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Giant Cell Tumor of Bone

Detailed Fine Structural Analysis of Different Cell Components

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Summary. We describe here the ultrastructure of the *multinucleated giant cells* and the *mononuclear stromal cells* in one case of giant cell tumor of bone. The most frequently occurring stromal cell ("*stromal cell type 1*") was of fibroblast-like appearance with an irregular outline of both cells and nuclei. Characteristic features of the cytoplasm were the abundance of rough surfaced endoplasmic reticulum and the well developed Golgi apparatus; particulate glycogen was present in the ground cytoplasm of some cells. A second stromal cell type ("*stromal cell type 2*") was also recognized, albeit more infrequently. It was macrophage-like with many mitochondria and lysosomes, but with a poorly developed endoplasmic reticulum.

The composition of the fixative was found to influence considerably the appearance of the *multinucleated giant cells*, especially of their endoplasmic reticulum and Golgi-associated structures. Vacuolar elements with tail-like expansions were frequently observed in the vicinity of the Golgi apparatus; their functional significance and relationship to the Golgi apparatus and the endoplasmic reticulum is enigmatic. Bodies with the appearance of conventional lysosomes occurred in limited numbers – while mitochondria were abundant. The structural organization and distribution of the endoplasmic reticulum varied between different regions of the cells. Nuclei were of irregular shape with deep infoldings of their limiting membranes and peripheral condensation of the chromatin.

The possible nature of mononuclear cells in the neoplastic process is discussed. The morphologic evidence favors the conclusion that the fibroblast like stromal cell is neoplastic; convincing neoplastic properties are lacking for the type 2 cells. Although similarities exist between multinucleated giant cells and osteoclasts, distinct differences between the two cell types were noted. The origin, function and possible neoplastic nature of the giant cells is discussed on the basis of the findings presented.

Key words: Giant cell tumor – Ultrastructure – Bone tumors.

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Introduction

Giant cell tumor of bone is a locally destructive lesion, often with typical clinical characteristics. It was first described by Nelaton (1860), and since then the biological properties of the lesion have been discussed. The presence of giant cells is one characteristic feature of giant cell tumor of bone. The function, biogenesis and neoplastic importance in the tumor of such giant cells has been discussed at length. As long ago as 1930, Johnson pointed out that there was no general agreement in the literature as to the biological importance and origin of giant cells: "the number of ideas on this subject nearly equals the number of authors who have written about the question".

Careful light microscopic examinations have failed to distinguish between the various types of mononuclear stromal cells in the tumor; furthermore, their importance in the neoplastic process and their histogenesis have been a matter of dispute. It is well established that most true giant cell tumors are benign; few are truly malignant and metastasize.

Although during the last two decades several reports concerning the ultrastructural appearance of giant cell tumors have been published, agreement concerning the fine structural characteristics and significance of the various cells constituting these tumors has not been reached. Thus, there is need for detailed fine structural studies of these lesions. In order to try to further elucidate the structure, histogenesis, interrelationships, and functions of the various cell types in giant cell tumors we have undertaken fine structural, histochemical and biochemical studies of these tumors.

In the present study, which is the first in a series of interrelated investigations, we present a case of giant cell tumor of bone, applying light and electron microscopic techniques in the analysis of the different cells constituting the tumor tissue.

Materials and Methods

Small pieces of tumor tissue were excised for fixation which was carried out either in a fixative composed of 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and 0.1 M sucrose (Arborgh et al., 1976), for 12 to 24 h, or in 2% osmium tetroxide buffered with *s*-collidine. The glutaraldehyde-fixed material was postfixed in *s*-collidine buffered osmium tetroxide (2%). After the completion of fixation, the small tissue pieces were dehydrated in alcohol solution; uranyl acetate was included in the 100% alcohol to obtain en bloc staining. Epon 812 was used as embedding medium. After polymerization, thin sections were cut on an LKB ultramicrotome, stained with lead citrate, and examined in a Jeol 100 C electron microscope.

Light microscopic examinations were performed on paraffin embedded material fixed in 5% paraformaldehyde (buffered to pH 7.2 with 0.15 M cacodylate buffer), and on toluidine blue stained 1μ thick sections prepared from Epon embedded tissues.

Case History

34 year old male first admitted to the hospital due to pain in the left knee. X-ray examination showed an osteolytic destruction which was mainly located in the condylar region of the left

femur. The lesion was curetted and the resulting cavity packed with heterologous bone ("Kieler Knochen"). Two years later, the patient sustained a pathological fracture through the tumor region. Open biopsy was performed and another thorough curettage was carried out; the cavity was filled with a mixture of autologous and heterologous bone. The histopathological diagnosis was "*giant cell tumor of bone*", Jaffe type II. Material for complete light and electron microscopy was obtained only on the occasion of the second operation.

Morphological Observations

A. Light Microscopy of Paraffin Sections

The tumor tissue had a greatly varied appearance (Fig. 1). In some areas, it was highly cellular with only small amounts of intercellular substance. In other parts, the tumor was rich in collagen, the number of cells being fairly small. As a rule, two main cell types could be found: mononuclear "stromal cells" and multinucleated giant cells. The mononuclear cells showed – in the cellular areas – some degree of cellular and nuclear atypia and a moderate number of mitoses. The giant cells appeared in most areas of the tumor, often in close relation with capillaries (Fig. 1). The cells were usually irregularly shaped, differed considerably in size, and contained variable numbers of nuclei (2–20).

B. Light Microscopy, Epon Sections

In the toluidine blue-stained sections, more detailed information about the structural characteristics of the tumor cells could be obtained. The mononuclear cells (Figs. 2, 3, 6) were irregular in shape, often elongated, and contained a nucleus with marginated chromatin. One or two prominent nucleoli were usually present. In some areas, lipid droplets were abundant in the cytoplasm of the cells (Figs. 3–6). The number of lipid droplets in the cytoplasm varied considerably between different areas of the tumor tissue and all transitions between droplet-filled and droplet-lacking cells were encountered. As illustrated in Figure 6, occasional "pale areas" with granular and membranous structures were observed in the tumor tissue. Clearly defined subpopulations of mononuclear cells, which in the light microscope could be separated from each other on the basis of size, shape or structure of cytoplasm or nucleus, were not revealed.

As shown in Figures 3 and 4, the giant cells had an abundant cytoplasm with prominent granularity, and sometimes showed the presence of vacuoles in the cytoplasm. The cell shape was mostly irregular, in some areas with long interconnecting bridges between the various portions of the cells (Fig. 4). The close spatial relationship between giant cells and capillaries or blood cells was often evident (Fig. 4).

The nuclei varied greatly in number, size and shape. The chromatin structure was inhomogeneous with a tendency toward margination. Nucleoli were prominent and varied considerably in size.

C. Electron Microscopy

1. Mononuclear Stromal Cells. In low magnification survey electron micrographs, cellular areas of tumor tissue with tightly packed and closely apposed cells alternated with areas containing abundant intercellular collagenous matrix. There was no difference in the general appearance of the mononuclear cells in the crowded portions when compared to areas with widely separated cells. Regions of tumor tissue with structures corresponding to the pale areas observed in the light microscope were readily identified. The fine structural correlate of these pale areas appeared to be swollen and seemingly degenerated cells or portions of cells often containing large dense bodies of lysosome-like structure (cf. Figs. 6 and 7).

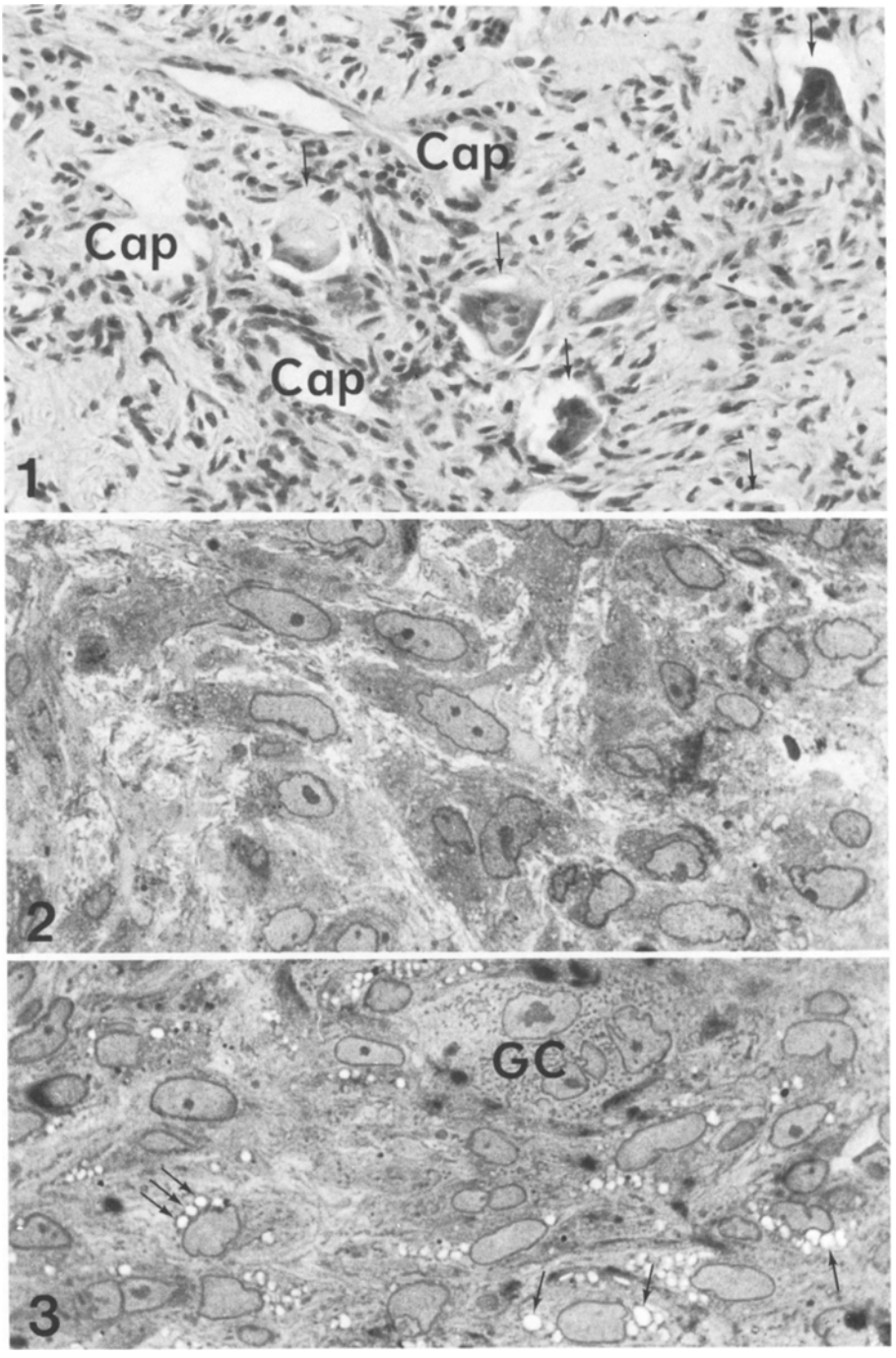
On the basis of the fine structure, the mononuclear cells could be divided into two groups, hereafter referred to as type 1 and type 2 stromal cells. The *type 1 stromal cells* were of common occurrence. They might be unassociated with other cells, or be arranged in compact groups (Figs.7–9). Their size and shape varied considerably; usually, they were ovoid with long, irregular extrusions. The cytoplasm in these cells was abundant, containing a well developed – mainly rough surfaced – endoplasmic reticulum (ER) (Figs. 9–13). In solely osmium-fixed tissues, the cisternae of the ER were usually expanded, with a finely granular content (Fig. 10). The cisternal widening was also observed after primary fixation in glutaraldehyde (Figs. 11 and 13) but was not that pronounced. With the later fixation, the cytoplasm also seemed to be more "dense" than after primary osmium fixation.

The Golgi regions were prominent in most cells and were often located close to the nucleus (Figs. 8, 10, 12). Organelles, which on the basis of their fine structure were presumed to belong to the lysosomal system, seemed to be rather few, often appearing in small clusters (Figs. 10 and 12). Ferritin or hemosiderin-like electron dense granules were sometimes revealed in high concentrations within the lysosome-like organelles (Figs. 10 and 12). Vesicles and vacuoles of presumed endocytotic origin were rarely seen. The number and distribution of mitochondria in the type 1 cells varied, in some areas being sparse, in others very frequent.

Fig. 1. Portion of tumor tissue with abundant capillaries (*Cap*) and multinucleated giant cells (*arrows*) with variable size, shape and number of nuclei. Buffered paraformaldehyde; paraffin; section staining with H&E. ×250

Fig. 2. High magnification light micrograph of tumor tissue fixed in glutaraldehyde followed by OsO_4, embedded in Epon, and stained with toluidine blue. The area illustrated is composed of haphazardly oriented loosely interwoven mononuclear "stromal" cells. Nuclei are elongated with folded bordering membranes and show margination of chromatin and presence of one or two prominent nucleoli. Cellular outlines are irregular with formation of slender processes. The amount of cytoplasm surrounding the nuclei is highly variable and appears coarsely granular. Collagen is comparatively sparse. ×960

Fig. 3. Material prepared as in Figure 2. Portion of tightly packed cells, one of which is a typical giant cell (*GC*). The mononuclear stromal cells have indistinct borders and show presence of large fat droplets (*arrows*) in their cytoplasm. Nuclei are similar to those illustrated in Figure 2. ×900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Cap
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2
GC
3

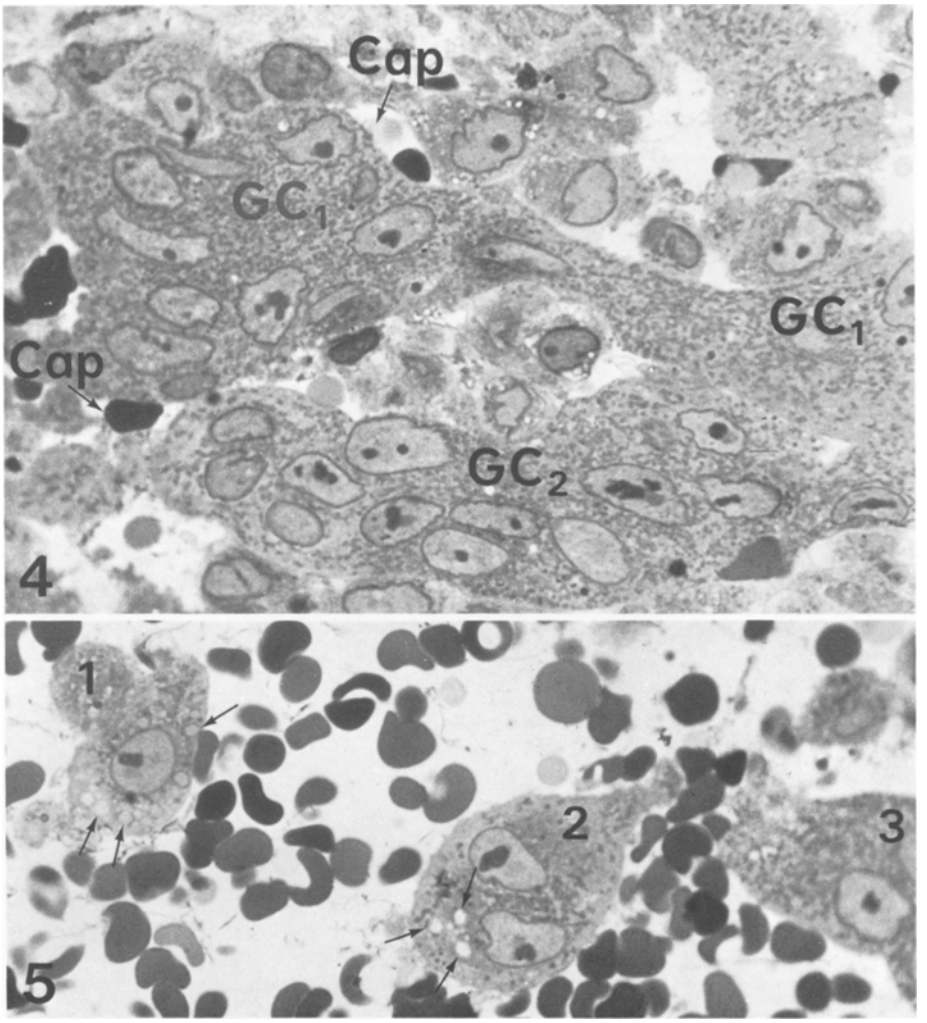


Fig. 4. Tissue prepared as in Figure 2. Portions of two multinucleated giant cells (GC_1 and GC_2) surrounded by capillaries (*Cap*) are illustrated. GC_1 is composed of two bulky, nuclei-containing portions bridged by a thin stalk of cytoplasm. Note variability in size and shape of both nuclei and nucleoli. Up to 3 or 4 nucleoli are present in individual nuclei. ×1000

Fig. 5. Tissue prepared as in Figure 2. Cell marked "*2*" is binucleated with an appearance of nuclei and cytoplasm similar to that in the multinucleated cells in Figures 2 and 3. The same applies for the mononuclear cell indicated by "1". "*3*" is a portion of a multinucleated giant cell. Note presence of lipid droplets (*arrow*) in cells "1" and "2". ×950

In some areas of the tissue, the cells contained numerous fat droplets in the cytoplasmic ground substance (Fig. 7), while in other areas such droplets were few or absent. Large numbers of glycogen particles were revealed in the cell sap of occasional type 1 cells (Fig. 11). Furthermore, material with a collagen-like appearance was occasionally seen in the ground cytoplasm (Fig. 9).

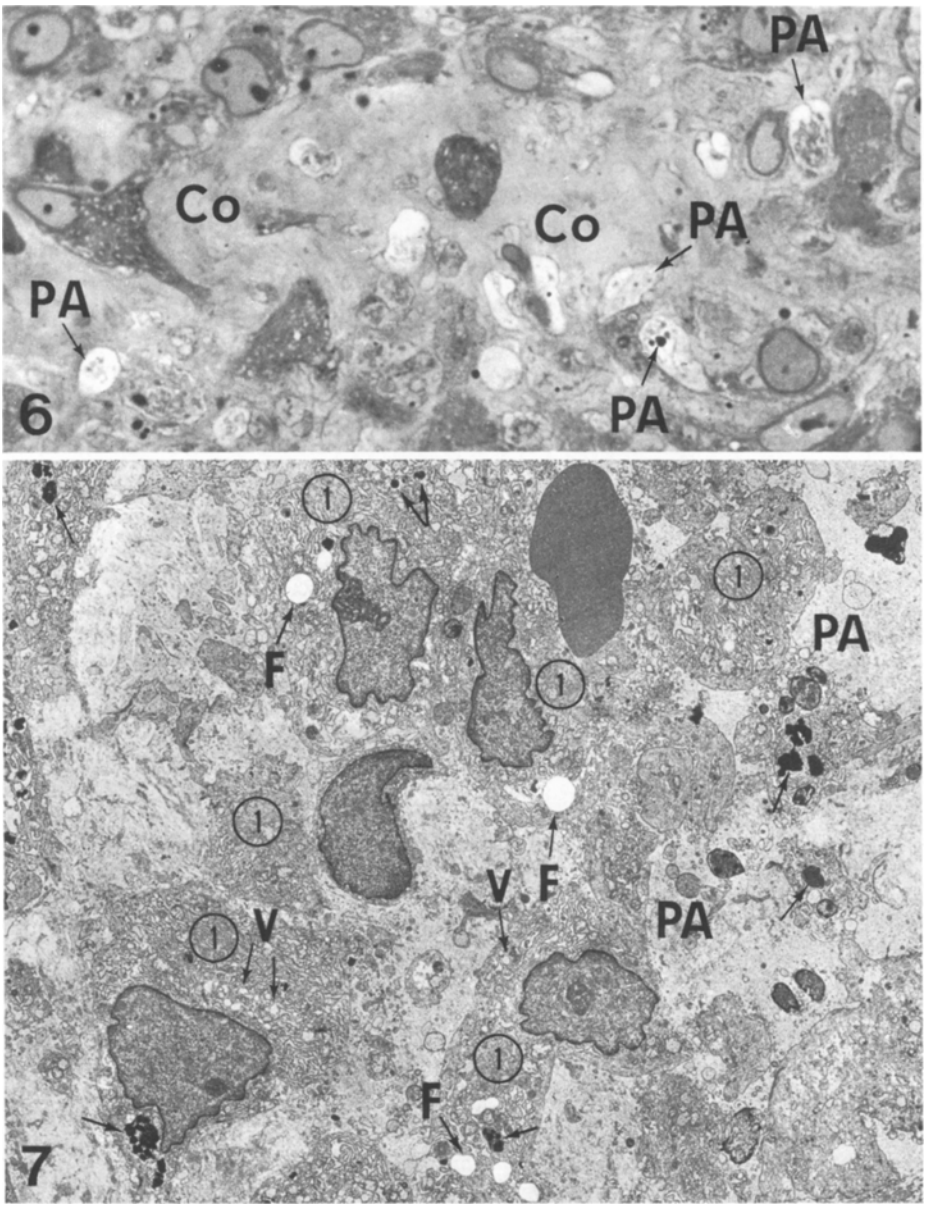


Fig. 6. Material prepared as in Figure 2. From area rich in collagen (Co). PA indicates pale areas of the tumor tissue, containing granular material and membrane fragments. ×950

Fig. 7. Low magnification electron micrograph showing several mononuclear "stromal" cells type 1 (*1*) with abundant endoplasmic reticulum. Dense bodies in the cytoplasm (*arrows*)—believed to represent lysosomes— vary considerably in size and shape. Cellular and nuclear outlines are irregular. Some cells contain fat droplets (*F*). Intracellular vesicles are indicated by V. Pale areas, PA. OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×2700

Most of the cells had a well developed system of microfilaments, usually distributed haphazardly throughout the cytoplasm (Figs. 8, 12, 15). Free ribosomes were rarely observed.

The nuclei of the type 1 stromal cells showed pronounced polymorphism, and were bordered by deeply folded nuclear membranes. Sometimes, nuclear lobulation was prominent (Fig. 12) resulting in images of several isolated nuclear fragments in the same cell (Fig. 13). The chromatin was characteristically condensed towards the periphery of the nucleus. One or two prominent nucleoli were often observed. In some cells, the irregular nuclear shape resulted in the formation of "pseudo"-inclusions (Fig. 8).

The *type 2 stromal cells* were infrequently encountered in the tumor. They were irregularly shaped, forming abundant thin peripheral projections (Figs. 11 and 14). Generally, they contained numerous mitochondria and lysosome-like bodies, while the ER was sparsely developed. The Golgi apparatus was fairly prominent with tightly packed cisternae. Fat droplets and glycogen particles were absent from the cell sap which contained a moderate number of free ribosomes and irregularly dispersed microfilaments. The nucleus had an irregular outline with its chromatin focally condensed to a coarse irregular pattern throughout the nucleoplasm. Nucleoli were rather large and prominent.

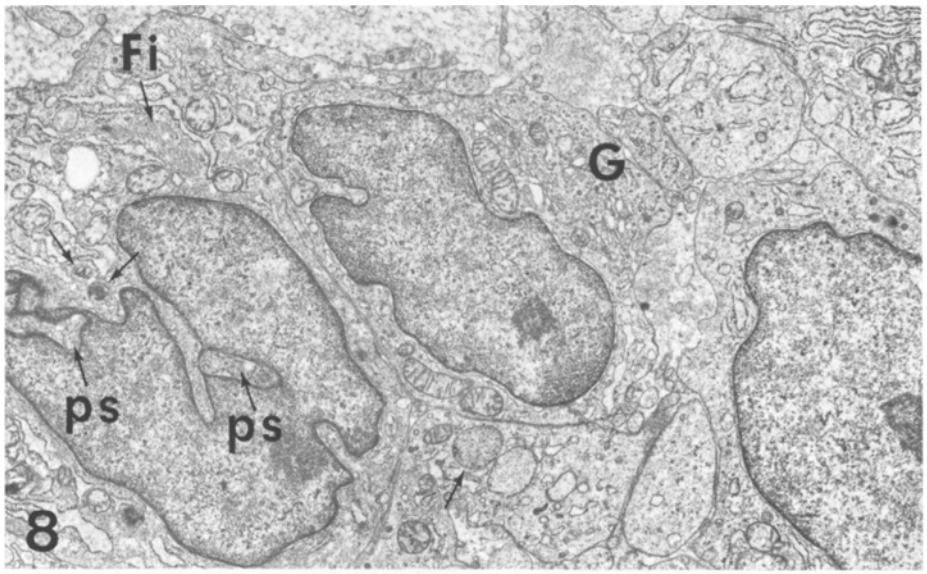
Critical features in the distinction between type 1 and type 2 cells were the prominent, well developed ER in the former and the sparse ER combined with abundant lysosome-like bodies and mitochondria in the latter. Mononuclear cell elements other than stromal cells type 1 and 2 have only been noted occasionally and mainly include endothelial cells.

2. Multinucleated Giant Cells. Among the different cytoplasmic organelles, mitochondria were most conspicuous (Figs. 16 and 17). They occurred in large numbers and exhibited variable size and shape in the sections. Crystalline inclusions or other mitochondrial aberrations were never seen. In general, the mitochondrial matrix was homogeneously dense in tissues fixed primarily in glutaraldehyde while in osmium-fixed tissues irregular pale areas were present in the matrix, and the mitochondria appeared slightly swollen (Figs. 16 and 17).

In tissue fixed primarily in glutaraldehyde, prominent endoplasmic reticulum (ER) was noted in peripheral portions of some cells (Fig. 16). It formed meandering, roughly parallel cisternae sometimes oriented perpendicularly to the plasma membrane of the giant cell. These cisternae were often concentrated in regions

Fig. 8. Part of area with tightly packed type 1 cells. One of the nuclei shows deep infoldings giving rise to nuclear "pseudoinclusions" (*ps*). Dense bodies are marked by *arrows*. *G*, large Golgi area; *Fi*, filamentous area in the ground cytoplasm. OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×6500

Fig. 9. Moderately collagen-rich with type 1 cells characterized by highly irregular shapes and well developed rough endoplasmic reticulum. Most of the cisternae of the latter are widened. Plasma membranes are usually tightly applied in areas where cells abut on each other. Observe that collagen-like material is located both in the extracellular space (*Co*) and intracellularly (Co^x). One cell contains a large residual body (*RB*). Dense bodies are indicated by *arrows*. OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×5725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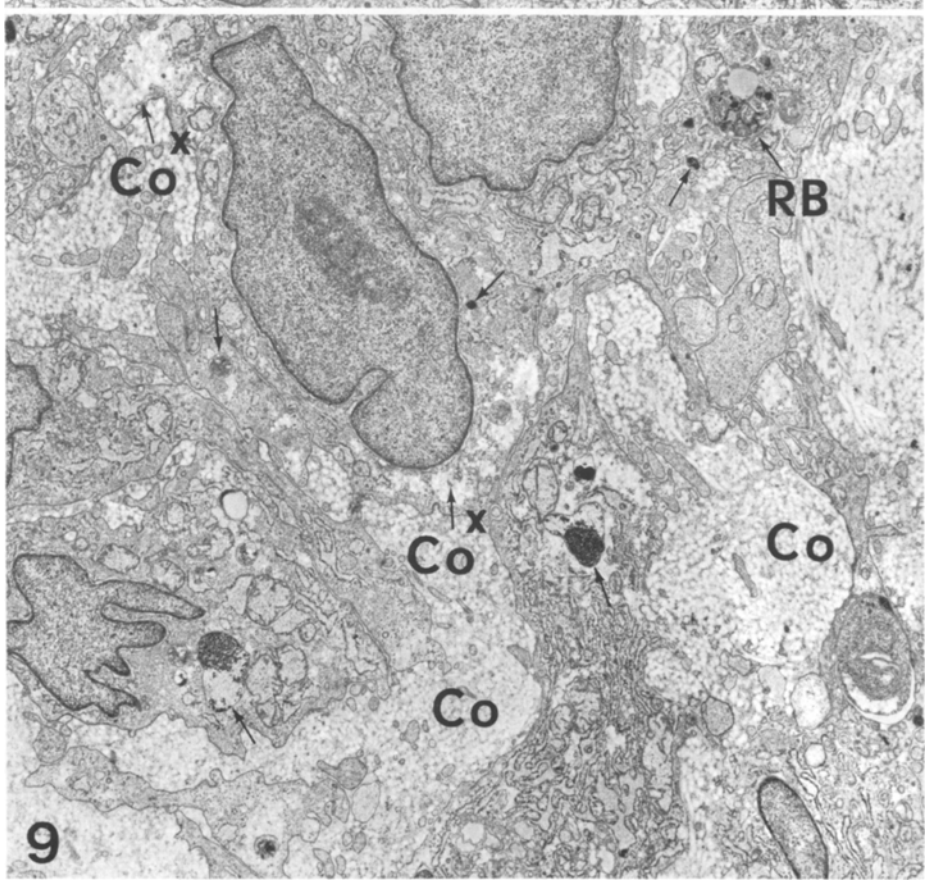
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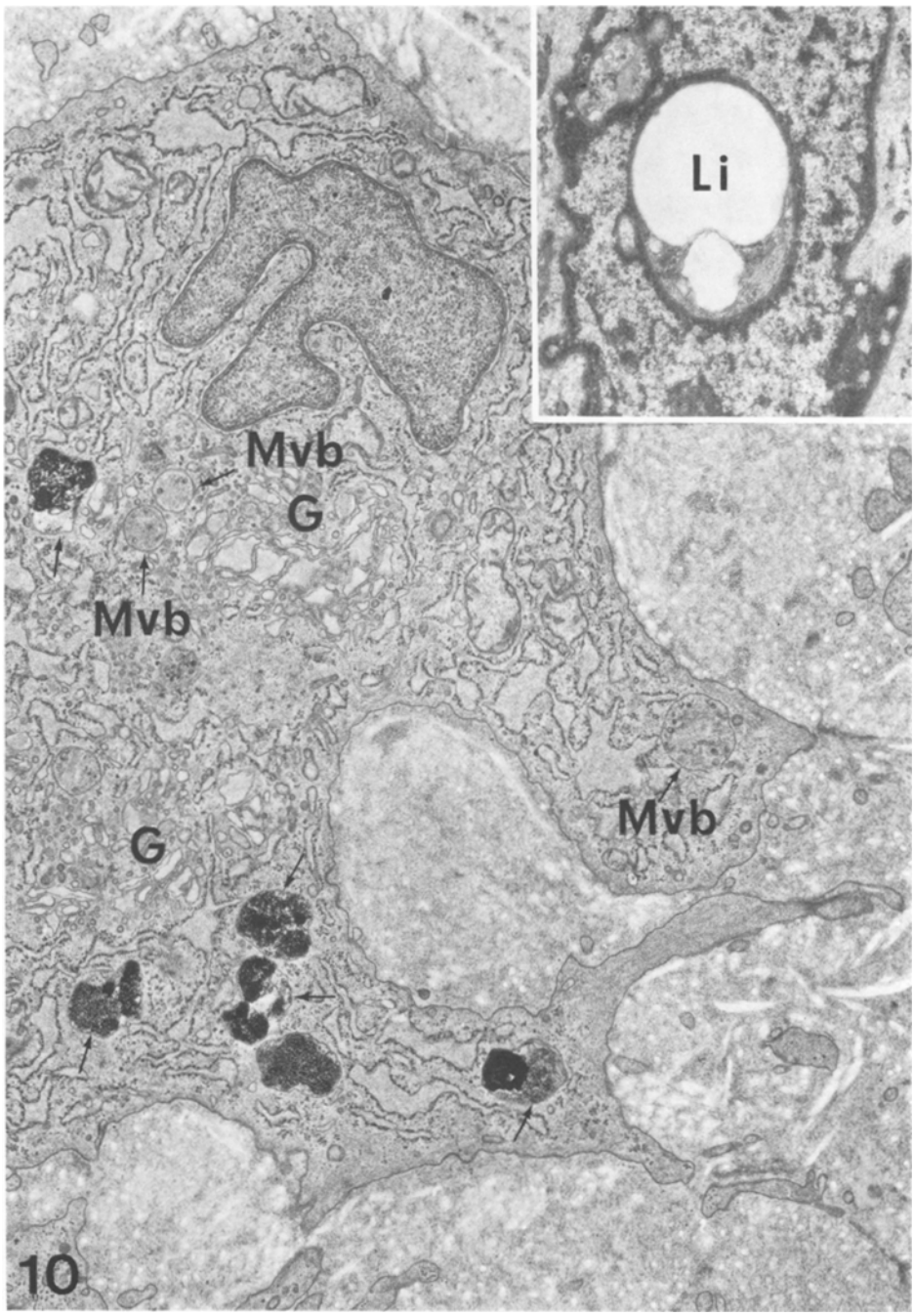

Fig. 10. Portion of type 1 cell forming slender extrusions into the collagen-rich stroma. Note large Golgi areas (*G*), abundant rough surfaced endoplasmic reticulum and presence of numerous large dense bodies filled with hemosiderin-like material (*arrows*). Mvb, multivesicular bodies; OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×11,550. *Inset*. Part of nuclei of type 1 cell with large lipid containing pseudoinclusions

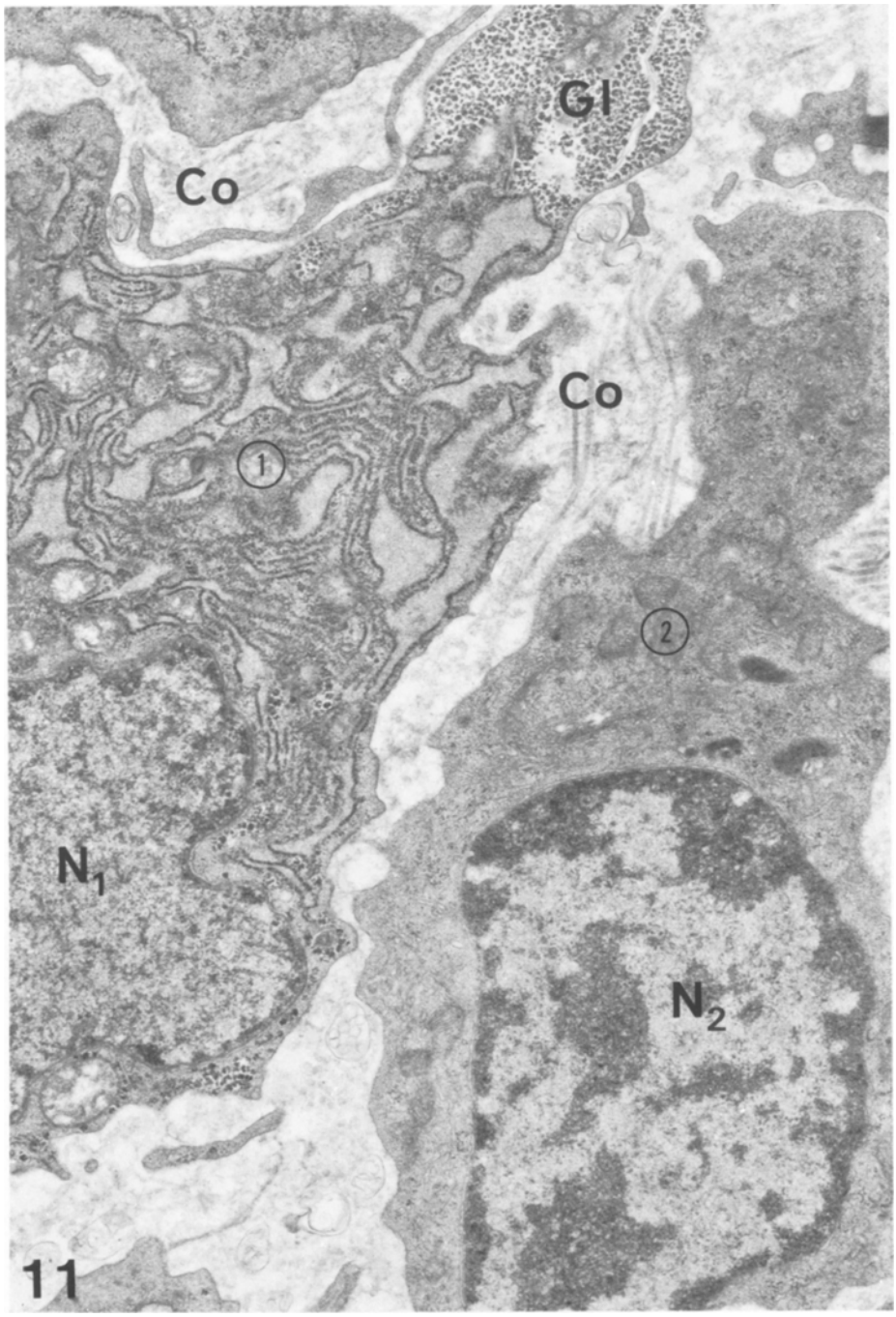

Fig. 11. Adjacent mononuclear stromal cells, one type 1 (*1*) and one type 2 (*2*) are illustrated. The nuclei (N_1 and N_2) differ in shape and distribution of chromatin material. Endoplasmic reticulum dominates the cytoplasm of the type 1 cell while mitochondria form the major cytoplasmic constituent in the type 2 cell where endoplasmic reticulum is very sparse. Note the presence of particulate glycogen (*Gl*) in the type 1 cell. *Co*, collagen. Glutaraldehyde; OsO_4; Epon; section staining with lead citrate and uranyl acetate. × 15,000

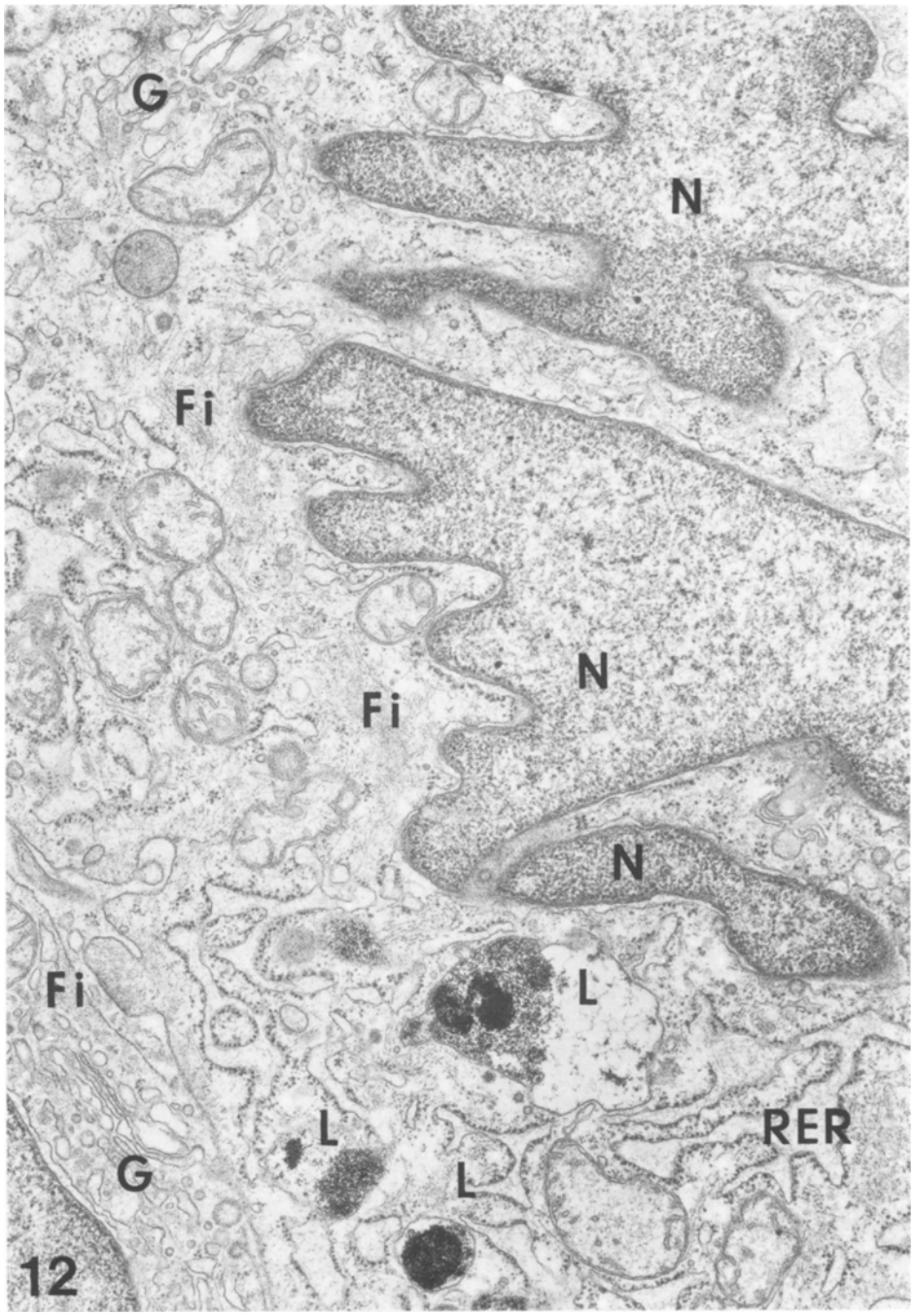

Fig. 12. Portion of giant cell tumor tissue with a stromal cell type 1. The nuclei (*N*) are extremely lobulated with condensation of chromatin in its peripheral regions. In the cytoplasm microfilaments (*Fi*) are abundant. The rough endoplasmic reticulum (*RER*) is in some areas well developed with irregular widened cisternae. A few lysosome-like bodies (*L*) with hemosiderin-like content can be seen in the lower part of the cell in the centre. *G*, Golgi area. OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×20,000

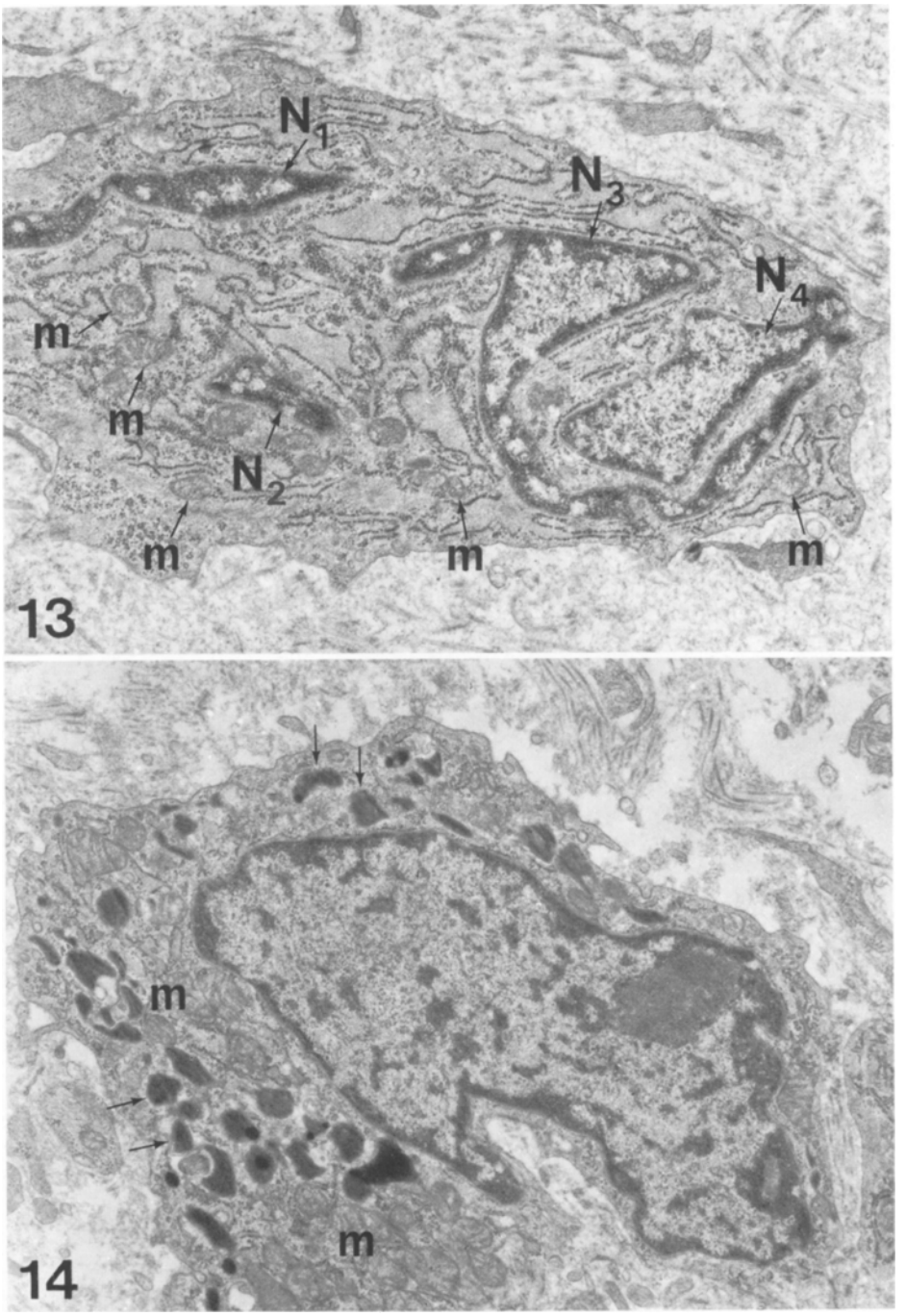

Fig. 13. Portion of type 1 cell with well developed rough surfaced endoplasmic reticulum. There are at least 4 separate nuclear images in the picture (N_1 through N_4). Mitochondria (*m*) are few and small. Glutaraldehyde; OsO_4; Epon; section staining with lead citrate and uranyl acetate. $\times 13{,}000$

Fig. 14. Picture illustrating a type 2 mononuclear stromal cell. The cytoplasm contains an abundance of dense bodies (*arrows*) and mitochondria (*m*). Glutaraldehyde; OsO_4; Epon; section staining with lead citrate and uranyl acetate. $\times 11{,}500$

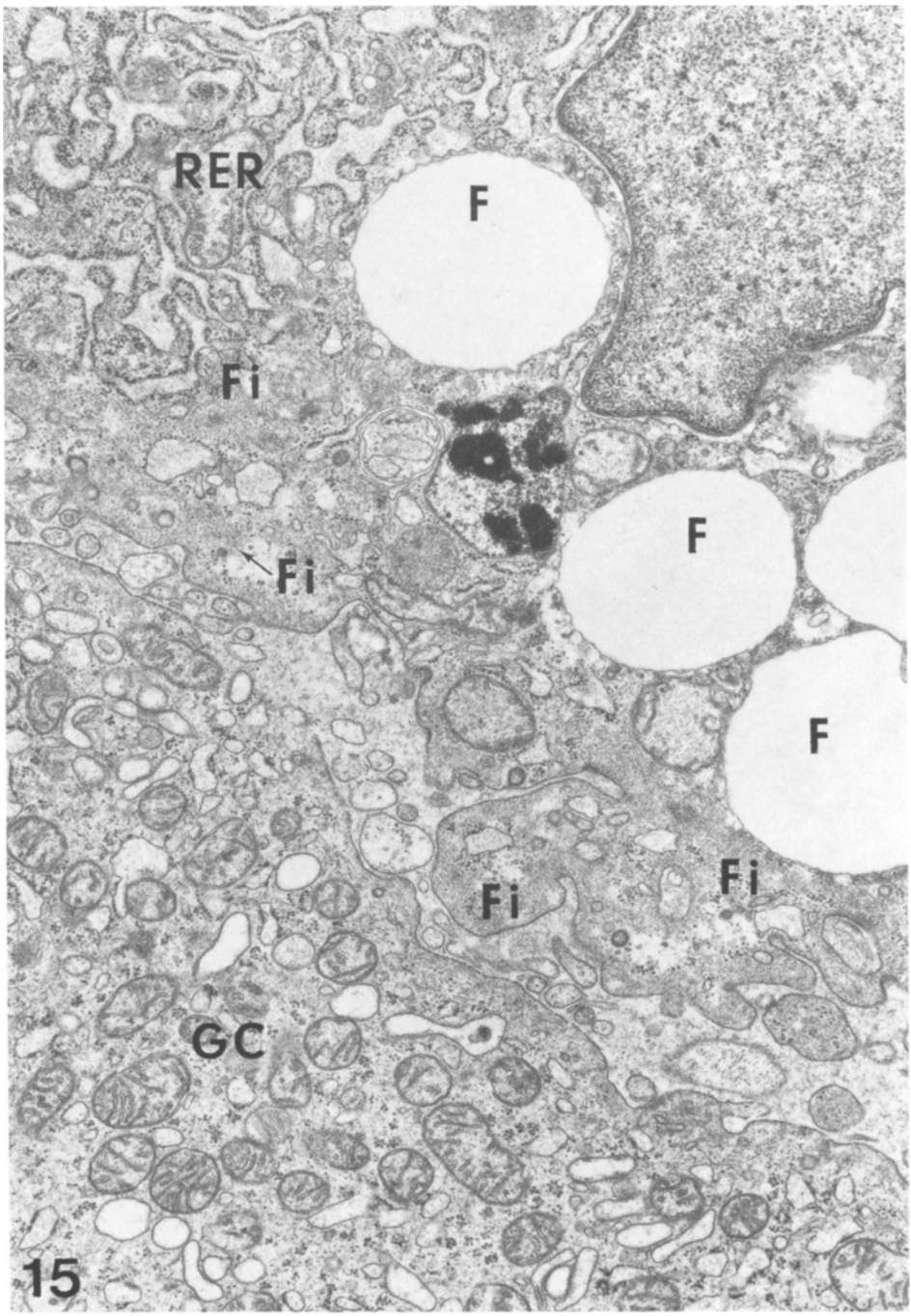

Fig. 15. Portions of a giant cell (*GC*) adjacent to a type 1 mononuclear stromal cell (right upper area of the picture). The cells are separated by an extracellular space of variable width. Mitochondria, along with vesicular and vacuolar elements predominate in the cytoplasm of the giant cell, while rough surfaced endoplasmic reticulum (*RER*) and fat droplets are conspicuous in the type 1 cell. *Fi*, microfilaments. OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×15,200

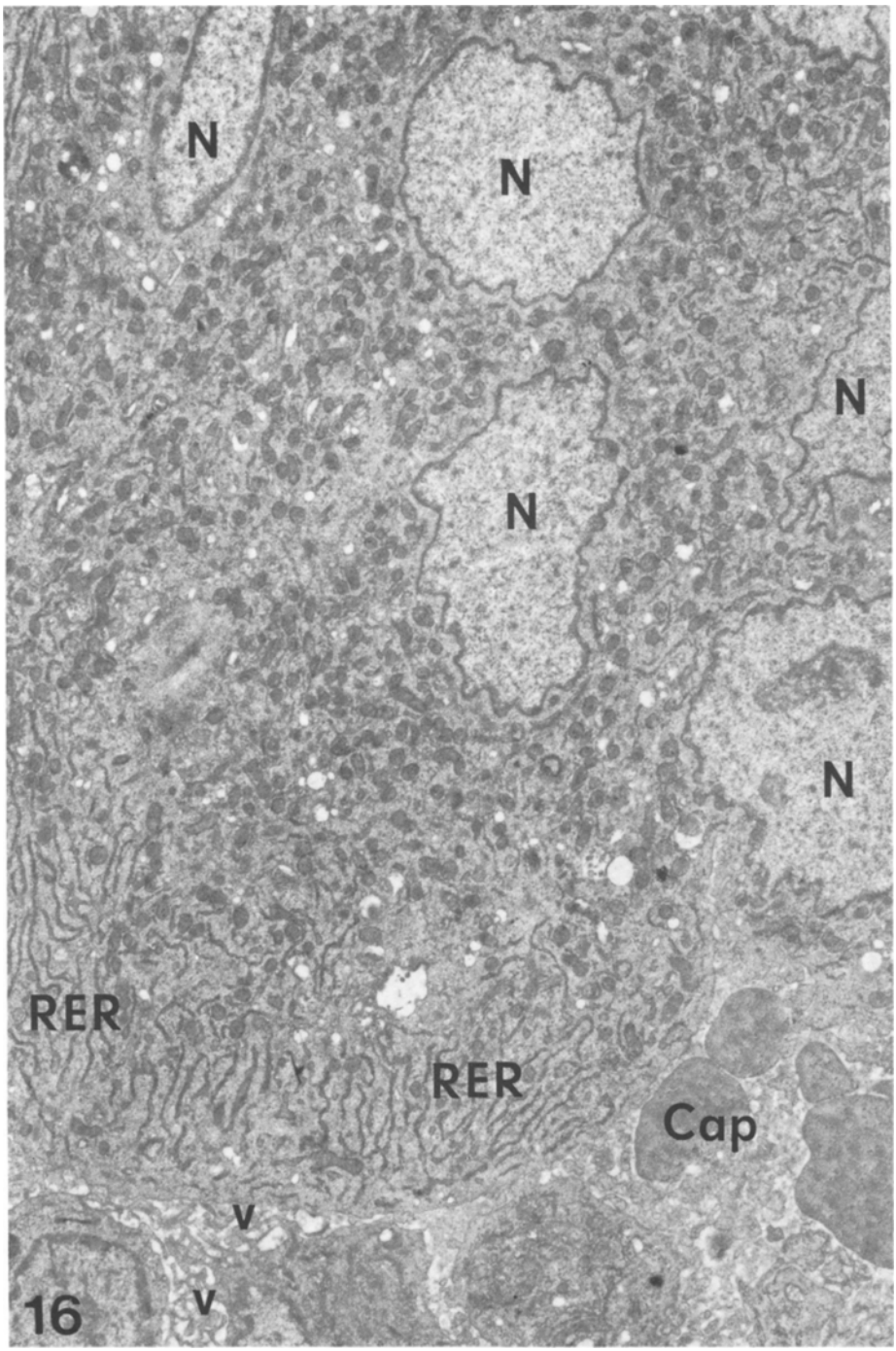

Fig. 16. Survey electron micrograph of a portion of a giant cell with several nuclei (*N*). The cytoplasm contains tightly packed mitochondria. Rough endoplasmic reticulum (*RER*) is concentrated in the periphery of the cell. Adjacent cells form numerous microvillous-like projections (*v*). *Cap*, capillary. Glutaraldehyde; OsO_4; Epon; section staining with lead citrate and uranyl acetate. $\times 6000$

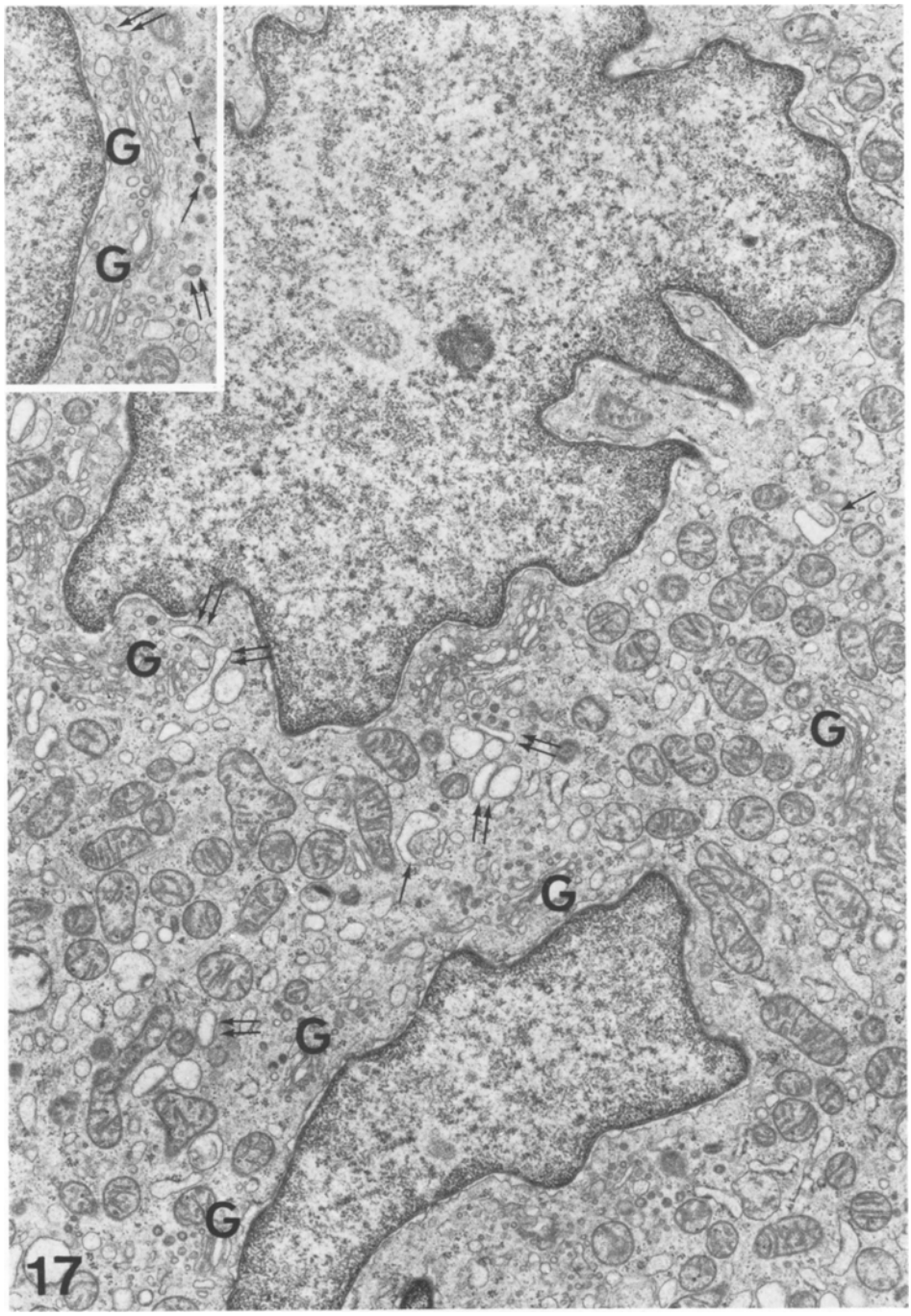

Fig. 17. Portion of giant cell with wide-spread Golgi areas (*G*) in the vicinity of the nuclei. The Golgi areas are made up of cisternae surrounded by vesicles (some of which are coated), and vacuoles of variable size and shape. Some vacuoles appear to flatten out to attain a sausage-shaped structure (*double arrows*). Similar elements supplied with a narrow tail (*single arrows*) occur in areas of cytoplasm away from the Golgi regions proper. Rough surfaced endoplasmic reticulum is sparse and mitochondria numerous. *Inset* illustrates the relationship between coated vesicles (*single arrows*) and the Golgi membranes. In some areas, coated membrane fragments show continuity with smooth surfaced membranes (*double arrows*) as though coated vesicles were budding out from or fusing with these membranes. OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×13,000; *inset* 18,000

located close to capillaries. The membranes bordering the cisternae were closely applied, creating a narrow, slit-shaped lumen, and were studded with ribosomes on their cytoplasmic side. In central portions of the cells, ER seemed to be more sparse and appeared as flat, short, rough-surfaced elements interspersed randomly among the numerous mitochondria. Smooth-surfaced ER of conventional type was rarely encountered. Vesicular or vacuolar shapes of easily identified ER were not usually seen.

The images of the ER in tissues primarily and solely fixed in osmium tetroxide were at variance with those in the glutaraldehyde-fixed material. Thus, in the central portion of the cells, rough-surfaced ER often showed the appearance of expanded, short tubular cisternae with irregular shapes and pale lumens (Figs. 17 and 18). Ribosomes were frequently lacking in some portions of the bordering membranes.

The Golgi regions of the multinucleated giant cells were often large (Figs. 17–19). They were usually located in perinuclear regions and sometimes seemed to completely surround the adjacent nucleus. In tissues fixed primarily in glutaraldehyde, the Golgi regions were composed of flattened smooth-surfaced cisternae arranged in parallel arrays and surrounded by moderate numbers of vesicles and vacuoles.

The appearance of the Golgi elements was somewhat different in primarily osmium tetroxide-fixed cells, in that vacuoles and expanded smooth-surfaced tubular elements—which could be seen also in glutaraldehyde-fixed material—were more abundant, and that the cisternae showed irregular dilatations and expansions (Figs. 17–19). In many areas, smooth-surfaced vacuoles with a finely granular content appeared to form part of the Golgi apparatus (Fig. 19) and transitions between sausage-shaped cisternae and such vacuoles were frequently observed (Fig. 17). It also appeared that vacuolar structures with tail-like expansions (Figs. 17 and 18) were part of the Golgi apparatus or represented transitions between the Golgi complex and the Golgi-associated ER. Coated vesicles were often observed in the Golgi regions (Figs. 17 and 19). Occasionally, these vesicles seemd to be budding out from smooth-surfaced Golgi membranes (Fig. 17, inset) although the appearance did not exclude fusions of Golgi elements with coated vesicles.

Single membrane limited, often irregularly shaped bodies containing variable amounts of granular, membraneous and dense materials, and believed to represent lysosomes, occurred in limited numbers throughout the cytoplasm. Some of these bodies contained hemosiderin- and ferritin-like material. They were not concentrated in the vicinity of the Golgi regions, and a typical GERL was not observed. The lysosome-like bodies were of identical appearance irrespective of whether glutaraldehyde or osmium tetroxide was utilized for the primary fixation.

Bodies with a structure corresponding to that of autophagic or digestive vacuoles, often containing well preserved cellular fragments were occasionally noted in the cytoplasm of the giant cells. Sometimes, clusters of such vacuoles were present (Fig. 18).

The cytoplasmic ground substance contained a fairly well developed system of microfilaments, while microtubules were inconspicuous. Condensation of

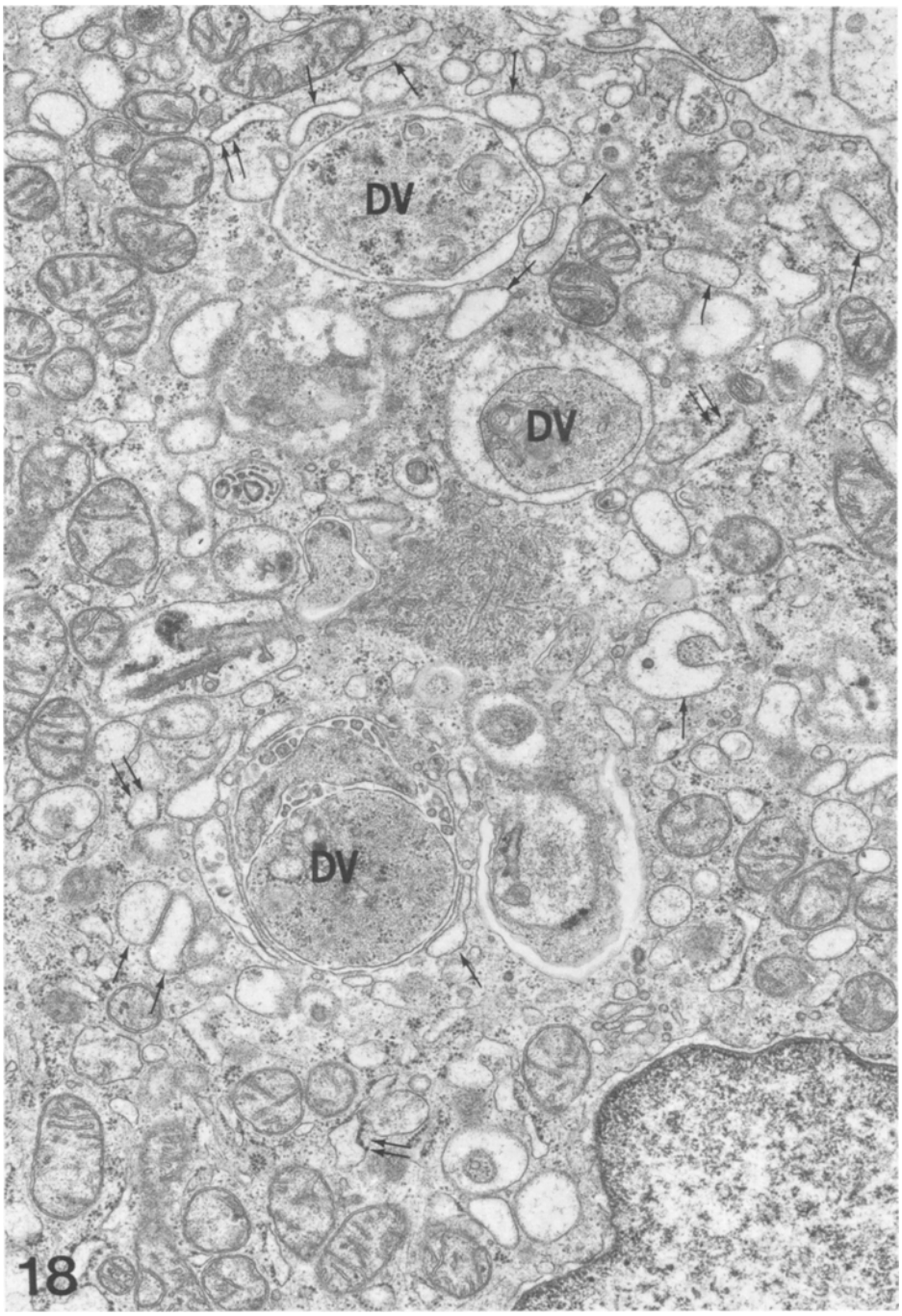

Fig. 18. Area of a giant cell with several digestive vacuoles (*DV*) located both in the perinuclear region of the cytoplasm as well as in the cell periphery. The vacuoles contain free ribosomes, vesicular elements and membraneous material of variable degree of degradation. Several tubular and vesicular formation (*arrows*) are distributed throughout the cytoplasm. Note that ribosomes are attached to many of the irregularly shaped vacuolar elements (*double arrows*). OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×18,300

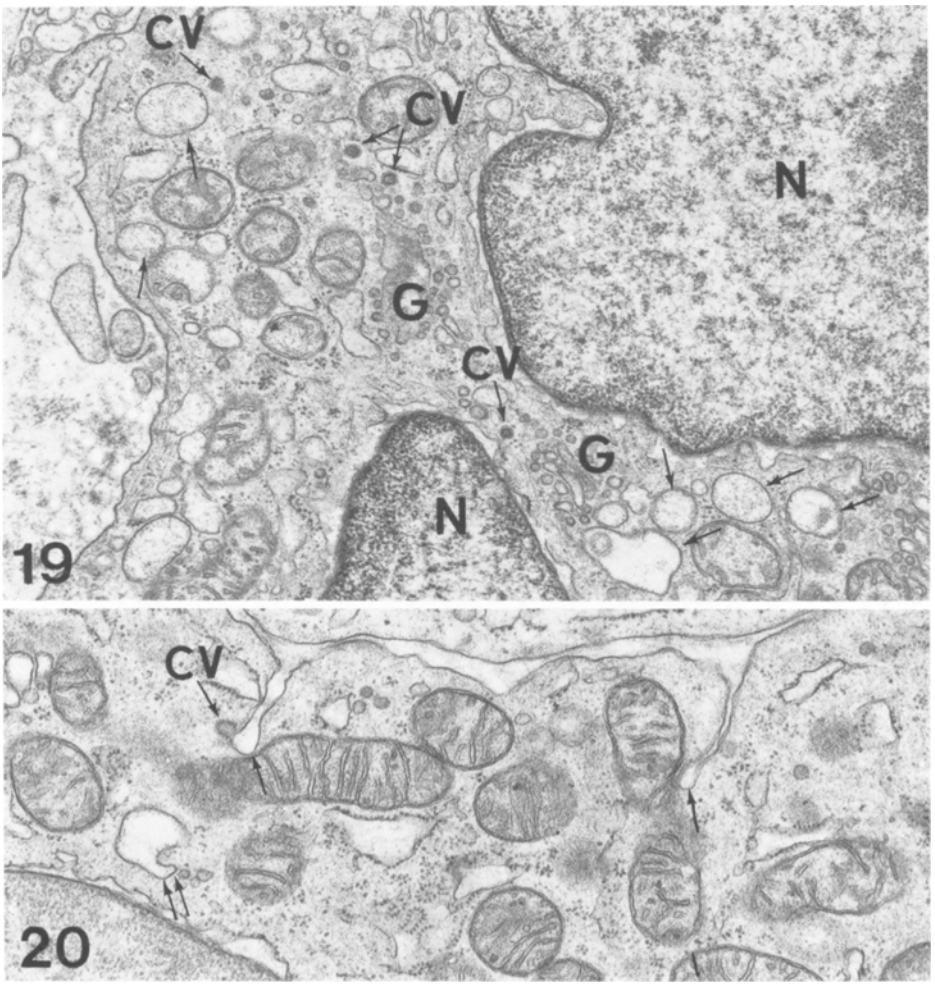

Fig. 19. Detail of a giant cell with portions of two nuclei (*N*). The Golgi areas (*G*) are prominent and are surrounded by small vesicles, some of which are coated (*CV*). Such coated vesicles can also be found near the cell surface. Irregularly shaped vesicles sometimes containing electron dense granules are marked by arrows. OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×18,300

Fig. 20. Peripheral region of a multinucleated giant cell. The bordering plasma membrane forms deep infoldings (*arrows*). *CV* indicates a coated membrane fragment, probably representing fusion or fission between a coated vesicle and the plasma membrane. One of the vesicular structures in the cytoplasm has a slender projection (double arrows) directed towards a group of small vesicles. OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×25,000

microfilaments to form a distinct subplasmalemmal "ectoplasmic layer" was not observed. Microfilaments were best revealed in tissues fixed in glutaraldehyde and postfixed in osmium tetroxide.

The nuclei of the multinucleated giant cells were irregularly shaped with deep infoldings and extrusions of the nuclear membrane (Fig. 17). The chromatin

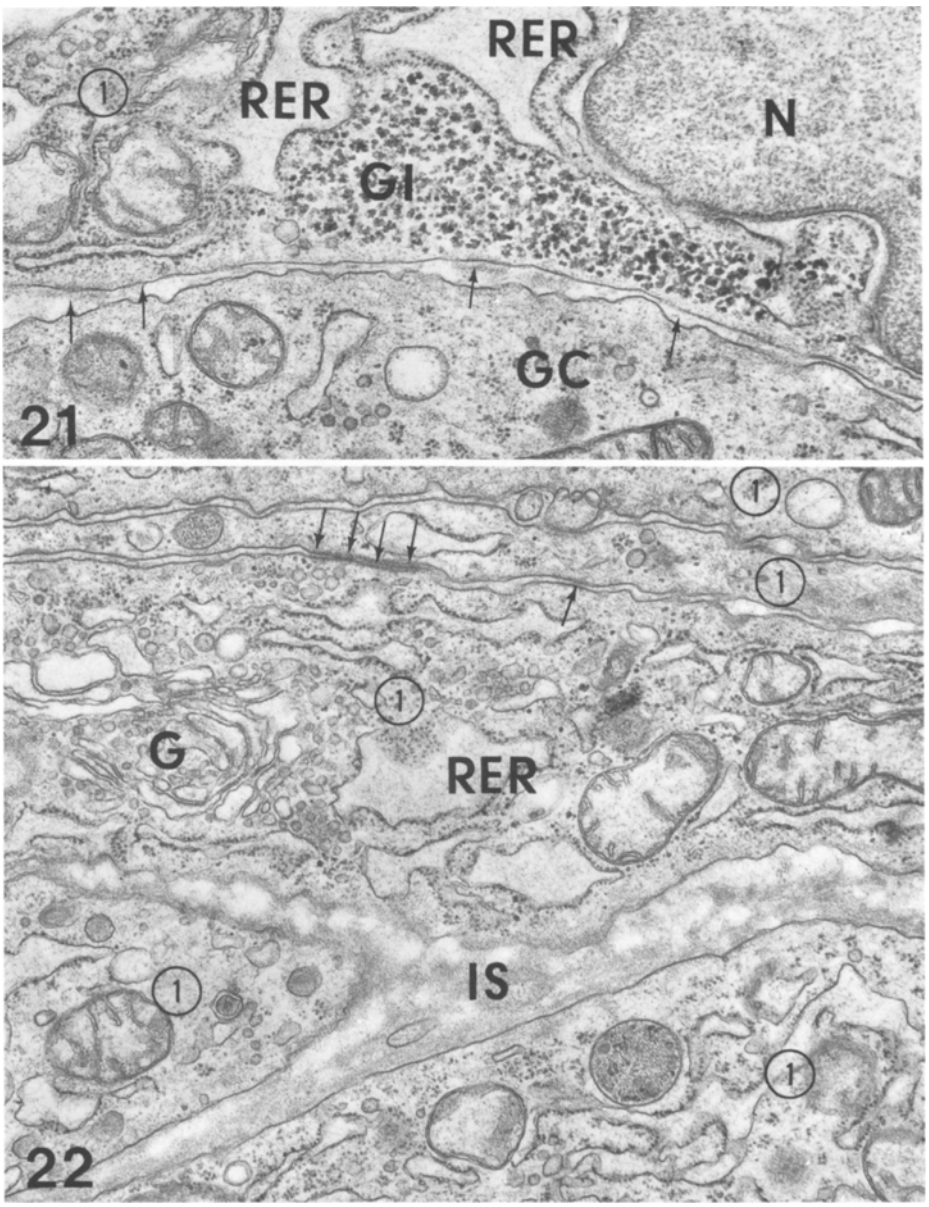

Fig. 21. Picture illustrating a giant cell (*GC*) in close connection with a type 1 stromal cell (*1*). The intercellular space (*arrow*) is very narrow. Observe the accumulation of glycogen (*GI*) and the prominent rough endoplasmic reticulum (*RER*) in the stromal cell. *N*, nucleus. OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×27,400

Fig. 22. Area of tumor tissue, showing closely packed type 1 mononuclear stromal cells (*1*). Between two of the cells, there seems to be a plasma membrane attachment (*arrows*) of the "tight junction"-type. *RER*, rough endoplasmic reticulum; *G*, Golgi area; *IS*, intercellular space. OsO_4; Epon; section staining with lead citrate and uranyl acetate. ×23,250

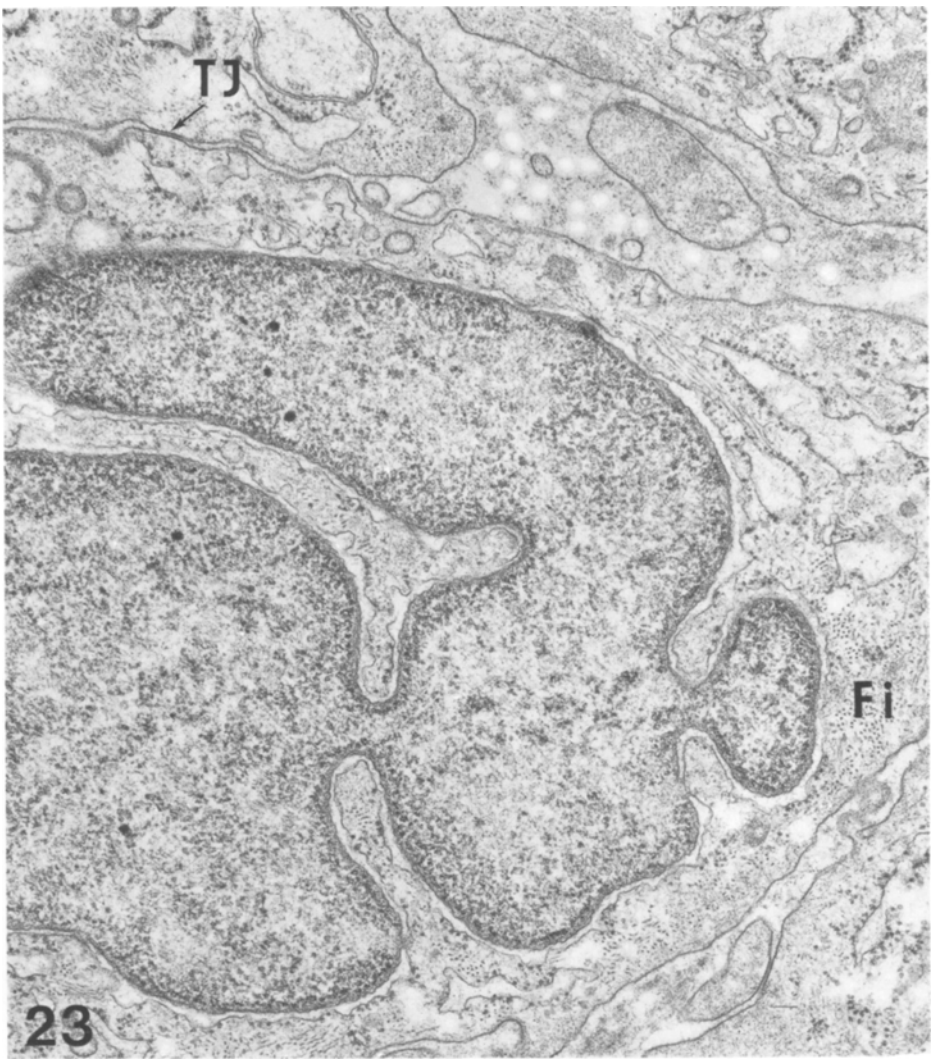

Fig. 23. Adjacent portions of type I stromal cells with a tight junction (*TJ*) between two of the cells. The nucleus in the centrally located cell is lobulated. Transversely sectioned filaments are marked Fi. OsO_4; Epon; lead citrate. ×30,000

was generally condensed towards the peripheral part of the nucleus. Large nucleoli were frequently encountered. Crystalline, virus-like or filamentous inclusions were not present in the nuclei. Fixation differences in the appearance of the nuclei were not conspicuous.

Usually, the plasma membrane was smooth or showed a wavy configuration. Microvillous-like projections were present in some of the cells but a true brush border was never encountered.

Figures 21–23 illustrate the interrelationship between various cells. The amount of intercellular substances between mononuclear stromal cells is

reduced in some areas and tight junctions are formed (Figs. 22 and 23). Similar sparsity of intercellular substance could also be seen between giant cells and mononuclear stromal cells (Fig. 21).

Discussion

In an extensive report by Schajowicz (1961) the morphological and histochemical properties of various cases of giant cell tumor of bone were discussed and compared with the clinical course. As in earlier reports (Jaffe et al., 1940; Morton, 1965; Murphy et al., 1956), Schajowicz by light microscopy noted that the tumor was composed of a mixture of mononuclear and multinucleated cells surrounded by a sparse collagen-poor extracellular substance. In general, the tissue was rich in newly formed vessels. The author considered the mononuclear stromal cells to be the principal tumor cells, and suggested that they were of mesenchymal origin. In subsequent light microscopic studies only one type of mononuclear stromal cell has been observed (Hutter et al., 1962; Mnaymneh et al., 1967; Oberling et al., 1967; Oliva et al., 1974). The appearance of the stromal cell nucleus was considered to be similar to that of the giant cell (Dahlin et al., 1969; Hutter et al., 1962; de Marchi et al., 1969; Mnaymneh et al., 1967; Schajowicz, 1961).

On the basis of electron microscopic investigations, it was possible to separate the mononuclear stromal cells into subgroups (Horie, 1961). However, agreement as to the specific characteristics of each group has not been reached. Horie (1961) described one *histiocyte-like cell* type and one *fibroblast-like stromal cell.* Iwashita (1965) also found two types of stromal cells, one being rich in ER and poor in mitochondria; in the other cell type ER and mitochondria were limited to certain areas of the cell. A *third cell type* was described in a report by Hanaoka et al. (1970). They reported the existence of a cytoplasm-poor cell, inseparable from the lymphocyte, on a morphological basis. They also discussed the probable existence of "intermediate" cell types.

In 1972, Steiner et al. published a report on the ultrastructure of giant cell tumors of bone and related the structure of the tumor to its localization in the skeleton. The authors separated the "principal" mononuclear tumor cell – with fibroblast-like appearance – from "less frequent mononuclear cells" (resembling lymphocyces, monocytes, histiocytes and transitional stages between these cells). They also claimed to have identified intermediate forms between the principal mononuclear cells and the less frequently occurring cells.

Recurrence of genuine giant cell tumor of bone is common, mainly due to the fact that radical excision is difficult to perform with preservation of function. In such recurrences, the biological behaviour of the tumor is as a rule unaltered, provided the tumor has not been irradiated. The tumor studies in the present report belonged to the category of non-irradiated recurrencies with preserved structural and biological characteristics and can be considered to represent a common type of generally benign genuine giant cell tumor of bone.

Mononuclear Stromal Cells

We were only able to define one type of mononuclear stromal cell by light microscopy, even when thin Epon sections were studied with high resolution. By applying electron microscopic techniques, however, two mononuclear stromal cell types could clearly be distinguished. This failure of light microscopy to separate the two cell types from each other is evidently due to inability to distinguish between different subcellular organelles. Pale areas might be misinterpreted as a special type of pale cells but evidently result from degenerative alterations in the general population of stromal cells. The predominant cell – referred to as type 1 – is similar to that earlier described as fibroblast-like (Hanaoka et al., 1970; Steiner et al., 1972). It resembles the fibroblast both in shape and cytoplasmic content. Thus, the type 1 cell is spindle-shaped often with very irregular outline, richly developed ER – indicating a high degree of protein synthesis –, and prominent Golgi regions. Fibroblasts are furthermore known to contain lipid droplets and hemosiderin pigment in their cytoplasm, as did the type 1 stromal cells.

The nuclei in the type 1 stromal cells were very irregular with deep infoldings and tendency toward pronounced lobulation. These nuclear modulations seem to be more prominent than in the common fibroblast. This irregularity is indicative of a high metabolic activity of the cell. Similar functional conclusions could be drawn from the presence of large and prominent nucleoli. The formation of "pseudoinclusions" in the nuclei is apparently the result of extensive irregularities in nuclear shape; true intranuclear inclusions have never been seen in this tumor. Nuclear alterations of the type seen in the type 1 cells are often observed in tumor cells and the findings are consistent with the view that the type 1 cells are truly neoplastic. It should be kept in mind, however, that prominent ER and the aforementioned nuclear modulations can also be encountered in rapidly growing non-neoplastic fibroblastic tissues, for instance during wound repair. Whether these stromal cells take part in the formation of collagen or not is uncertain. However, the cells are often closely surrounded by bundles of collagen, and some of them contain fibrillar material which is also reported to occur in fibroblasts. Consequently, our findings are consistent with the view that the type 1 cell is involved in the process of extracellular collagen deposition. Occasionally, the cells seemed to encompass truly collagen-like material in the cell sap.

The type 2 cell was much less frequent than the fibroblast-like cell (type 1). The fine structure of these cells was very similar to that of connective tissue macrophages. Thus, the cell surface was very irregular and mitochondria and lysosomes were abundant. The type 2 cells therefore appear to correspond to the "less frequent stromal cells" described by Steiner et al. (1972) and the type 2 stromal cells observed by Hanoka et al. (1970). There is no convincing evidence to suggest – on morphologic grounds – that these cells are neoplastic in nature.

Our observations appear to preclude the existence of cells other than types 1 and 2 in the tissues of the giant cell tumor studied. Intermediate forms between

type 1 and type 2 cells have not been identified in the sections studied. This does not exclude the possibility that the cells could be closely related and even have developed from the same "stem" cell.

Multinucleated Giant Cells

The structure of the giant cells in giant cell tumor of bone and its relationship to the biological properties of the tumor has been discussed on the basis of light and electron microscopic observations (Jaffee et al., 1940; Schajowicz, 1961; Hanoka et al., 1970; Steiner et al., 1972). Further, the mode of formation of the cell has been a matter of considerable debate (cf. Johnson, 1930). The similarity in appearance between osteoclasts and giant cells has led some authors to conclude that the giant cell participates in resorptive processes in bone and that it is responsible for the bone destruction resulting from the growth of this tumor (Schajowicz, 1961). Some authors have questioned whether osteoclasts and giant cells differ at all (Schajowicz, 1961; Göthlin and Ericsson, 1976).

Judging from our results, it is clear that there is a certain resemblance in structure between osteoclasts and giant cells (Göthlin and Ericsson, 1976). However, the cells are not identical. Thus the surface structure differs in that osteoclasts mostly exhibit a brush border, while the giant cells in the present case never showed such formations. Endocytosis seemed to be limited in the giant cells. Furthermore, the hydroxy-apatite crystals often present in tubular invaginations and vesicular and vacuolar structures in the superficial regions of osteoclasts were never seen, and there was no "ectoplasmic layer" in the tumor giant cells as has been reported for osteoclasts (Lucht, 1972; Göthlin and Ericsson, 1976). These differences in appearance can be taken to speak in favor of the assumption that the cells are not of the same type. However, it can not be excluded that the morphological differences could reflect functional modulations of the cells depending, for instance, on the composition of the extracellular milieu. Besides, if tumor giant cells are truly neoplastic, their lack of structural specializations such as a brush border might be taken as an indication of defective differentiation.

Conspicuous differences in the appearance of the ER were noted between giant cells fixed primarily in glutaraldehyde and those fixed solely in osmium tetroxide. These differences are apparently solely fixation-induced ("fixation artefacts") which must be taken into consideration when interpreting the results. Cells fixed in osmium tetroxide alone showed an irregular dilatation—and perhaps fragmentation—of the short segments of rough ER present in central portions of the cells and associated with the Golgi regions. With regard to the latter, flattened cisternae in the glutaraldehyde-fixed cells seemed to correspond to vacuoles and empty tubular elements in the osmium-treated material.

The digestive phase of the phagocytic process occurs within the lysosomal system, which appeared to be less well developed than in osteoclasts. Typical secondary lysosomes were rather infrequent in the giant cells. However, some

digestive activity was evidently occurring in the cells as shown by the presence of "digestive vacuoles" containing well recognizable cytoplasmic organelles. These vacuoles might either signify heterophagy or autophagy. It can not be stated on the basis of morphology alone whether other structures, such as the membrane-bound tubular system found in the giant cells, belong to the lysosomal vacuole or not. This problem has to be further explored in ultrahistochemical investigations.

The nuclear structure of the giant cell characterized by margination of chromatin, occurrence of prominent nucleoli and irregular outline did not differ markedly from that of type I mononuclear stromal cells. The nuclei of osteoclasts are more regular in outline and do not have as prominent nucleoli as the giant cells. The appearance of the nuclei in the giant cells suggest that these cells are highly active in synthetic metabolic functions.

As to the histogenesis/origin of the giant cell our findings are not unambiguous. Thus, we have considered the possibility of amitotic division as proposed by Schajowicz (1961). The pronounced "lobulation" of nuclei, sometimes seen, might indicate a formative stage in amitotic division of nuclear segmentation.

Another possible mode of giant cell formation would be the fusion of mononuclear cells with each other and with multinucleated cells. The close association between mononuclear stromal cells type 1 indicates a connection between the cells and could support the "fusion" theory. Fusion between giant cells may also occur. However, we presently lack morphological evidence for this mechanism. Nor have we seen any "incorporation" (phagocytosis) of mononuclear cells into the multinucleated giant cells, Judging from the close cell to cell association as described above, there seems to be a more intimate relationship between the multinucleated cells and the fibroblast-like stromal cells type 1 than the type 2 cells. If, indeed, the multinucleated cells are created through fusion between one specific type of stromal cells, this cell would most likely be the fibroblast-like one (type 1). However, the ultrastructural similarity between stromal cells type 1 and the multinucleated cells is not so striking as to strongly suggest such a relationship. Indeed, since the bone tissue contains marrow cells with multipotential properties, the giant cell could equally well develop from these marrow-cells directly as from mononuclear stromal cells. Electron microscopy alone is evidently not sufficient for solving problems concerning the genesis of the tumor giant cells, partly due to the static nature of this technique, and partly due to difficulties in pinpointing absolute morphologic criteria for different cell types under variable functional conditions. It appears likely, however, that histo- and cytochemical methods—at the electron microscope level, and combined with autoradiographic and tissue culture techniques—should contribute to the elucidation of the mode of formation and origin of the multinucleated giant cells.

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