Nuclear Inclusions in Paget's Disease of Bone

Abstract. *The ultrastructure of bone cells was examined in biopsies from 18 patients with Paget's disease of bone and from 60 patients with a variety of other bone diseases. A characteristic nuclear inclusion was found in the osteoclasts of each patient with Paget's disease. The nuclear inclusion most closely resembles viral nucleocapsids of the measles type.*

Paget's disease of bone affects up to 3 percent of the population over age 40. In its most severe form it causes pain and characteristic deformities. The initial pathologic lesion is a focal increase in bone resorption, followed by an apparently compensatory increase in bone formation. A striking clinical feature of the disease is the long latent period before symptoms of bone pain or deformity ensue.

Despite knowledge of the existence of Paget's disease for 100 years the etiology remains unknown. Marked variability in the size of osteoclasts and the presence of large numbers of nuclei in some osteoclasts are characteristics of Paget's disease (*1*). In order to explore the possibility that there might be a primary abnormality in the bone-resorbing cells we examined the ultrastructure of bone cells in biopsies obtained from patients with Paget's disease.

Eighteen patients with classical radiological features of Paget's disease were studied. Six patients had not received any previous therapy, while 12 had received either salmon or human calcitonin, mithramycin, sodium fluoride, or disodium etidronate for periods ranging from 1 week to several years prior to biopsy. Biopsies in 13 patients were obtained from an iliac crest under local anesthesia with a Bordier biopsy needle. In five patients undergoing corrective orthopedic surgery, pagetic bone was obtained from the tibia or femur. Iliac crest or alveolar bone biopsies obtained from 60 other patients were studied for comparison. These patients had the following disorders: renal osteodystrophy, vitamin D–resistant rickets, primary hyperparathyroidism, fibrous dysplasia, osteomalacia, fibro-osseous dysplasia, traumatic bone cyst, multiple myeloma, cementoblastoma, and medullary carcinoma of the thyroid. Normal alveolar bone from five patients was also evaluated.

The bone specimen was fixed immediately in phosphate-buffered neutral 5 percent glutaraldehyde at 4°C for 2 hours, and then either postfixed in phosphate-buffered 1 percent osmium tetroxide or decalcified in 10 percent ethylenediaminetetraacetate, *p*H 7.3, and postfixed. After dehydration tissue blocks were embedded in Epon and thin-sectioned for electron microscopy. Thin sections were stained with uranyl acetate and lead citrate (*2*).

Evaluation of the ultrastructure of bone cells revealed that the nuclei of osteoclasts from pagetic patients contained characteristic nuclear inclusions not seen in any other cell type (Fig. 1). These inclusions were present in 100 percent of Paget's disease biopsies but in none of the osteoclasts or any other cells from the other patients' biopsies. The nuclear inclusions were present in 20 to 40 percent of osteoclasts and when present, in about one-fourth of the nuclei. The form of the inclusions was variable. The most common type was a random distribution of microfilaments which occupied 15 percent or more of the nuclear area (Fig. 1). Less frequently, the non-membrane-bound microfilaments were packed in paracrystalline array and occupied about 5 percent of the nucleus (Fig. 1a). Intermediate configurations were also found. The nuclear inclusions did not occur in, or appear attached to, the nucleolus. In biopsies from five patients, an occasional nucleus (about one in five) appeared deeply folded with marginated chromatin and diminished karyoplasm (Fig. 1d). These nuclei appeared to be degenerating. Microfilaments could be seen not only in the center of such a nucleus but also in gaps in the membrane and in the adjacent cytoplasm of the osteoclast (Fig. 1c).

The individual microfilaments in the nuclei of the pagetic osteoclasts measured approximately 15 nm in diameter and were highly variable in length owing to differences in orientation. At higher magnification they were seen to have an electron-lucent central core with an internal diameter of about 5 to 7 nm. When tightly packed in the nucleus in paracrystalline array, a cross section of the inclusions showed a hexagonal arrangement of the microfilaments (Fig. 1b). In the cytoplasm, the microfilaments were present in short segments either in loose bundles or packed in strands of about four filaments.

The primary importance of the nuclear inclusions we have observed is that they were found regularly in osteoclasts of all patients with Paget's disease. Identical structures have also been found in the osteoclasts of 23 patients with Paget's dis-

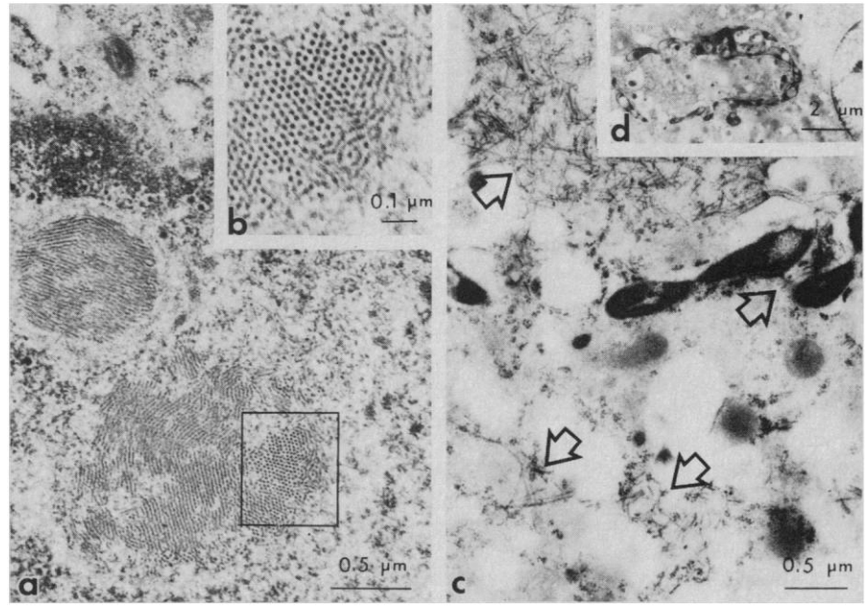

Fig. 1. Nuclei of osteoclasts containing typical inclusions from patients with Paget's disease. (a) Two nuclear inclusions illustrating different packing arrangements of loose bundles of microfilaments, cut in cross section or in longitudinal section. The upper left inclusion illustrates a distinct, but non-membrane-bound, separation from the karyoplasm, while the filaments of the lower inclusion merge with the surrounding karyoplasm (×21,400). (b) Higher-power view illustrating hexagonal packing of filaments in cross section and tubular appearance of some of the strands (×49,400). (c) Edge of ring-shaped nucleus of degenerating osteoclast apparently releasing microfilaments which are seen both inside the nuclear remnants and in the cytoplasm as well as within an opening in the ring-shaped structure itself. Gaps between nuclear elements are penetrated by typical osteoclast cytoplasm (×17,100). (d) Low-power view of same nucleus as (c), showing the appearance of the degenerating nucleus containing microfibrils (×3220).

ease by Rebel *et al.* (*3*). They postulated the presence of a papovavirus, but 20 serums from our patients with Paget's disease showed no increase in papovavirus antibody titers (*4*). Rebel *et al.* could not find similar nuclear inclusions in biopsies from patients with a variety of other bone diseases. Since two laboratories now have identified these inclusions in all cases of Paget's disease studied (41 patients) and not in any (72 patients) without Paget's disease, it is probable that they are not normally found in osteoclasts. The only other instance of similar intranuclear microfilaments observed in bone cells has been reported by Welsh and Meyer in two patients with giant cell tumors which contain innumerable osteoclasts (*5*).

The significance of the nuclear inclusions we have observed in the osteoclasts of patients with Paget's disease is not known. Since nuclear bodies of several types have been found in many different human and animal cells, it is possible that the inclusions are an indication of some unidentified pathologic process. Bouteille *et al.* (*6*) have concluded that "simple" nuclear bodies may be nuclear organelles associated with cellular hyperactivity. In Paget's disease, there is great cellular activity, and we did observe "simple" and "complex" nuclear bodies in osteoclasts and other bone cells. However, the nuclear inclusions found only in the osteoclasts of Paget's disease and giant cell tumor do not resemble any of the nuclear bodies described by Bouteille *et al.* (*6*).

The possibility that the pagetic inclusions are a handling or processing artifact is remote, since all the bone biopsies were handled in an identical manner and none were found in the nonpagetic specimens. The inclusions are unlike prenecrotic nuclear changes (*7*), although some of the osteoclasts and their nuclei appeared to be degenerating. In nine patients with renal osteodystrophy in whom osteoclastic degeneration was noted this was not accompanied by the nuclear inclusions seen in pagetic patients. Since mitoses were not observed in any of the pagetic osteoclasts, it seems unlikely that the microfilaments are associated with the mitotic spindle which contains microfilaments of 28 nm in diameter (*8*). Neither are the nuclear inclusions pseudonuclei, as can be seen by observing the nuclear remnants of the ring-shaped nuclei with the enclosed microfilaments. Likewise, the nuclear inclusions of multiple sclerosis or lupus erythematosis are morphologically distinct from those seen in Paget's disease (*9*).

The ultrastructural findings in the osteoclasts of patients with Paget's disease resemble those that have been reported in nerve cells from patients with subacute sclerosing panencephalitis (*10*). The clinical course of this central nervous system disorder is not unlike that of Paget's disease in which localized lesions may remain latent over many years. Evidence exists which implicates a measles-like virus as the etiologic agent responsible for the disorder (*11*). The typical inclusions that we have observed are intranuclear, the same size, tubular, hexagonally packed, and sometimes found in single strands. The primary difference is that they appear "stiffer," with less undulations than measles nucleocapsids.

In addition to the morphology, two other features suggest a viral presence in the osteoclasts. The presence of degenerating nuclei and the apparent release of the microfilaments into the cell cytoplasm is a sequence of events known to occur with viral infections. The formation of multinucleated cells occurs after measles infection, and, although osteoclasts normally contain multiple nuclei, in Paget's disease there may be increased numbers of nuclei. Since neither budding nor particles typical of mature virions were seen, proof of a viral nature of these microfilaments will await the isolation of an agent or identification of a viral antigen.

Although the origin and significance of the nuclear inclusions in Paget's disease remain to be determined, the finding of characteristic nuclear inclusions in pagetic osteoclasts suggests that these will be important markers in studies of the etiology of Paget's disease.

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Thyroid Hormones: Effect of Physiological Concentrations on Cultured Cardiac Cells

Abstract. *Cultured cardiac cells prepared from newborn rat heart will respond in vitro to physiological concentrations of* L*-triiodothyronine. The cells are grown in a culture medium that contains hypothyroid calf serum. A dose response relationship of* L*-triiodothyronine indicates that this system may be a useful model for elucidation of the mechanism of thyroid hormone effects on the heart.*

Thyroid hormones have a variety of biological effects in numerous organ systems. In spite of extensive studies in vivo (*1*), the mechanism of action of the thyroid hormones on the heart remains to be defined. By using cultures of GH_1 cells (*2*), a clone derived from a rat pituitary tumor, it has been shown that L-thyroxine (T4) and L-triiodothyronine (T3) modulate the rate of prolactin and growth hormone production as well as the rate of GH_1 cell replication (*3*, *4*). Because the cellular receptors for thyroid hormones, which appear to mediate these biologic responses, are localized in the cell nucleus (*5*, *6*), cultured GH_1 cells are useful for studying the molecular aspects of thyroid hormone action with particular regard to control of pituitary function.

Although there have been several studies of the effects of thyroid hormone on isolated cardiac tissue (*7*), no myocardial system responsive to physiological concentrations of thyroid hormones in vitro has been described. We have now developed such a system.