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The Osteoclasts of Hen Medullary Bone Under Hypocalcaemic Conditions

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Summary. Osteoclasts of medullary bone after several days of hypocalcaemic diet are substituted on the trabecular surface by active osteoblasts. The fate and the ultrastructure of the osteoclasts withdrawn from medullary bone surfaces in the course of a low calcium diet has been studied in serial semithin and ultrathin sections. The cytoplasmic surface of osteoclasts located in marrow compartments presents blebs and protrusions and the whole cell is often irregularly branched in several directions. A large amount of granular endoplasmic reticulum is accumulated at the cell periphery; often the cisternae are distended to form vesicles with an inner core of dense material. Osteoclasts seem to divide into mono or polynucleated smaller units.

Key words: Osteoclasts – Medullary bone – Bone cells

Introduction

Osteoclasts are the well-known cells involved in bone resorption. Their ultrastructure and the correlation between structure, activity and the level of circulating calcitonin and parathyroid hormone have been extensively investigated both in mammals and birds (Kallio et al. 1972; Lucht 1972a, b, 1973; Holtrop et al. 1974; Gothlin and Ericsson 1976; Holtrop and King 1977; Miller 1977, 1978). Nevertheless, during a more general study on the effect of a prolonged hypocalcaemic diet on medullary and cortical bone of chickens, we observed unusual aspects and behaviour of the osteoclasts and decided to perform a detailed investigation on these cells after a prolonged hypocalcaemic diet.

The material used for observation is the medullary bone of femurs of laying-hens kept on a low-calcium diet. This tissue under physiological conditions acts as the source of as much as 40% of the calcium utilized during the calcification of the egg shell (Mueller et al. 1974). Under hypocalcaemic diet all the calcium necessary for shell calcification derives from the skeleton (from both medullary and cortical

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bone) (Taylor and Moore 1954; Taylor and Bélanger 1969) where a considerable erosion takes place, especially at the endosteal surfaces (Urist 1959). Under hypocalcaemic diet an alternation of different cellular population is observed (Zambonin Zallone and Mueller 1969). During the first 48 h of diet, the predominant cells on the trabecular surfaces are active osteoclasts stimulated by the hypocalcaemia. In the following days they are replaced by a large number of osteoblasts that lay down a new network of trabeculae, which, due to the hypocalcaemic conditions, calcify only partially. The level of PTH in the course of the diet increases significantly and the newly formed bone matrix has a chemical composition different from control birds (de Bernard et al. 1980). Most osteoclasts withdraw from the trabeculae, remaining adherent only to fully calcified surfaces (Zambonin Zallone and Mueller 1969; Zambonin Zallone and Teti 1977). We have carried out an ultrastructural investigation on the medullary bone in the course of a low calcium diet, with the aim of investigating the ultrastructure and behaviour of the osteoclasts after their detachment from the trabecular surfaces.

Materials and Methods

16 White Leghorn hens, whose egg deposition had been controlled for at least 15 days, were removed from the commercial laying ration (3% calcium) and put on a low-calcium diet (0.1% calcium) (Zambonin Zallone and Mueller 1969). They were sacrificed after 8 days of treatment. This day was chosen because it is known that cell population and structure of medullary bone are strongly affected by the position of the egg in the oviduct (Bloom et al. 1941, 1958; Miller 1977), but after 5–6 days of diet, egg-laying is interrupted probably by a pituitary cut-off mechanism in the production of gonadotropins in hens fed a calcium deficient diet (Taylor et al. 1962). For this reason, after 6–7 days of treatment the cell population of medullary bone is conditioned only by the effect of the diet. After sacrifice, the femurs were promptly dissected out and samples from the mid-diaphysis and metaphysis fixed in buffered glutaraldehyde and postfixed in osmium tetroxide. After dehydration and embedding in Araldite Durcupan Fluka the specimens were sectioned with either a diamond or a glass blade on a Sorvall Mt-2 Ultramicrotome. 5–6 grids of ultrathin sections were collected regularly every 10 semithin 1 μm sections, in order to follow shape and structure of the cells at different levels.

Results

The overall picture presents some differences between diaphysis and metaphysis. After eight days of diet the trabecular network of the diaphysis is thinned and consists mainly of partially calcified osteoid matrix, surrounded by active osteoblasts. In the metaphysis, where normally medullary bone consists of a dense trellis of very thin trabeculae interposed within the meshes of the thicker trabecular network, the effect of the diet is an impressive rarefaction of the medullary bone lattice. Osteoclasts in both metaphysis and diaphysis are present, adherent to fully calcified surfaces or detached from bone. A detailed study on bone tissue modification produced by the diet is in preparation. Only the data relevant to osteoclasts will be reported here.

Observed at trasmitted light the osteoclasts display a very irregular aspect. In the metaphysis more than in the diaphysis they are often completely detached from the few medullary trabeculae still present and are located in marrow compartments

not occupied by cells of the haemopoietic lineage. Occasionally they still adhere to a calcified area of a trabecula through a cytoplasmic extension, while the cell body protrudes into the marrow spaces. They vary in size from small osteoclasts with a few nuclei to very large ones with ten to thirty. The cytoplasmic surface presents blebs and protrusions and the whole cell is irregularly branched in several directions (Figs. 1, 2). Sometimes a branch is continuous with the rest of the cytoplasm only via an extremely narrow bridge (Fig. 2B). Numerous mononucleated elements are always seen in close proximity to the osteoclasts and often connected to the latter by cytoplasmic processes (Figs. 1, 2). Under light microscope these cells show a scarce cytoplasm and clear nucleus, and lie always close between the branches of osteoclasts detached from bone. Only seldom can a small number of the above cells be observed around a resorbing osteoclast. Their shape varies considerably. In general only a thin rim of cytoplasm envelops the round or oval nucleus, but the cells can also be quite long and narrow (Fig. 2, A and C).

Under the electron microscope, the osteoclasts examined present some special features. The structure of the resorbing osteoclasts does not differ from that already known from the literature (Lucht 1972a, b; Holtrop and King 1977; Gothlin and Ericsson 1976). As to the osteoclasts lying in the marrow, totally or partially detached from bone, a large part of their cytoplasm, generally close to the plasma membrane, is frequently occupied by regularly parallel cisternae of the granular endoplasmic reticulum (Fig. 3). Sometimes these cisternae are distended into round vesicles of varying size with an inner core of dense material and an outer ring more transparent to the electron beam (Fig. 4). The smaller vesicles are continuous with flat E.R cisternae, while the larger ones (0.2–0.5 μm in diameter) are regularly rounded and seem free of connections (Fig. 5). They have been found in nearly all the osteoclasts observed, from one to five in every cell section, and are consistently located in the external part of the cell. As usual, mitochondria and dense bodies are present in large numbers, together with many free polyribosomes and Golgi complexes. Microtubules and microfilaments are also seen especially close to the cell surface, which is always extremely irregular. Areas extending into a dense network of long interlacing microvilli alternate with large cytoplasmic blebs and smooth-surfaced areas. In general, the cytoplasm close to the plasma membrane is devoid of organelles, but not exceptionally cisternae of the endoplasmic reticulum are found inside the blebs and microvilli. A large number of coated vesicles is often seen in the cell close to the plasma membrane.

The ultrastructural features of the mononucleated cells surrounding the osteoclasts are quite constant. These elements present a nucleus with dispersed chromatin, a large Golgi area, few cisternae of the granular reticulum, many free ribosomes and some mitochondria. Often large vesicles, filled with flocculent or irregularly dense amorphous material are seen as well as autophagosomes containing residues of ribosomes and/or granular endoplasmic reticulum. A pair of centrioles is also a constant feature of these cells (Figs. 6, 7). Desmosomes, zonulae adhaerentes and tight junctions are frequently observed between them, whereas zonulae adhaerentes only are found between these cells and osteoclast processes (Fig. 7). Sometimes they are connected with the osteoclasts by a thin cytoplasmic bridge (Fig. 8).

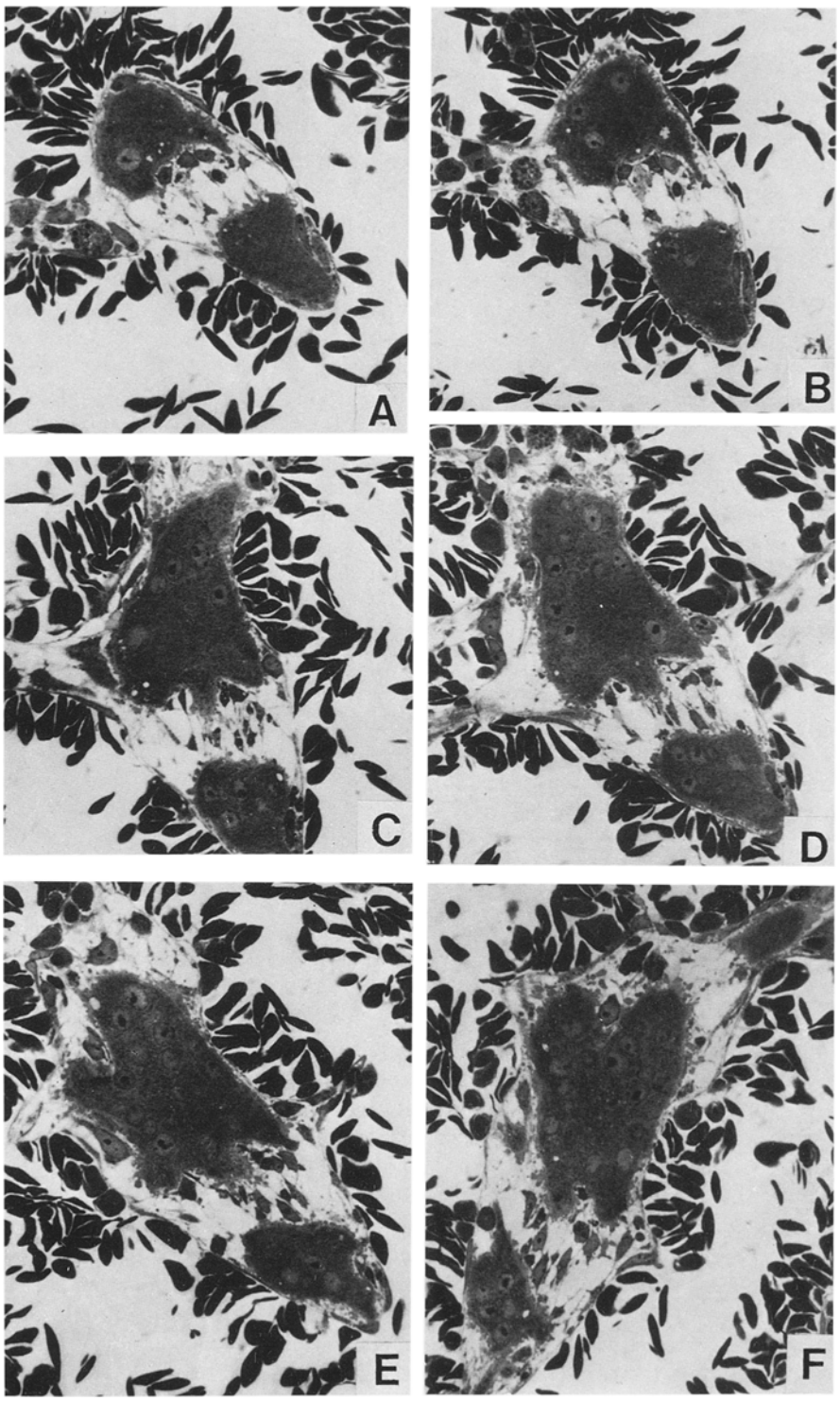

Fig. 1. Sections at different levels of two branches of an osteoclast. Note the irregular surface of the cell and the small mononucleated elements lying between the two branches. ×600

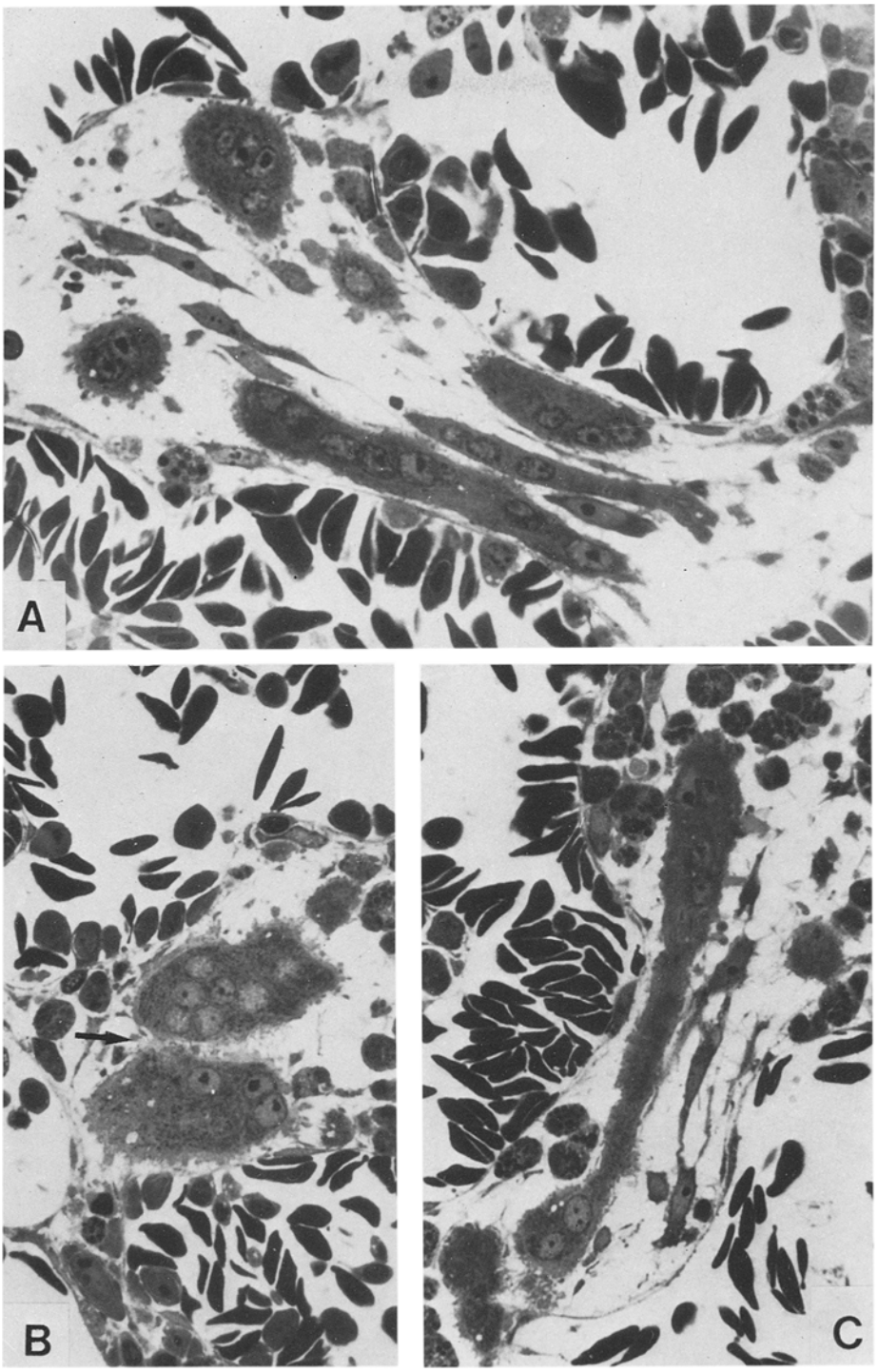

Fig. 2. **A** Several small osteoclasts free in the marrow, far from trabecular surfaces. The round ones present blebs all around the cell body, the nuclei of the others are arranged in a row. Mononucleated cells are also visible. ×1,100. **B** Two branches of an osteoclast connected by a narrow cytoplasmic bridge (*arrow*). ×1,000. **C** Free osteoclasts and nearby mononucleated cells. ×1,000

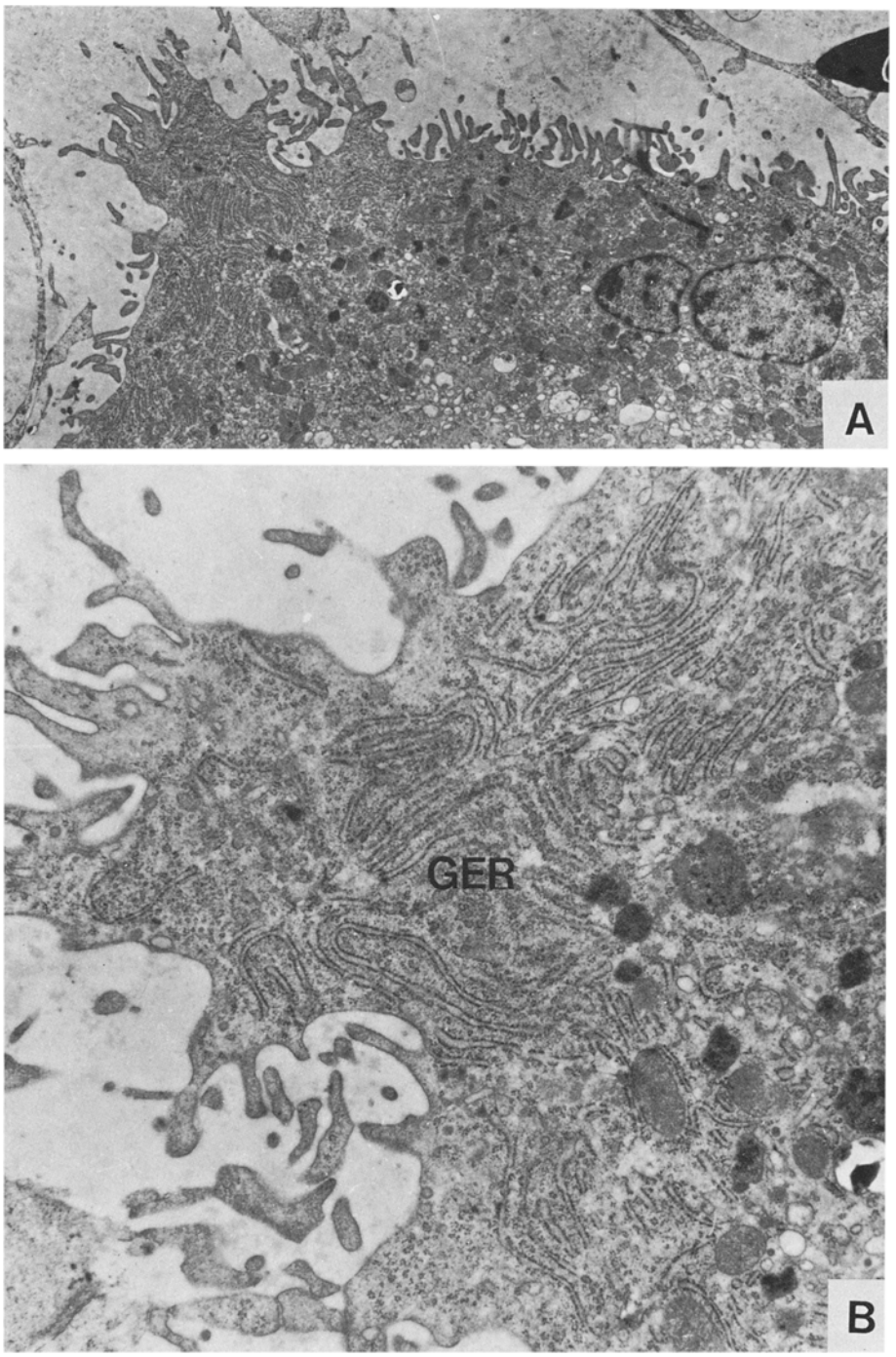

Fig. 3. Cytoplasmic surface of an osteoclast facing the marrow. A large amount of granular endoplasmic reticulum (*GER*) is present close to the cell surface. **A** ×4,650. **B** detail at ×13,500

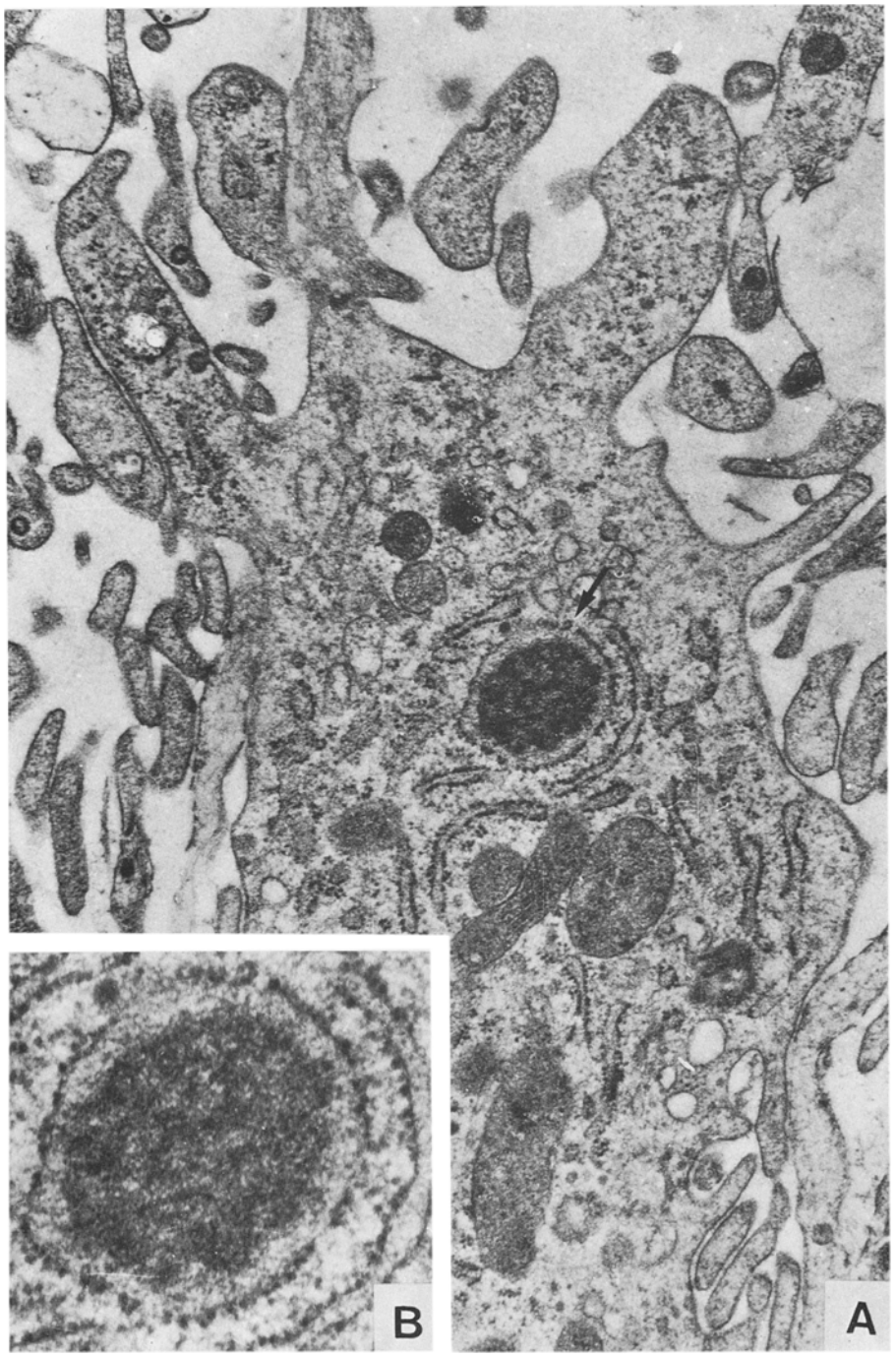

Fig. 4. Osteoclast surface facing the marrow: a cisterna of the granular endoplasmic reticulum is distended to form a vesicle containing dense heterogeneous material (*arrow*). **A** ×24,000. **B** detail of the vesicle: the dense core seems to consist of filamentous material; ×57,000

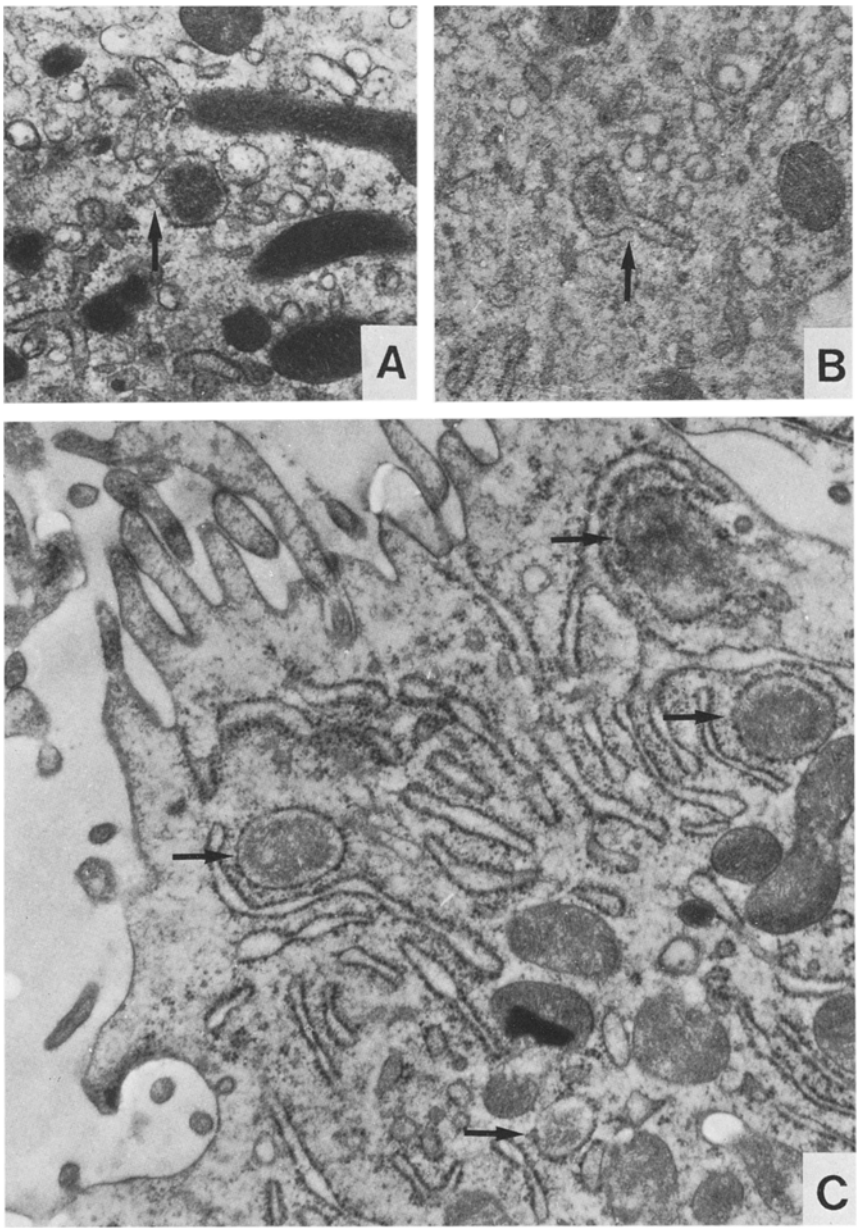

Fig. 5. Dense vesicles of the GER in osteoclasts: in **A** and **B** they are connected with flat E.R. cisternae (*arrows*). In the vesicles, an outer portion more transparent than the central core is always present. They are consistently located in a part of the cell rich in endoplasmic reticulum cisternae, close to the cytoplasmic surface. ×19,000

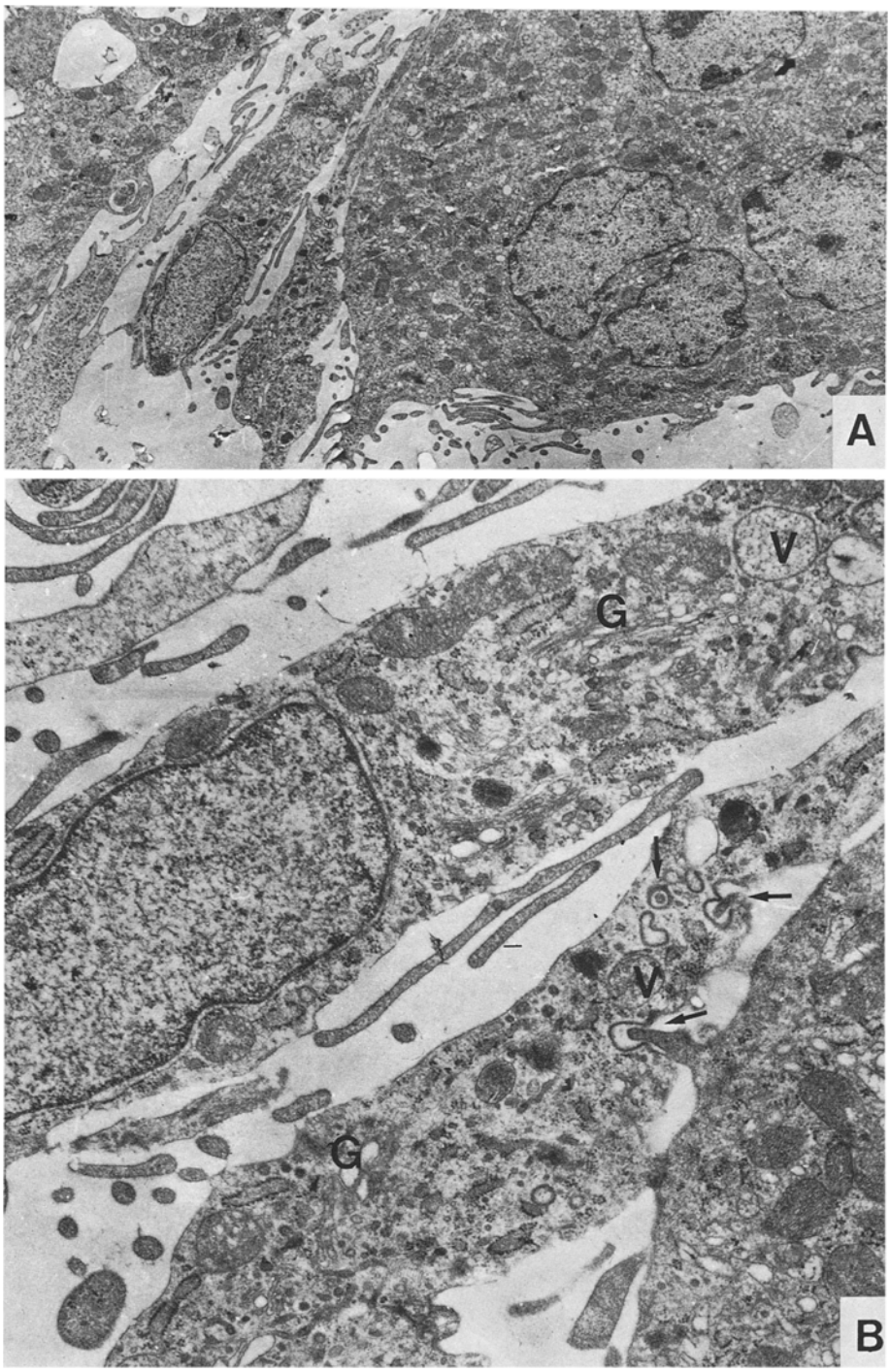

Fig. 6. A Two mononucleated cells lying between the branches of a single osteoclast. ×4,500. **B** Both cells contain Golgi areas (*G*), few mitochondria and vesicles with flocculent material (*V*). Note (*arrows*) two microvilli of the osteoclast (bottom right hand corner) projection into coated invaginations of the membrane of the adjacent small cell. ×17,000

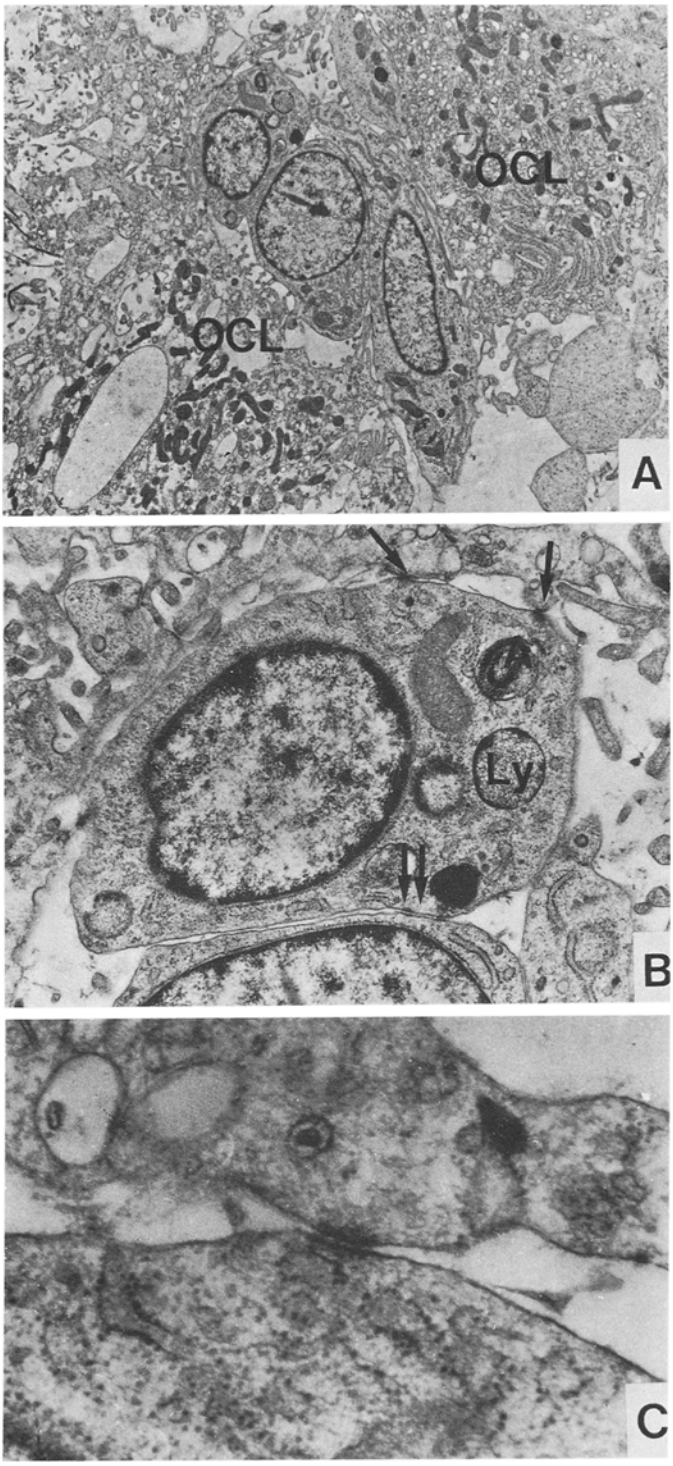

Fig. 7. Three mononucleated cells lying between two branches of an osteoclast. **A** ×3,500. **B** Note the dense vesicles, probably lysosomes (*Ly*), and an autophagosome (*A*). Zonulae adhaerentes can be seen between the small cell and the osteoclast (*single arrow*) and between the two small cells (*double arrow*). ×12,000. **C** Detail of the junction between osteoclast and mononucleated cell in another section of the same area. ×43,000

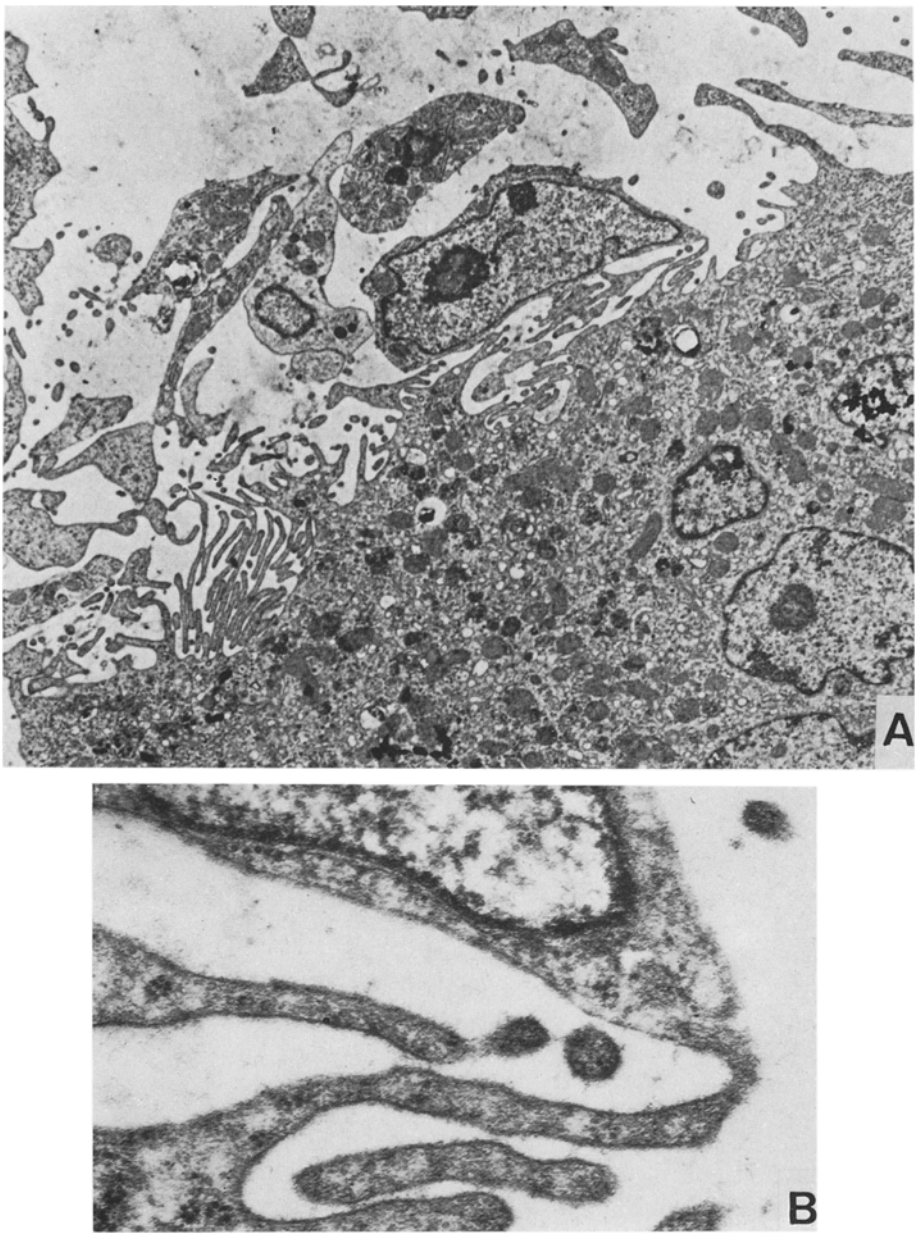

Fig. 8. Mononucleated cell connected to an osteoclast by a thin cytoplasmic bridge. **A** ×5,600. **B** ×43,700

Discussion

Luk et al. (1974) found microvilli on the cell surfaces facing the marrow in osteoclasts separated by a narrow gap from the wall of resorptive lacunae, and they suggested that the osteoclasts were withdrawing, the microvilli indicating the direction of movement. Hancox and Boothroyd (1961), by means of microcinephotography of tissue cultures, observed osteoclasts migrating out of bone or lying isolated in the medium. In this condition they displayed an energetically active, undulating border and produced blebs and extrusions of varying shape and size. Large osteoclasts seemed to tear themselves apart into smaller units which wandered away autonomously; small units, conversely, tended to coalesce into large cells. Jones and Boyde (1977) also, with the use of scanning electron microscopy, showed that osteoclasts display a very complicated form. Extensively branching, elongated cells were identified by these authors as motile, non-resorbing osteoclasts. Our findings are in line with these observations but the following remarks are apposite. After eight days of hypocalcaemic diet, a condition of hyperparathyroidism is established (de Bernard et al. 1980), the medullary bone trabeculae are mainly constituted of poorly calcified osteoid matrix and are lined by osteoblasts; osteoclasts seem to have temporarely exhausted their function in that bony region. During regular laying cycles, the number of osteoclasts does not change; these cells are apposed to bone surfaces with cyclic variations of their resorptive activity; ruffled borders are present during egg shell calcification and disappear at its completion, while extensive, interdigitated cell processes appear along the peripheral surface of the osteoclast away from the bone (Miller, 1977). In our material, since the cell population on the trabecular surfaces in the course of the hypocalcaemic diet comprises mainly osteoblasts (Zambonin Zallone and Mueller 1969; Zambonin Zallone and Teti 1977), the period of inactivity for the osteoclasts is unusually long and could account for the ultrastructural changes observed. While an altered rate of enzyme secretion could explain the presence of dense material inside the G.E.R. (Watari 1974), it is difficult to explain the augmented quantity of G.E.R. membrane.

The presence of mononucleated cells around the osteoclasts gives rise to speculation. These cells are joined to osteoclasts by cytoplasmic extensions or by zonulae adhaerentes. Actually they could be either cells in the act of fusing with or detaching from the osteoclasts. Labelling studies have demonstrated that osteoclasts originate by fusion of precursor cells. When ^{3}H-thymidine is given in vivo, labelled nuclei appear in osteoclasts only after several hours and remain there for a variable length of time (Kember 1960; Young 1962). This indicates that precursor cells incorporate ^{3}H-thymidine prior to fusion, thus forming osteoclasts, and that the osteoclast has a continuous turnover of its nuclei. Recent experiments with cell markers, osteopetrotic animals and parabiotic system suggest that the precursor cell for the osteoclast is a blood-borne element, probably a monocyte (Gothlin and Ericsson 1973; Hall 1975; Walker 1975; Loutit and Sanson 1976; Marks and Walker 1976; Jotereau and Le Douarin 1978; Simmons and Kahn 1979; Teitelbaum et al. 1979), but the ultimate fate of the osteoclasts is still a matter of speculation. There is no direct evidence that nuclear shedding is accompanied by osteoclast death and no direct evidence, either, for the fate of shed nuclei. In vivo

observations like ours cannot provide a sufficient basis for a hypothesis which is why an in vitro approach with cultures of osteoclasts isolated from medullary bone is at present being undertaken (Teti et al. in press).

References

Bloom W, Bloom HA, McLean FC (1941) Calcification and ossification. Medullary bone changes in the reproductive cycle of female pigeon. Anat Rec 81:443–475

Bloom HA, Domm LV, Nalbandov AV, Bloom W (1958) Medullary bone of laying chickens. Am J Anat 102:411–453

de Bernard B, Stagni N, Camerotto R, Vittur F, Zanetti M, Zambonin Zallone A, Teti A (1980) Influence of calcium depletion on medullary bone of laying hens. Calcif Tissue Int 32:221–228

Gothlin G, Ericsson JLE (1973) On the histogenesis of the cells in fracture callus. Electron microscopic autoradiographic observations in parabiotic rats and studies on labelled monocytes. Virchows Arch BZ 12:318–329

Gothlin G, Ericsson JLE (1976) The osteoclast. Review of ultrastructure, origin and structure-function relationship. Clin Orthop 120:201–228

Hall BK (1975) The origin and fate of osteoclasts. Anat Rec 183:1–12

Hancox NM, Boothroyd B (1961) Motion picture and electron microscopic studies on the embryonic avian osteoclast. J Biophys Cytol 11:651–661

Holtrop ME, King GJ (1977) The ultrastructure of the osteoclast and its functional implications. Clin Orthop 123:177–196

Holtrop ME, Raisz LG, Simons HA (1974) The effect of parathyroid hormone, colchicine and calcitonin on the ultrastructure and activity of osteoclasts in organ culture. J Cell Biol 60:346–355

Jones SG, Boyde A (1977) Some morphological observations on osteoclasts. Cell Tissue Res 185:387–397

Jotereau FV, Le Douarin NM (1978) The developmental relationship between osteocyte and osteoclasts: A study using the Quail-Chick nuclear marker in endochondral ossification. Dev Biol 63:253–265

Kallio DM, Garant PR, Minkin C (1972) Ultrastructural effect of calcitonin on osteoclasts in tissue culture. J Ultrastruct Res 39:205–216

Kember NF (1960) Cell division in endochondral ossification. A study of cell proliferation in rat bones by the method of tritiated autoradiography. J Bone Joint Surg 42B:824–839

Lucht U (1972a) Osteoclasts and their relationship to bone as studied by electron microscopy. Z Zellforsch 135:211–228

Lucht U (1972b) Cytoplasmic vacuoles and bodies of the osteoclast: An Electron microscope study. Z Zellforsch 135:229–244

Lucht U (1973) Effect of calcitonin on osteoclasts in vivo. An ultrastructural and histochemical study. Z Zellforsch 145:75–87

Luk SC, Nopajaroonsri C, Simon GT (1974) The ultrastructure of the endosteum: A topographic study in young adult rabbits. J Ultrastruct Res 46:165–183

Loutit JF, Sansom JM (1976) Osteopetrosis of microphtalmic mice: A defect of the haematopoietic stem cell? Calc. Tissue Res 20:251–259

Marks Jr SC, Walker DG (1976) Mammalian osteopetrosis – A model for studying cellular and humoral factors in bone resorption. In: The biochemistry and physiology of bone. Vol IV. Calcification and Physiology (GH, ed) Chap 6. New York Academic Press

Miller SC (1977) Osteoclast cell-surface changes during the egg-laying cycle in Japanese quail. J Cell Biol 75:104–118

Miller SC (1978) Rapid activation of the medullary bone osteoclasts cell surface by parathyroid hormone. J Cell Biol 76:615–618

Mueller WJ, Schraer H, Schraer R (1974) Calcium metabolism and skeletal dynamics of laying pullets. J Nutr 84:20–26

Simmons DJ, Kahn AJ (1979) Cell lineage in fracture healing in Clinic Bone Graft. Calcif Tissue Int 27:247–253

Taylor TG, Bélanger LF (1969) The mechanism of bone resorption in laying hens. Calcif Tissue Res 4:162–173

Taylor TG, Moore JH (1954) Skeletal depletion in hens laying on a low calcium diet. Brit J Nutr 8:112–124

Taylor TG, Morris TR, Hertlendy F (1962) The effect of putuitary hormones on ovulation in calcium deficient pullets. Vet Rec 74:123–125

Teitelbaum SL, Steward CC, Kahn AJ (1979) Rodent peritoneal macrophages as bone resorbing cells. Calcif Tissue Int 27:255–261

Teti A, Pace G, Zambonin Zallone A (1981) Isolamento di osteoclasti da osso follicolinico di Uccelli mediante sedimentazione a gravità unitaria. Boll Soc Ital Biol Sper 53:8–14

Urist MR (1959) The effect of calcium deprivation upon the blood adrenal cortex, ovary and skeleton in the domestic fowl. Rec Prog Hormone Res 15:455–477

Walker DG (1975) Control of bone resorption by haematopietic tissue. The induction and reversal of congenital osteopetrosis in mice through use of bone marrow and splenic transplant. J Exp Med 142:651–663

Watari N (1974) Intracisternal inclusion bodies induced in animals. Their transformation and significance. J Electron Microsc 23:255–268

Young RW (1962) Cell proliferation and specialization during endochondral osteogenesis in young rats. J Cell Biol 14:357–370

Zambonin Zallone A, Mueller WJ (1969) Medullary bone of laying hens during calcium depletion and repletion. Calcif Tissue Res 4:136–146

Zambonin Zallone A, Teti A (1977) Modificazioni della struttura delle ossa in galline ovaiole a dieta ipocalcica. Boll Soc Ital Biol Sper 53:1073–1076

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