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Non-Ossifying Fibroma of Bone

A Histochemical and Ultrastructural Characterization

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Summary. The microscopic morphology, histochemistry and electron microscopy of a non-ossifying fibroma of the upper metaphysis of the femur in a sixteen-year-old girl, is presented.

The authors describe a fibroblastic cell type as the basis of the neoplasia which transforms itself into a foam cell loaded with lipids. Histochemically it is characterized by high activity in alkaline phosphatases, ATP-ases, fructose 1–6 diphosphatase and NADH-NADPH tetrazolio reductases. Electron microscopy identifies extremely active fibroblastic cells with a synthesis of proteic and lipid material which transforms them into foam cells. The lipids appear irregularly enveloped in laminar systems. There is also a deposit of hemosiderine in certain fibroblasts.

Non-ossifying fibroma differs from the metaphyseal fibrous bone defect as the fibroblasts show a higher activity for alkaline phosphatase and lipids are stored in the cytoplasma.

The present report deals with an histochemical and electron-microscopical study of a case of non-ossifying bone fibroma (Jaffe and Lichtenstein, 1942). It demonstrates the morphological characteristics of a tumor displaying a peculiar variety of alkaline phosphatase positive fibroblasts in whose cytoplasm, lipids are also produced and progressively stored.

Case Report

A sixteen-year-old girl (Clinical Record No. 29265) with no prior history of trauma complained of a pain which had been present in her hip for a period of three and half months. Roentgenograms revealed a radiolucent multioculated area involving the entire upper metaphysis of the femur with a secondary extension to subtrocantereal and cervical areas of the bone. The cortex delimiting the lesion was thinned but there was no discontinuity or rupture of the periostium (Fig. 1). A complete skeletal survey and extensive laboratory studies revealed no other abnormality.

A surgical section of the tumor presents an osseous cavity filled with firm reddish-brown tissue. After curettage the cavity was packed with autogenous cancellous bone chips. The post-operative course (20 months to date) has been excellent with no recurrence of symptoms.

The material from the present case is studied in comparison with a histochemical and electron microscopical analysis of two cortical bone defects as well as with a regeneration tissue scraped from the osseous cavity of the femur in a female patient who had been operated on two months before (simple curettage) for an osteoclastoma (giant cell tumor).

For *optical microscopy* fragments of tumor tissue were paraffin embedded and stained with hematoxilin-eosin, P.A.S., trichromic of Masson and reticuline of Gomori. Small analogous

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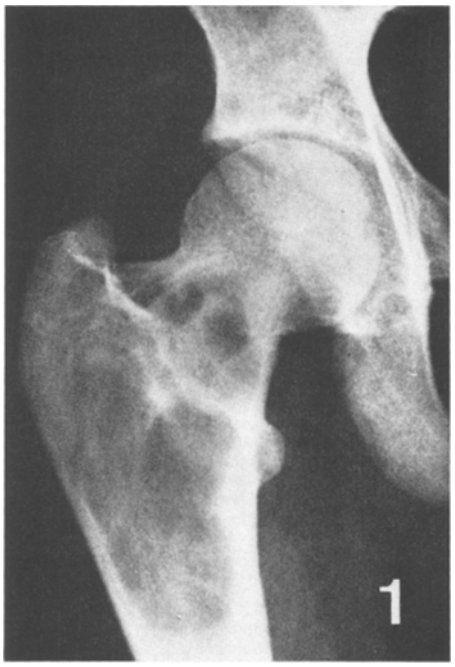


Fig. 1. Roentgenogram showing a radiolucent multiloculated area involving the upper metaphysis of the femur

fragments of tissue were frozen in carbon dioxide snow and sections were performed in an IEC cryostate without prior fixation. As previously described (Llombart *et al.*, 1970), the following histochemical techniques were employed: Alkaline and Acid Phosphatase; Glucose-6-phosphatase; Fructose-1-6-diphosphatase; ATP-ase (pH 9.4); Succinic dehydrogenase activated with menadione; Isocritic dehydrogenase; NADH and NADPH tetrazolium reductase. In addition, histological checks were made for Sudan III, for cholesterine by the Schultz-Liebermann techniques, hemosiderine (Pearl's reaction) and P.A.S. Nitro-blue tetrazolium was used as hydrogen acceptor. Enzymatic activity was evaluated as follows: none (−), low activity (+), moderate activity (++), and intense activity (+++).

Electron Microscopy. Several fragments of different areas of the tumor were cut into approximately 1 mm blocks and fixed in glutaraldehyde and osmium tetraoxide (OsO_4); the tissue blocks were then rapidly dehydrated in a graded solution of etanol-acetona, immersed in propilene oxide and embedded in Durcupan ACM (Fluka A.G.). Thin sections of tissue cut in an LKB ultramicrotome were placed on copper grills and stained with uranil acetate or lead citrate before being examined under a Jeol JEM-100 B electron microscope.

Results

A. Macroscopical Pathology and Histology

The tumor is made up of consistent fibrous connective tissue, with shells of sclerotic bone between tumoral areas. The microscopial picture of the tumor consits of bundles of firm connective tissue. Most cells are spindle-shaped, packed in close unions and associated with abundant collagen material. Occasionally they adopt a perivascular disposition with cells radiating from the perivascular area.

Most of the tumoral cells possess spindle-shaped contours. The nuclei are elongated with dense chromatine and small nucleolus. There are sparse cytoplasma with some vacuolated inclusions, Sudan positive of lipid nature. From cells, filled with lipoid like material and resembling macrophages, are associated in nests. A transition can be observed between spindle fibroblast and lipophage-like cells of a more xanthomatous nature with a progressive accumulation of Sudan positive lipids in the cytoplasm. No multinucleated giant cells could be detected. Interstitial hemorrhages are rare and deposits of hemosiderine exist in the interstices of the stroma as well as, on occasions, in certain mononucleated fibroblast. Bone formation is completely absent in the lesion itself.

B. Histochemistry

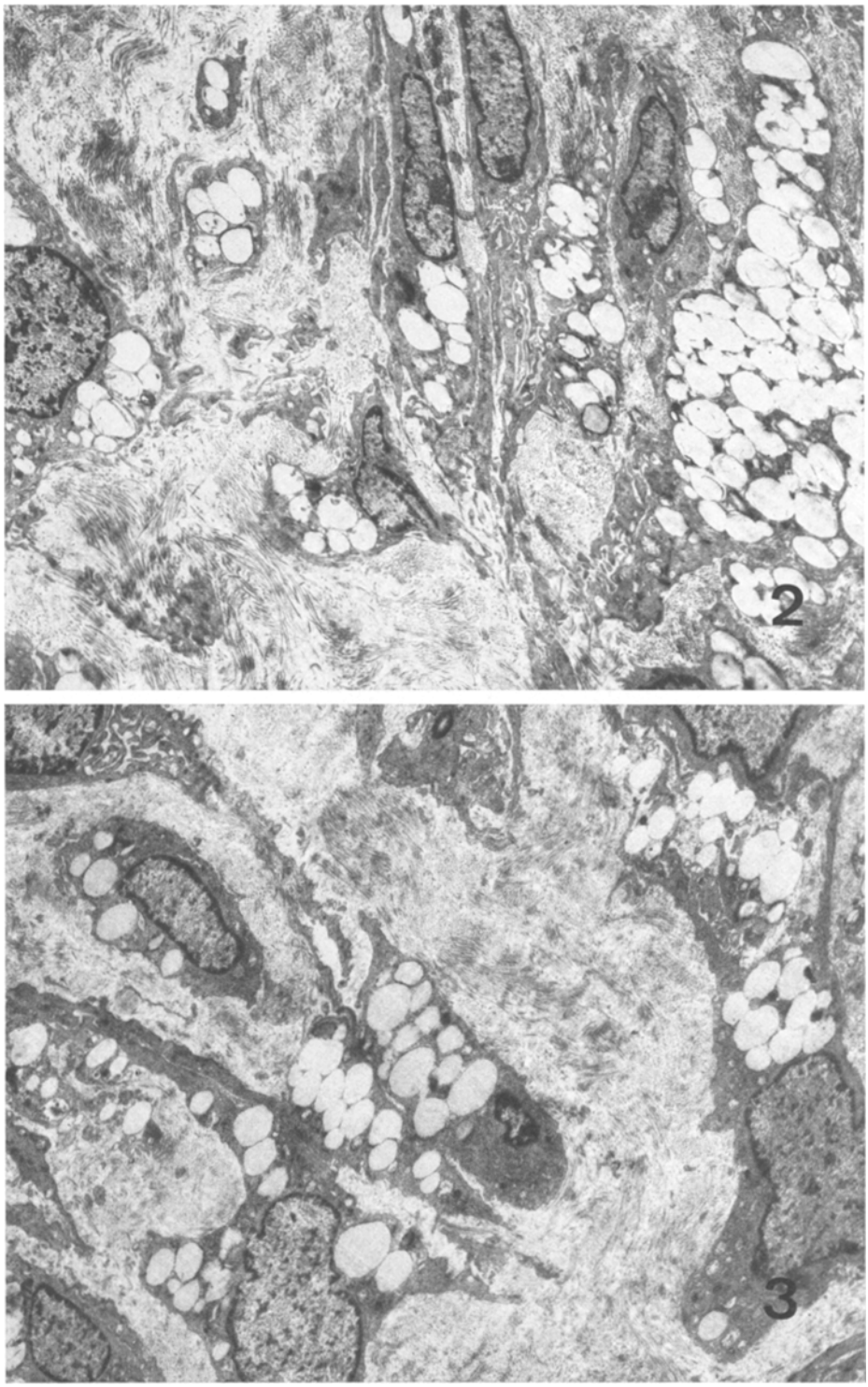
The tumoral cells possessed strong enzymatic activity for alkaline phosphatase and ATP-ase, pH 9,4. The presence of fructose 1–6 diphosphatase was also evident. There was a total absence of glucose 6 phosphatase and very low acide phosphatases, which were only evident in certain fibroblasts. All dehydrogenase systems were present on the cytoplasma of the neoplastic cells, but at different degrees of intensity. A high activity was evident for NADH and NADPH diaphorases.

Sudan positive lipids abound in the tumor and, at times, can be detected as cholesterine rich lipids. Cytoplasmic sudanophilia are more striking in the foam cells but are also visible, as small lipoid droplets, in the fibroblasts. No PAS positive material could be found. A deposit of a low quantity of hemosiderine particles exists in the interstitium of the stroma as well as in the cytoplasm of some tumoral fibroblasts.

C. Ultrastructural Findings

The tumor is provided with a great many cells associated to an abundant fibrillar stroma and a number of capillaries. Low power electron micrographs show two cell patterns as seen under optical microscope (Figs. 2 and 3). One of these cells is spindle-shaped with an elongated cytoplasma, irregular profiles and sharply delineated borders resembling fibroblasts. The second type of cell is more polygonal in shape and contains a large number of lipid droplets inside the cytoplasma. Between the fibroblast-elongated and lipid rich foamy varieties, there exists an intermediate or transitional type of cell, also of a more or less polygonal contour, whose cytoplasma contains a large number of organelles, but in which, at the same time, small droplets of irregularly distributed lipids are to be seen. The nucleus is either elongated or round-oval according to the amount of lipid stored in the surrounding cytoplasma. No junctions or desmosomes exist between any tumoral cells but the cytoplasmic processes are sometimes close to one another.

Extracellular space is formed by a large number of more or less mature fibrillar structures of collagen, with typical periodicity, associated in relatively compact bands of fibers, with amorphous material filling the interstices. In some areas, there is a perivascular distribution of the neoplastic cells. Tissue is rich in capillaries possessing a continous endothelium in close contact to perithelial cells. No inflammatory elements are present in the tumor. Only very occasional lymphocites can be detected within the stroma in the perivascular areas. When studied by electronic



Figs. 2 and 3. Ultrastructural aspects of tumoral fibroblasts, some of them located with lipids. Extracellular spaces with large number of fibrillar collagen. × 3000

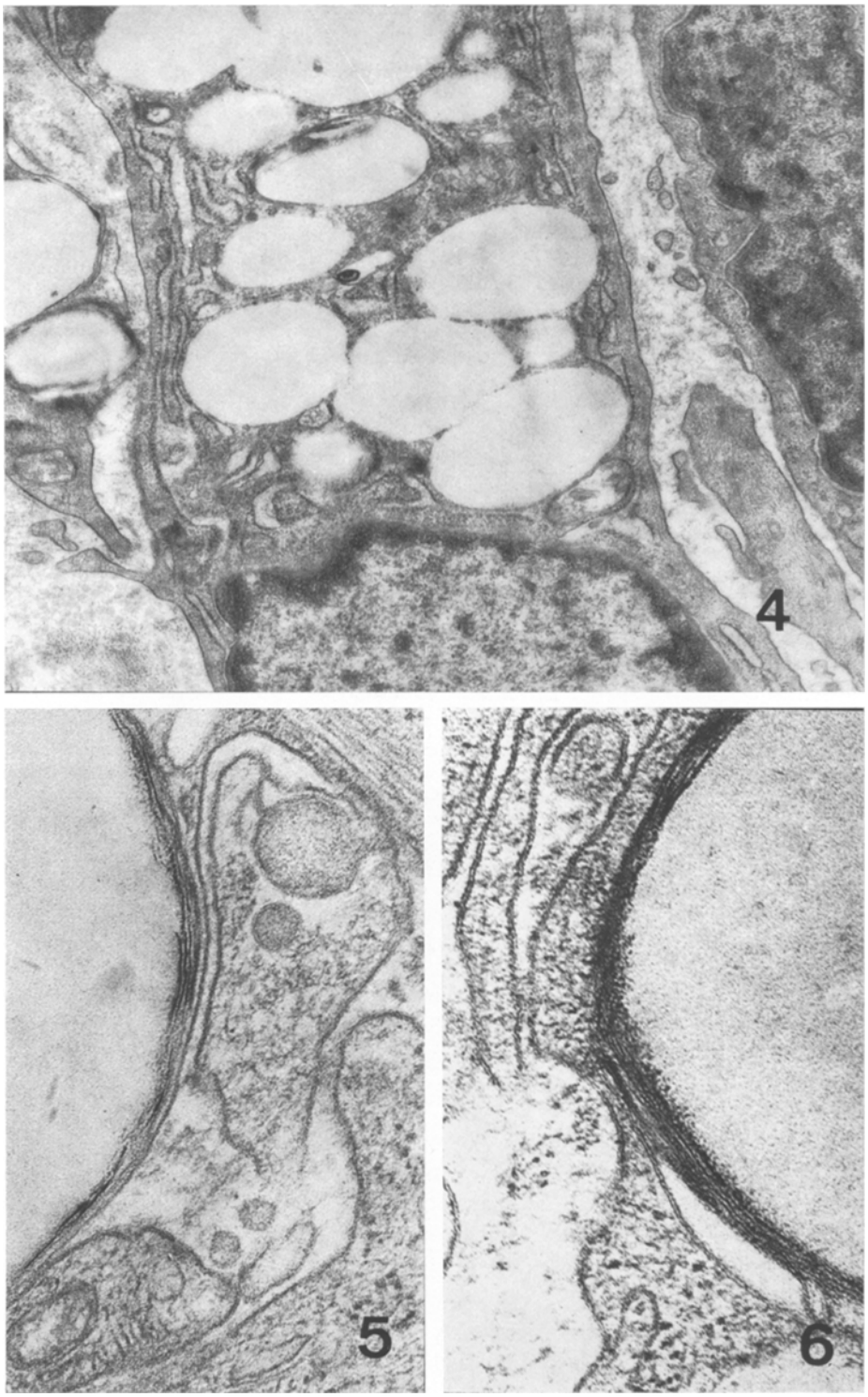


Fig. 4. Tumoral fibroblast with highly developed granular endoplasmic reticulum in synthetic activity and in close association with lipid droplets. × 10000

Figs. 5 and 6. Two close views of vacuoles in which filaments and membranes of the agranular endoplasmic reticulum engulf lipid droplets. × 60000

means, hemosiderine inclusions are commonly found in the interstices. Certain intracellular deposits are also to be seen in the fibroblast of the tumor. There has been no sign of cells of a histiocytic structur.

Fibroblasts are dominant in number and situated in close contiguity to other cellular elements of the neoplasia which appear to derive from the former. This fibroblast is the basic structure of the present lesion. It possesses an elongated nucleus provided with occasional infoldings of the membrane; the chromatin is dense at the periphery, the nucleolus usually prominent and highly active. The cytoplasma is well developed, with abundant organelles, and large at the perinuclear area. Digitations, projections and irregular elongations of the cytoplasma are evident. The cytoplasmic organelles are typical of the mesenchymatous fibroblastic cell. There is an extremely well-developed granular endoplasmic reticulum (REG) as well as cisterns and profiles of a granular endoplasmic reticulum (RE) which dilate at times and are filled with an amorphous homogeneous material. Golgi complexes, usually prominent, are located in the perinuclear area. Many filaments, either irregularly distributed or in compact bands, fill almost all the hialoplasma of the cell (Fig. 4).

Most of the cells are actively secretory, as they possess a large number of REG profiles in close association to free ribosomes in continuity with the dilated cysterna of the RE located at the periphery of the cytoplasma near the cell membrane. No extrusion of secretory material could be detected, but close contiguity exists, at times, between these cytoplasmic areas and the extremely immature young fibers (with a periodicity for collagen) located in the extracellular spaces and filling the interstices inside an amorphous material. The present structures possess a marked resemblance to the already well-known production of pre-collagen material elaborated inside the REG of the fibroblast in mesenchymatous tissues.

Lipid droplets of a low dense homogeneous material are also present in a great many tumoral cells. The transition from mesenchymal fibroblast-like cells to the more polygonal lipid rich foamy structures takes place through an intermediate type of cell in which lipids are manufactured and progressively stored. The lipid rich cell shows a complete loading of the cytoplasma by droplets in close contiguity to one another. There exists an occasional fusion of droplets to form larger vacuolated structures, but most of them remain isolated from one another, there by retaining structural independence (Fig. 4). We have detected a relation between the RE and lipid droplets, since the latter seem to be manufactured inside the membranes of this endoplasmic reticulum. The lipid inclusions are sometimes encircled by a filamentous matrix adopting a more or less defined laminar structure (Fig. 5 and 6) engulfing the lipid droplet. This filamentous laminar formation is in continuity with the RE.

As in the more mesenchymal fibroblastic tumoral cells, secretory activity persists in those cells that have adopted a more foamy appearance. There exists, therefore, morphological evidence for a progressive transition of the tumoral cells from the fibroblast variety to the more foamy lipid rich polygonal cells in the same area investigated.

Hemosiderine particles are seen in fragmented fashion between the fibrillar stroma as dense homogeneous bodies. Some fibroblasts with highly developed REG, having electron-dense inclusions mimicking hemosiderine particles inside the mitochondria are also present.

Discussion

The matrix-cell of the non ossifying fibroma is the fibroblast. Jaffe (1958) also defends the theory that the basic element of the tumor is the bone marrow fibroblast and that this cell may undergo lipid inhibition and adopt a foamy appearance. Our electron-microscopic studies confirm this hypothesis, since the foam

cells are not of a lipophagic nature (histiocytic cells) but mesenchymal fibroblast with a progressive intracytoplasmatic overload of lipids.

The histochemistry of the tumoral fibroblast is also characteristic, showing an extremely high content for alkaline phosphatase and ATP-ase, which are supposed to be enzyme exclusively active in the osteoblast and in boneforming tumors (Jeffree and Price, 1965; Schajowicz, 1972; Peydro and Llombart Bosch, 1972). Our own experience in two unpublished cases of fibrous cortical bone defect with similar histochemical techniques confirm a lower activity for alkaline phosphatase present in the fibroblast of this lesion. Nevertheless, Jeffree (1972) has shown how the activity of the fibroblasts in alkaline phosphatase such as fibrous dysplasia, non-osteogenic fibroma, or osteosarcoma depends essentially on their proliferative capacity. The more rapidly these fibroblasts proliferate the higher is their alkaline phosphatase content.

The fibroblast involved in non-ossifying bone fibroma also possesses a peculiar morphology when observed by electron microscope. In the cytoplasma there is evidence for protein and lipid synthesis. Sometimes, both kinds of material are elaborated simultaneously in the same cell at the REG and, independently, in agranular endoplasmic reticulum. The cells usually possess a highly developed REG, whose cisterns might be dilated and filled by secretory material as previously described by Movat and Fernando (1962) with reference to activity in fibroblast. There exists a close association between the REG filled with the homogeneous dense structure and the presence of a highly developed fibrillar stroma with collagen fibers showing typical periodicity; sometimes, immature collagen fibers may be seen very near to the cell membrane. As is characteristic in the case of normal fibroblasts, there are also abundant filaments irregularly distributed inside the cytoplasma and filling the matrix of the cell. Other typical fibroblast structures (spindle-cell form), with elongated nucleus and active nucleolus, irregular contour, presence of perinuclear Golgi) are found as well in most of the tumoral cells. The structure of these fibroblasts is comparable to the structures seen by us in regenerative situations of the bone and in metaphyseal fibrous defects also analyzed by electronic microscopy. These latter cells correspond to fibroblasts in synthesis activity. Their most striking difference from present tumoral fibroblasts and the other quoted lesions, is their high lipid content.

The lipid droplets are of a variable electron density and are also irregularly distributed throughout the cytoplasma. The progressive accumulation of lipids appears to be associated with a morphological change in the cell contour, from spindle to polygonal or stellate form. Cells adopting a polygonal shape correspond to those considered as foam cells under optical microscopy. There is no evidence for a coalescence of lipid droplets inside the cytoplasma, and no signet ring cells are visible. There is evidence for transition between fibroblast and lipid loaded cells. Furthermore, most of the fibroblast shows one or more lipid droplets inside the cytoplasma.

Lipids in foam cells are not associated with any capillary absorption not do they have a degenerative origin. They are progressively elaborated inside the agranular endoplasmic reticulum and stored in the cytoplasma. A transition between lipid droplets and the membranes of the endoplasmic reticulum is apparent and almost all droplets are enveloped by a laminar structure which is progressively broken,

when the droplet volume increases. Napolitano (1963) and Fawcett (1966) have reported that lipid droplet in fat tissue did not appear to be limited by a membrane, being stored within the cell on the cytoplasmic matrix, but Wood (1967) and Imaizumi (1969) found that, in chick bone marrow and mouse mesenterium cells, the lipid droplets are not enclosed by membrane but by a highly ordered filament complex in parallel rings around each droplet. In the present case, the lipids are also apparently surrounded by filaments proceeding from or in continuity with the endoplasmic reticulum.

We think that non-ossifying bone fibroma is formed by mesenchymal fibroblast of the bone marrow, which is progressively transformed into foam cells as they synthesize lipids inside the endoplasmic reticulum and are secondarily stored as droplets encircled in part by filamentous rings.

The present cells are not of the histiocytic nature described in other tumoral conditions. The normolipemic plane xanthoma studied by Zemel *et al.* (1970) is formed by cells with a large quantity of lipids stored in the cytoplasma, without being definedly enveloped, and surrounded by numerous active lysosomes. The ultrastructure of the malignant fibroxanthoma of the skin (Merkow *et al.*, 1970) is also very different from the present case as the fibroblast-like cells are loaded with lipids but also possess a great many lysosomes, which reveal the histiocytic character of the cells. We have not found any evidence to allow us to consider the foam cells of the tumor as histiocytes. Moreover, no resemblance exists between the tumor and benign or malignant histiocitosis analysed by electron microscopy (Ritter, 1965; Imamura *et al.*, 1971; Imamura and Muraya, 1971; Bessis, 1972). On the basis of our study, we have no reason to consider non-ossifying fibroma as a histiocytic fibroxanthoma, a theory defended various decades ago by several authors (Phelip, 1935; Bahl, 1936; Burman and Zimberg, 1938).

Hemosiderine particles have been seen in the stroma of the tumor more or less in association with the collagen fibers, but the most striking fact is the existence of electron dense material of micellar nature mimicking hemosiderine inside the mitochondrial of some fibroblast, as has been described in the case of the reticular cells of the bone marrow (Bessis, 1972).

The support of the clinical evidence contributed by Skrede (1970) we believe that non-ossifying fibroma of bone should be considered as a benign tumor proceeding from the bone marrow fibroblast displaying a progressive overload of lipids in the cytoplasma and, in this way, differentiated from other pathological conditions associated with a proliferation of fibroblasts, such as metaphyseal fibrous defects.

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