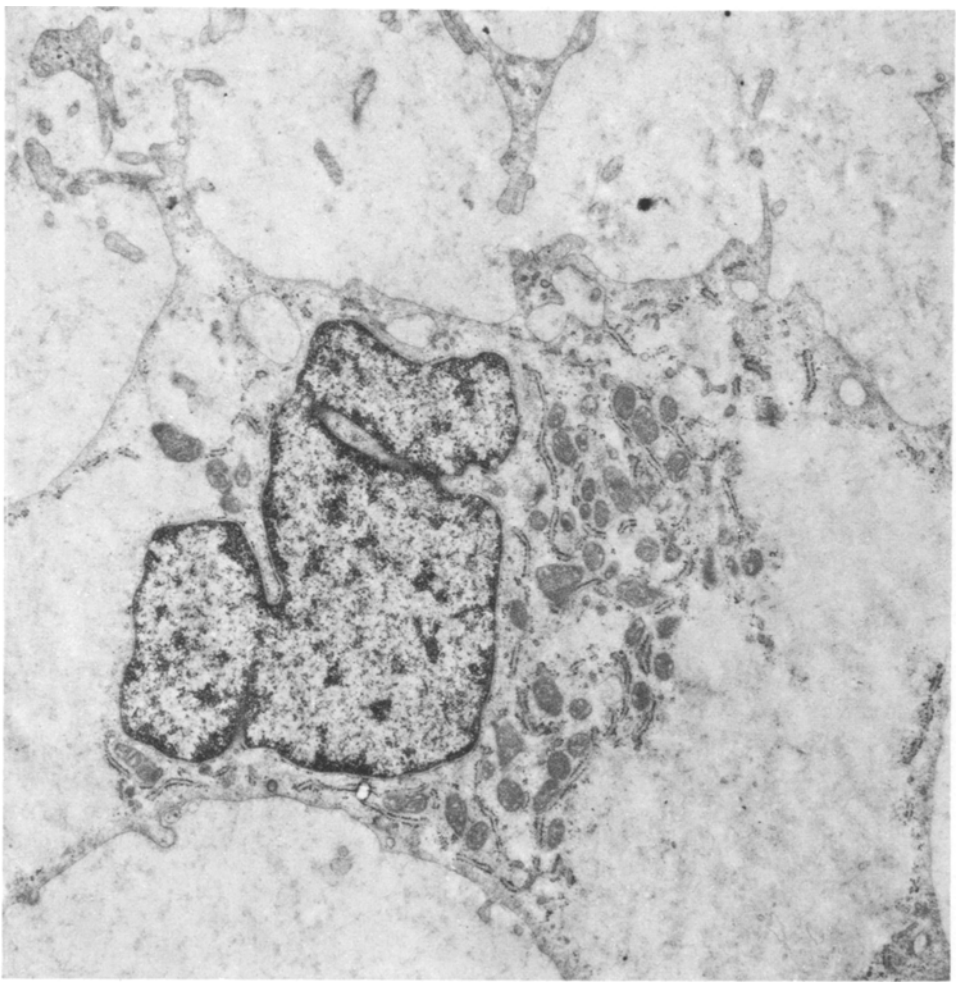

Fig. 9. Case 1. Clear-cell with an irregularly indented nucleus and perinuclear mitochondria and RER. The main part of the cytoplasm consists of a light-staining ground substance without organelles. Microvillous projections and delicate extensions are seen, the latter delimiting extracellular spaces (in low magnification appearing as "vacuoles"). × 7,000

The intercellular spaces contained very few collagen fibers. The capillaries within the tumor tissue had a normal appearance and were closely associated with the tumor cells.

Histochemical Study

Histochemical staining for characterization of the glucosaminoglycans and carbohydrates, such as glycogen, gave the same results in both cases. Staining

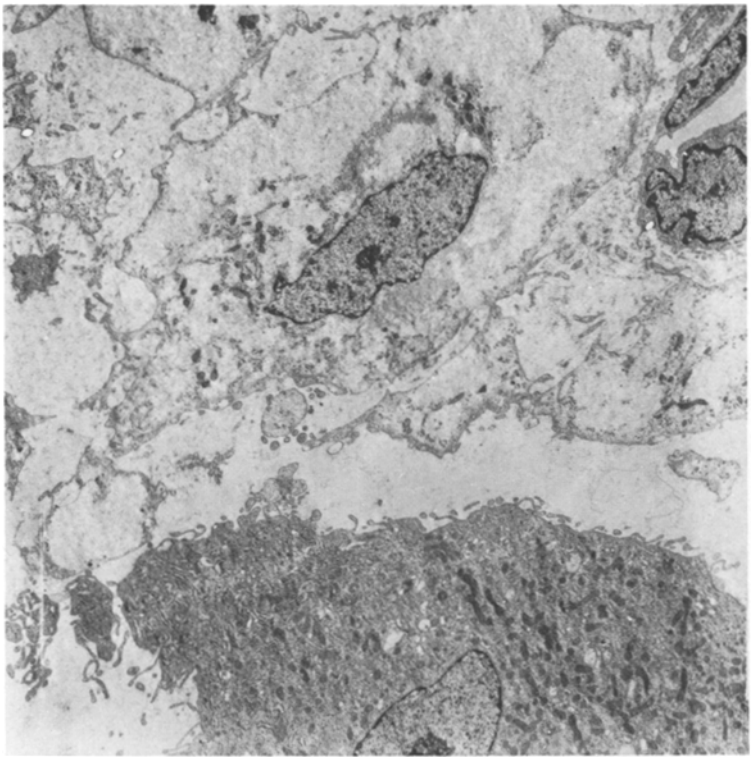

Fig. 10. Case 1. A clear-cell associated with a dark-staining multinucleated giant cell of osteoclast type (*below*) and a capillary (*top right*). ×3,000

with Alcian blue gave a positive stain, mainly of the intercellular matrix and the cytoplasmic borders, at both pH 2.5 and pH 1.0. Staining with toluidine blue gave a strong metachromatic reaction of the same structures at both pH 4.0 and pH 1.0, both results indicating the presence of sulphated glucosaminoglycans. The Alcian blue staining and the metachromasia after staining with toluidine blue were eliminated by prior digestion of the sections with testicular hyaluronidase. The critical electrolyte concentration after staining with Alcian blue at different concentrations of $MgCl_2$, according to the method of Scott, was 0.55 M. The latter two results are compatible with the presence of chondroitin 4- and 6-sulphate, but do not indicate the presence of keratosulphate.

The clear cells contained abundant finely granular, PAS-positive material, which was almost completely eliminated by prior digestion of the sections with diastase. With the ConA-HRP-DAB method the cytoplasm of the clear cells was stained intensely brown as was the cytoplasmic membrane; prior diastase digestion did not significantly impair the staining of the cytoplasm.

Oxidation with periodic acid (PA) prior to the ConA-staining only slightly reduced the intensity of the staining; diastase digestion prior to this PA-ConA sequence almost completely abolished the staining.

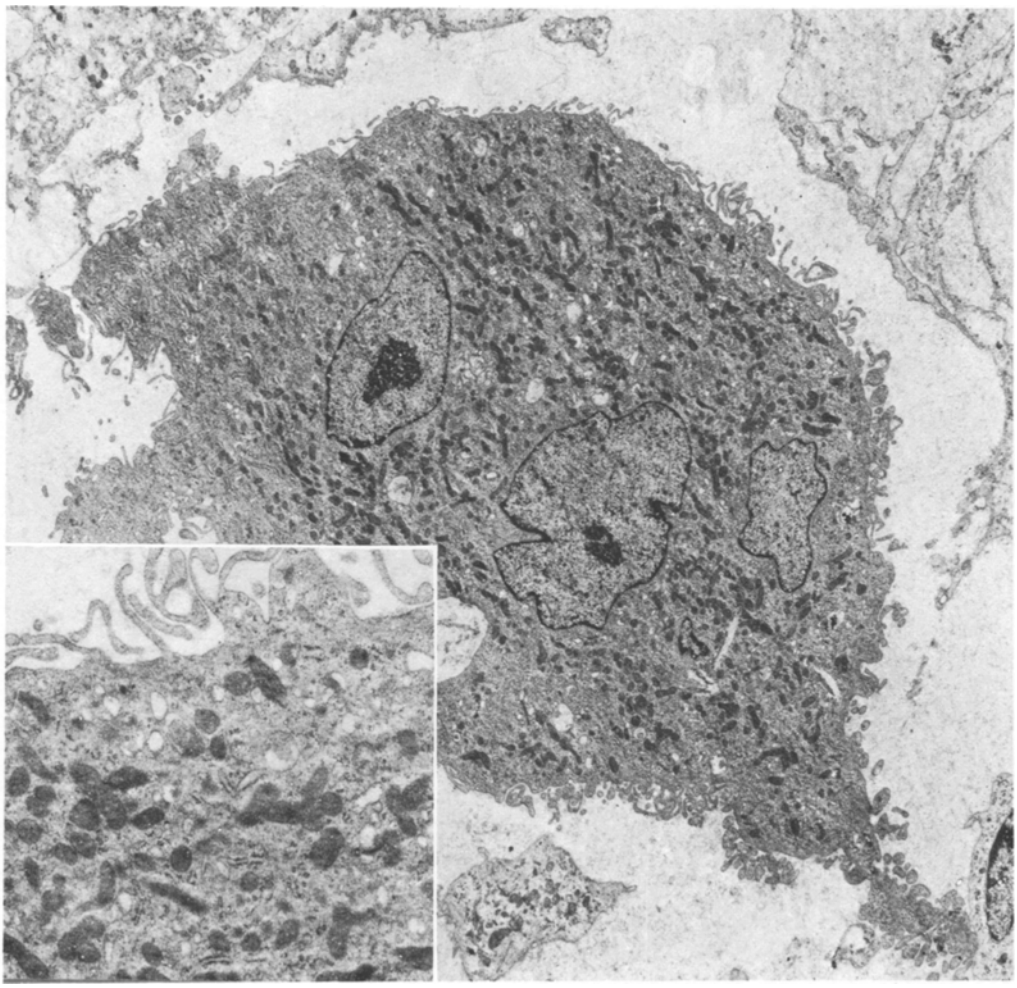

Fig. 11. Case 1. Survey electron micrograph of a giant cell with parts of four nuclei, two of which have distinct nucleoli. Inserted (*below left*) is a detail of the giant cell showing the characteristic ruffled border and numerous mitochondria. ×4,000 and 12,000 respectively

The pieces of tumor incubated with dialyzed iron (DI) and examined ultrastructurally showed a distinct and strong staining of the cytoplasmic membranes and the intercellular matrix. The intercellular matrix showed a granular or network-like stain. Within the cytoplasm of the tumor cells there were moderately stained areas, which in counterstained sections could be identified as dilated cisternae of rough endoplasmic reticulum and vesicles of Golgi zones (Fig. 12).

Pieces of tumor incubated with high iron diamine (HID) mostly showed staining of the intercellular matrix corresponding to that of DI-stained specimens. They also showed staining, although less prominent, of the cytoplasmic membrane, but no staining of the internal cytoplasm (Fig. 13).

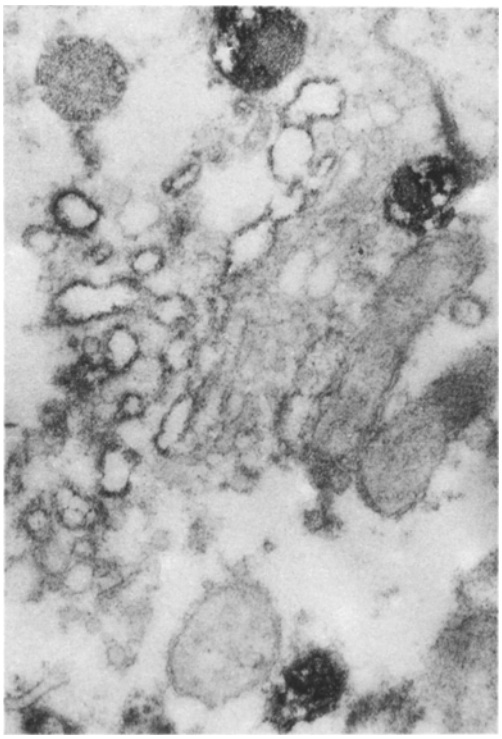

Fig. 12. Case 1. Smooth membrane vacuoles and vesicles in a Golgi zone positively stained after incubation in dialyzed iron, indicating the presence of acid glucosamonoglycans (no contrast staining). ×30,000

Thin sections stained with PATCH for ultrastructural demonstration of vic-glycols showed abundant positively stained material, apparently glycogen, diffusely or in patches in the cytoplasm, both within the clear areas and in the more organell-rich perinuclear areas (Fig. 14). This PATCH-positive material never formed a major of the clear areas.

Discussion

The two tumors had a roentgenographic and light-microscopic appearance consistent with the findings in the Mayo Clinic series of clear-cell chondrosarcoma (Unni et al. 1976). Seven out of twelve patients with roentgenograms available for review in that series had an osteolytic expansive lesion at the upper end of the femur, as had both patients in this series. Both tumors presented as well defined lesions with a benign roentgenographic appearance resembling that of chondroblastoma. In the Mayo Clinic series there were clear-cell chondrosarcomas resembling histologically, in some respects, benign bone lesions such as osteoblastoma, aneurysmal bone cyst and, most of all, chondroblastoma. Interestingly, in our case 2, the tumor showed areas with a somewhat osteoblastoma-like appearance. Apart from this, the two tumors showed the characteristic appearance of clear-cell chondrosarcoma: a solid tumor of clear vacuolated

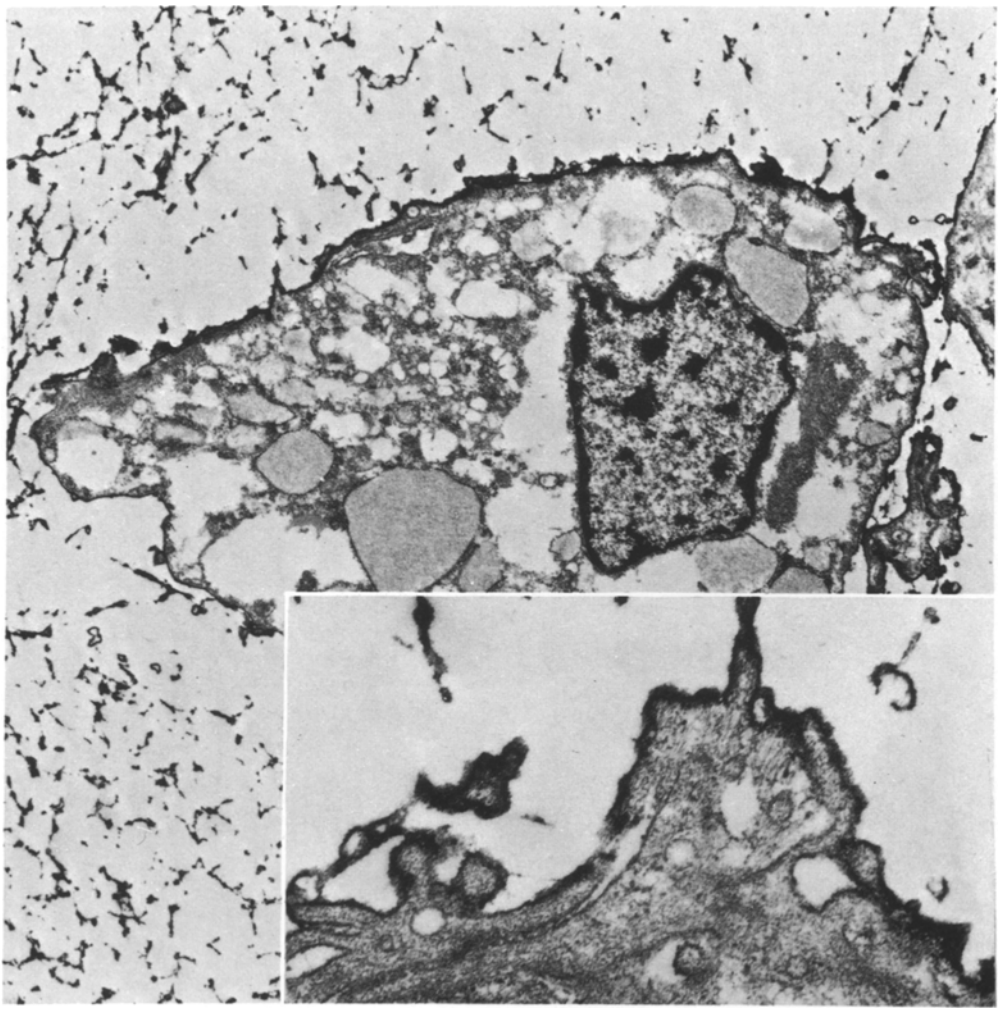

Fig. 13. Case 1. High iron diamine incubated tissue indicating the presence of sulphated glucosaminoglycans in the cytoplasmic membrane (*detail inserted*) and in the reticular extracellular matrix (contrast stained with uranyl acetate and lead citrate). × 7,000 and 30,000

rounded or polygonal cells with distinct cytoplasmic boundaries and scattered, relatively small, multinucleated giant cells of osteoclast type. The finding of more clearly chondromatous areas and the demonstration of sulphated glucosaminoglycans are further support for a cartilaginous origin of the tumor.

Histochemical analysis at the light-microscopic level showed the presence of abundant acid sulphated glucosaminoglycans, predominantly in intracellular spaces. The results of the examination with the Scott technique and enzyme digestion are compatible with the presence of chondroitin 4- and 6-sulphate,

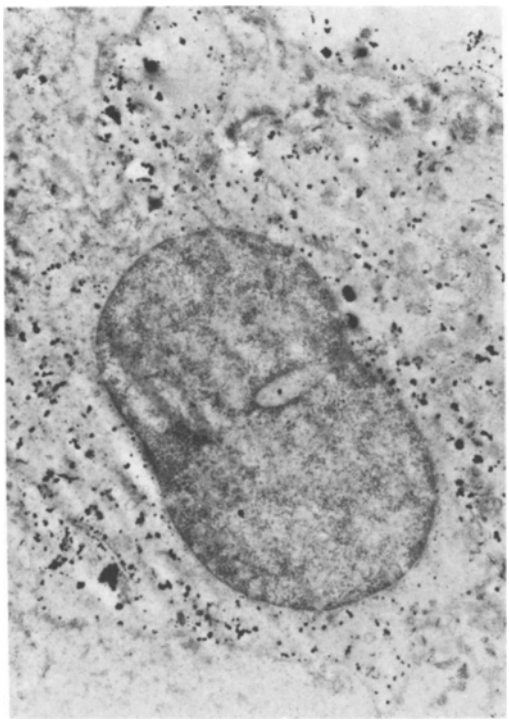

Fig. 14. Case 1. PATCH-SP-stained thin section of a clear cell showing the rather evenly distributed glycogen (non-osmificated, no contrast staining). ×7,000

while no keratosulphate was demonstrated. The PAS and ConA-HRP-DAB stainings (with and without prior PA-oxidation) performed on untreated and diastase treated sections indicate the presence of cytoplasmic glycogen (Katsuyama and Spicer 1978). The results of the histochemical study at the ultrastructural level indicate the presence of sulphated glucosaminoglycans within the intercellular matrix and intimately associated with the cytoplasmic membrane, whereas the internal cytoplasm contained non-sulphated acid glucosaminoglycans, very possibly hyaluronic acid.

In the brief ultrastructural description of the clear-cell chondrosarcoma by Le Charpentier et al. (1979), the tumor cells were described as "normal or tumorous chondroid cells" and it was stated that "the clear cytoplasm results from the abundance of glycogen". In the present study both the light- and electron-microscopic examination showed the abundant glycogen to be rather evenly distributed throughout the cytoplasm, while the characteristic vacuoles seen under the light microscope seemed to correspond to the areas lacking organelles and containing a low-density, granular material. The clear-cells showed besides the abundance of glycogen other features characteristic for chondroblasts: microvilli, cisternae of rough endoplasmic reticulum, and Golgi zones, as described in ordinary chondrosarcomas of bone (Schajowicz et al. 1974).

The multinucleated giant cells showed characteristically a ruffled border, abundance of mitochondria and numerous small cytoplasmic vacuoles and vesicles, and small uniform nuclei, i.e. features described as characteristic for osteo-

clasts of tumors such as osteoclastoma and osteoblastoma (Aparisi et al. 1977; Aparisi et al. 1978; Aparisi et al. 1979).

Neither of the two tumors has recurred or metastasized after surgical removal of the tumor; however the follow-up period is short. The studies by Unni and coworkers and by Le Charpentier and coworkers both indicate a favorable prognosis and slow tumor growth. Of altogether 20 cases previously known from the literature, only five have died from the tumor disease; interestingly, one of them had a recurrence of a dedifferentiated chondrosarcoma.

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Accepted July 10, 1980