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THE EFFECTS OF WHOLE-BODY IRRADIATION ON OSTEOCLASTS DURING ORTHODONTIC TOOTH MOVEMENT IN THE RAT

BY

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Submitted in partial fulfillment of the requirement for the Degree of Master of Science in Dentistry

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December 20, 1989

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Abstract
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Glenn R. Goodrich

Osteoclasts are thought to be of hematogenous origin. Their direct precursors are unknown, but many believe them to be descendants of the monocyte/macrophage cell line. The fate of the osteoclast is even more obscure. They are thought to have no set life span, existing by fusing and shedding nuclei until the physiologic need for them is fulfilled. Much of the current knowledge of osteoclasts has come from studies using irradiation to deplete their numbers and then following their recovery. Some of these studies have indicated possible subclasses of osteoclasts that can react differently to similar stimuli.

To examine the effects of the depletion of bone marrrow osteoclasts on orthodontic tooth movement, thirty adult, male

Fischer 344 rats, divided into two groups of fifteen were used in this study. Both groups received an orthodontic appliance. One group also received 750R of whole-body Gamma radiation. Just prior to sacrifice, each animal was bled for a peripheral WBC count and a differential cell count. Three animals from each group were sacrificed at 6, 8, 10, 12 and 14 days. The right femur and the incisor tooth attached to the orthodontic appliance were removed and prepared for histologic examination with a light microscope.

The results showed a drastic decrease in the peripheral WBC count. The differential cell counts were inconclusive. The histology of the long bone marrow spaces showed a drastic reduction in the cellularity of the bone marrow, lasting from 3 to 14 days. However, the histology of the alveolar bone lingual to the incisors showed no apparent differences between the irradiated and the non-irradiated animals. Both groups showed continued tooth eruption, tooth movement and similar numbers of osteoclasts. These results

seem to indicate that the depletion of osteoclasts in bone marrow by irradiation does not affect the activity of existing osteoclasts in the alveolar process. Longer observation periods may be necessary to determine the effect of irradiation on orthodontic tooth movement. Alternatively, osteoclasts in the alveolar process may be different from osteoclasts elsewhere in their ontogeny and function. Further experimentation is needed to answer these questions.

DEDICATED TO:

My parents, **Dr. Glenn C. Goodrich and Judith A. Goodrich**, for giving me the freedom to do what I wanted and the support and encouragement to follow it through.

AND

To my wife, **Donna**, who put up with me and the long hours, always encouraging me to go after my dreams and whose loving support kept me going when it all got to be too much. I love you dearly.

AND FAMILY,

To my wonderful kids, **Benjamin and Elizabeth**, who many nights didn't get to see Daddy come home. You both make everyday a unique and beautiful experience well worth living.

ACKNOWLEDGEMENTS

Any project of this magnitude and complexity cannot be completed by a single individual. Therefore, I wish to thank those persons who participated in this project.

Dr. Tzong-guang Tsay, you're the best! I appreciate all your long hours and help. Mostly, I thank you for your values of excellence and a job done properly.

Dr. Donald Enlow, for impressing on me the need for basic science research in a clinical field, and allowing me the time to do this project as I felt it needed to be done.

Dr. Ordean Oyen, for his help in intially formulating some of the concepts explored in this thesis and allowing me access to equipment I needed.

Dr. John Bradrick, who, without his help I never would have gotten a radiation source.

Mrs. Marcia Dunner, you also are the best! Thank you for your help in all the little things that loom so big.

Ms. Traci Mason, for your help in typing this manuscript and coming to my rescue when things started falling apart.

Finally, to my classmates, you made my two years with you some of the most memorable of my life. I wish you all good luck and prosperity.

Thierry DeCoster
Isaravadee Udomsiri-Visetsiri
Man-Ching Cheng
Laurie Brightman
Stephen Parker

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INTRODUCTION

Hematopoietic tissue has been implicated as the source of osteoclasts. The osteoclast precursors migrate from the bone marrow compartment out into the skeletal tissues where they mature into functioning bone resorbing cells. They are thought to have descended from a monocyte/macrophage precursor. The fate of osteoclasts is unknown, but current evidence seems to indicate that there is no specific life span. It is thought that osteoclasts form by fusion of precursor mononuclear cells and disappear due to nuclei being shed. However, the exact mechanisms of osteoclast differentiation, maturation, and activation are still largely unknown.

Irradiation has been used extensively to study osteoclasts. It is used to destroy the stem cell source. The usual dose is equivalent to the LD_{50} for the animal being studied. This provides for the destruction of the majority of stem cells and allows the animals to survive long enough to complete the study.

This study is designed to examine the relative numbers of osteoclasts and their morphology during the local sequence of events resulting in osteoclastic bone resorption using an orthodontic tooth movement model. Most of the osteoclast precursors were destroyed by a LD_{50} dose of whole-body Gamma radiation. It is hypothesized that the osteoclast precursors should die out because they lack a stem cell source due to the destruction of the bone marrow. Therefore, by eliminating most of the osteoclast precursors via whole-body irradiation, it will be possible to study, histologically, the fate of the existing osteoclasts and their precursors. Thus, this orthodontic tooth movement model system should provide the chance to study the elimination of osteoclasts in the alveolar bone of rodents. It is hoped that this will provide greater understanding of osteoclast fate, life span and the possibility of osteoclast subclasses, as seen by the differential response of osteoclasts at different sites in the skeleton.

REVIEW OF LITERATURE

Osteoclasts are very large cells that can range up to 100 um. in diameter. These cells contain from 2-100 nuclei per cell, but they usually contain between 10 and 20. They are associated with bone resorption and are usually found in close approximation to bone in "Howships Lacunae". Two characteristic histologic features identify an osteoclast; a ruffled border and a clear zone. The ruffled border is made up of many finger-like projections of the plasma membrane adjacent to bone. Chamber and Moore (1983), have shown that there are both motile (no ruffled border, usually monocytic) and non-motile (ruffled border, multinucleated) forms of osteoclasts in cell culture. Calcitonin caused cells to become non-motile. The "clear zone" is an organelle-free zone which is rich in actin-like filaments. The "clear zone" is thought to be involved in the attachment of osteoclasts to bone. Osteoclasts also contain large numbers of lysosomes, numerous pleiomorphic mitochondria and extensive golgi complexes. The lysosomes contain many enzymes that could be important in matrix degradation. Vaes

(1968) was the first to show thiol proteinases in resorbing bone media. Osteoclasts have also been shown to contain cathepsin-B_1 which can degrade collagen. Histochemically, osteoclasts have been shown to contain tartrate-resistant acid phosphatase. This form of acid phosphatase is specific to osteoclasts and can be used as a marker (Mundy and Roodman 1987). Osteoclasts have various receptors on their plasma membranes; they contain calcitonin receptors but they do not contain the F_c and C_3b receptors that macrophage/monocytes have, nor do they have PTH or 1,25 D_3 receptors (Shapiro et al. 1979, Hogg et al. 1980).

OSTEOCLAST ORIGIN

The osteoclast is the primary cell type which is believed to be responsible for bone resorption. The origin of these cells has been controversial ever since they were named by Kolliker in 1873. Kolliker proposed that osteoblasts fused to form osteoclasts.

Until the early 1970's, the predominant theory was the "monophyletic theory" which proposed that all bone cells are derived from a locally proliferating source. This theory gained most of it's support from morphologic studies by histologists. Bloom et al. (1941), used the medullary bone of laying pigeons to describe cellular transformation of spindle cells to osteoclasts, osteoblasts, and reticular cells. Heller et al. (1950), described the same morphologic findings in rats, guinea pigs, kittens and puppies that had been given toxic doses of parathyroid extract. They describe the fusion of spindle-shaped cells (what they called osteoblasts) into multinucleated osteoclasts. Also, osteocytes, when liberated from bone fuse with the osteoclast (Heller et al. 1950). Purely on these morphologic findings

seen under the light microscope they developed a theory for the interconvertability of bone cells. This idea fit the morphologic dogma of a resting cell (mesenchymal) followed by a transitional form and then a definitive functional cell type (osteoclast/blast). This dogma influenced the interpretation of many studies which were to follow, such as Kember (1960) and Young (1962a and b, 1963, 1964). These studies used autoradiography to follow the fate of labelled nuclei in rat bones. The results of their studies showed the label first appeared in mesenchymal cells, then in osteoblasts and finally in osteoclasts. This led to the idea that mesenchymal cells give rise to osteoblasts and/or osteoclasts. Young (1962b) called the source of bone cells, osteoprogenitor cells, and he called the process whereby osteoclasts, osteoblasts and osteocytes arise from these osteoprogenitor cells, modulation. It was also shown at this time that osteoclasts may arise from osteoblasts or chondrocytes, (Crelin and Koch 1967). These authors used mouse pubic bone explants exposed to ^{3}H-Thymidine <u>in vitro</u> to show that the radioactive isotopes first appeared in

chondrocytes then in multinucleated giant cells (chondroclasts/osteoclasts). They felt that as ossification of the cartilage occurred, chondrocytes were also transformed directly into osteoblasts and osteocytes. Tonna and Cronkite (1961) using mouse femora and ^{3}H-Thymidine showed that the label first appeared in non-specific osteogenic cells, then osteoblasts and finally in osteoclasts, 36 hours post labelling. This led them to believe that osteoclasts arise from osteoblasts since the first label appeared in pre-osteoblasts then definitive osteoblasts and then the nuclei of osteoclasts. It took 36 hours because the label needed to cycle through to the osteoclast phase. Studies showing two distinct mononuclear cell populations that had some characteristics of osteoblasts and osteoclasts were interpreted as evidence for the local development of these cell types. Scott (1963 a and b), using fetal rat tibias, ^{3}H-thymidine and electron microscopic autoradiography, showed the label being incorporated into two osteogenic cell types, a spindle cell type and a rounded cell type. The label appears in a large portion of the uninuclear cells that

are associated with developing bone trabeculae or nearby capillaries one hour after administration. This was interpreted as different developmental pathways for osteoclasts and osteoblasts but that they both developed locally from the same stem cell. The ultimate development of the monophyletic theory was as follows. Osteoclasts arise from osteoprogenitor cells; these osteoclasts then break up to form osteoblasts which then revert to osteoprogenitor cells. This last form of the theory arose from the observation that resorption is followed by formation, in most cases, and that osteoclasts shed nuclei after treatment with calcitonin (Rasmussen and Bordier 1973). This theory, which allowed for interconvertability of the osteogenic cells, seemed to have strong evidence in its favor. However, most of the data was morphologic and at a light microscopic level. Identification of stem cells at this level is extremely difficult if not impossible. Also, when the monophletic theory was predominant many of the important cell types had not been well characterized. The autoradiographic studies were mainly inferred information from studies tracing a label

over several time periods. Autoradiography was also in its developmental stages at this time.

The monophyletic theory was the predominant theory concerning the origin of osteoclasts until it was challenged by Fischman and Hay (1962). They showed osteoclasts arising from white blood cells emigrating into the regenerating limb stumps of newts. Thus, they reintroduced the theory first put forward by Jordan (1925) of a biphyletic origin of bone cells. This new theory suggested an extra-skeletal origin for osteoclasts, namely hemopoietic tissue. Other support soon followed which cast doubt on the old monophyletic theory. Toto and Magon (1966) showed that osteoclasts did not arise from osteoblasts in the periodontium. Using twelve Sprague-Dawley male rats, the authors examined the periodontium surrounding the mandibular molar teeth. With ^{3}H-thymidine as a label and parathormone as a stimulus for bone resorption, they followed the formation of cells. They observed osteoclasts forming by the aggregation of loose connective tissue cells and felt that neither osteoblasts nor osteocytes could have contributed to the formation of

osteoclasts.

Bently and Greer (1970), in their studies on rabbits, questioned the chondrocyte origin of bone cells. Also at this time Kahn and Simmons (1975), using a chimaera composed of Japanese quail limb and bone rudiments grown on the chorioallantoic membrane of chick embryos, found osteoclasts with chick-like nuclei, osteoclasts with quail-like nuclei and osteoclasts with a mixture of chick and quail-like nuclei. The cell lines could be differentiated by their chromatin patterns. They felt this supported the concept that osteoclasts are derived from mononucleated hematogenous cells such as phagocytes and monocytes. These authors also showed, using quail/chick chimaera, that precursors to osteoclasts are present in the blood prior to the onset of ossification in the host skeleton.

Ko and Bernard (1981) showed that osteoclasts form *in vitro* from bone marrow mononuclear cells in osteoclast-free bone. In 1980, Ash *et al.* showed evidence for osteoclasts arising from hematopoietic stem cells using the giant lysosomes found in beige mice. Walker (1972 b), using

osteopetrotic mice of the gray-lethal and microphalmic stock parabiotically joined to normal siblings, showed that osteopetrosis can be transmitted or cured by parabiosis. He postulated a possible humoral mechanism for the correction of the osteopetrosis. The cell which would be responsible for this is the osteoclast and Walker felt this indicated a hematopoietic origin for this cell type.

Also, Gothlin and Ericsson (1976) used parabiotically joined rats, irradiated one of the rats with a lethal dose of radiation to eliminate the hematopoietic tissues. The other rat was injected with a ^{3}H-thymidine label while the circulation was interrupted. Once the circulation was reestablished, the femur was artificially broken in the irradiated animal. At various time periods the animals were sacrificed and the healing callus looked at autoradiographically. They found only labeled osteoclasts in the irradiated animals whereas, the non-irradiated animals had all osteogenic cell types labeled. These results further indicates a hematopoietic origin for osteoclasts.

OSTEOCLAST PRECURSORS

Although it is generally accepted that osteoclasts arise from a hematogeneous origin, there is still a debate as to their precursors. There are currently three main theories as to the progenitor cell for osteoclasts (Marks and Popoff 1988). The oldest theory states that osteoclasts arise from mature monocytes and/or macrophages directly, by fusion. The second, states that osteoclasts arise from a common progenitor of moncytes and macrophages, most likely the monoblast or the promonocyte. The third, and most recent, is that osteoclasts arise from a cell line completely separate from the monocyte/macrophage lineage.

The first theory is supported by the evidence that monocytes and macrophages are often seen near sites of bone resorption and they have many morphologic characteristics in common. However, this theory has fallen out of favor for the following reasons. There are differences in the cell surface receptors, antigenic determinants, enzymatic activities and functional responses to various stimuli. (Marks and Popoff 1988). Also, Scheven et al. (1986, a) have shown that the

acid-phosphatase isoenzyme in osteoclasts is tartrate resistant and this is the most reliable cytochemical marker for these cells. However, the acid-phosphatase isoenzyme located in monocytes and macrophages has been shown to be tartrate sensitive (Wergedal, 1970 and Hammarstrom et al. 1971). Thus, the possibility of a direct fusion to form osteoclasts is less likely. Also, more recent work (Anderson et al. 1979) has shown that osteopetrotic mice given macrophage infusions are not cured of the osteopetrosis, nor did macrophage infusions cure the osteopetrosis in rats (Schneider and Byrnes 1983).

The second theory, that osteoclasts share a common, less differentiated precursor with monocytes and macrophages, draws most of it's support from several recent studies. Oursler et al. (1985) and Nijweide et al. (1985) developed monoclonal antibodies to quail and chick osteoclasts. Oursler et al. (1985) came up with antibodies to osteoclasts and members of the monocyte cell-line, whereas the Nijweide et al. (1985) experiment yielded two monoclonal antibodies that were reactive to osteoclasts, Kupffer's cells and

intestinal macrophages, but not to earlier stages of the mononuclear phagocyte cell-line. Thus, the fact that these monoclonal antibodies identified receptors on osteoclasts and cells of the macorphage/monocyte lineage suggests some common origin. However, these results are contradicted by studies by Horton et al. (1984, 1985, A and B) where they developed monoclonal antibodies to cell surface determinants on cells of the monocyte-granulocyte lineage. These monoclonal antibodies do not interact with osteoclasts isolated from mice or humans. These results do not preclude their common origin, but do cast some doubt on it. For example, it was thought that osteoclasts lacked the F_c receptor common on many phagocytic cells, yet Pierce and Lindskog (1986) have now identified these receptors only on the ruffled border region in osteoclasts. Thus, for some of the receptors to be accessable for identification, it may require certain conditions to be present.

The third theory, that osteoclasts arise from a stem cell completely separate from that of the monocyte/macrophage lineage must be examined. This theory gains support from

the premise that if osteoclasts arise from the monocyte/macrophage lineage, then they should have some cell markers in common. Namely, those that are basic to all leukocytes. The lack of these markers starts to cast doubt on the origin of osteoclasts from a hematopoietic stem cell. Horton et al. (1984) suggests a possibility of the osteoclast progenitor arising from a cell in the stroma of the hematopoetic compartment. Newer evidence which does show that osteoclasts share some of the same antigenic determinants with cells in the monocyte/macrophage lineage, tends to refute this theory. These common determinants have been shown in both the rat (Sminia and Dijkstra 1986) and the human (Athanasou et al. 1986).

OSTEOCLAST FATE

If the origin and precursors of osteoclasts seem obscure, their fate is even more so. To date, no significant study on the life span and fate of osteoclasts has been reported.

One of the first references to osteoclast life span was made by Hancox (1956) where he states that it is short, usually about two days.

Other authors have also felt that osteoclasts have a set life span. Fishman and Hay (1961) describe osteoclasts with pyknotic nuclei and hyperchromatic cytoplasms, characteristics of dying and degenerating cells. They described this phenomena in osteoclasts that had invaded regenerating newt limbs. They also assigned a life span of "normally less than two days" to these osteoclasts. However, they were not the first to observe these degenerating cells. Hancox himself (1949) described these same findings in osteoclasts.

There is another theory which has been put forward and seems to have gained greater acceptance at this point in

time. One of the first to expound on this theory was Young (1962), using rats and a ^{3}H-thymidine label, showed that osteoclasts progressively incorporate nuclei and then shed them. He felt that life span may be a poor term to use with this cell type and that the multinucleated cell may survive considerably longer than any individual nuclear element. However, he did find that the turnover rate of individual osteoclast nuclei was approximately 9 days.

This nuclei "life span" was also addressed by Jaworski et al. (1981) in which they looked at the haversian systems in dogs using a ^{3}H-thymidine label. The labeled nuclei were found to reside in the osteoclast for approximately 11.5 - 13.5 days. They also felt as Young did that life span is a poor term for osteoclasts and they suggest the term "transit time" to refer to the duration each nuclei resides in the osteoclast.

Other studies have borne out the initial work of Young, all concluding that osteoclasts have no specific set life span, but are constantly exchanging nuclei, both incorporating and shedding them as needed. This was shown

in Hall's (1975) review of the then current literature on the fate of osteoclasts in which he states that "there is no direct evidence that nuclear shedding is accompanied by death of the osteoclasts, and no direct evidence for the fate of the shed nuclei". However, most of these studies do set a "transit time" and/or a "half-time" to the duration of the nuclei remaining in the osteoclast.

Loutit and Townsend (1982), give a longevity of up to 30 - 40 days for the entire osteoclast but a "half-time" of between 5.93 - 7.99 days for the giant lysosomes found in the osteoclasts. In this experiment the irradiated osteopetrotic beige mice (bg,mi/bg,mi) were saved by infusion of bone marrow extract from normal CBA mice. The decline of osteoclasts in the irradiated animals was followed by the giant lysosome markers. These same authors (1982) found similar results in a repeat of this experiment where they used larger doses of irradiation (900-1000 rads) and rescued beige mice with spleen cells from osteopetrotic (mi/mi) mice. They found the "half-time" to be approximately 10 days in these animals.

Also, Zallone et al. (1982) using osteoclasts isolated mechanically from the medullary bones of laying hens, showed that entire osteoclasts can remain morphologically identifiable for at least three weeks but they made no attempt at determining the "transit time" for nuclei.

All of these experiments have required the injection of a radioisotope, spleen cells from another animal and/or other exotic procedures. However, they all point to an osteoclast with no set life span but with a "transit time" for individual nuclei of approximately 10 days. Yet, there have been no definitive studies to actually try to test this hypothesis, to date. The data, at present, may best be summarized by Dr. Baron (1988) when he states, "We are beginning to understand the function of osteoclasts, but their fate is totally unknown."

RADIATION

Since the development of the theory that osteoclasts are derived from hematopoietic stem cells located in the bone marrow, ionizing radiation has become an important tool in current investigations.

Ionizing radiation, when absorbed by a biological material, is absorbed as 97% heat and less than 3% chemical change (Boag 1971). It is, however, this 3% of chemical change that causes all the damage. These quanta of energy are absorbed by molecules leading to ionization and free radical production. It is these highly reactive molecules that lead to the biological damage that is observed.

Ionizing radiation can be divided into two classes, direct and indirect. Direct ionization occurs due to charged particals, like Alpha and Beta particles. Indirect ionizing radiation occurs due to x-rays, neutrons, uncharged particals and Beta or Gamma rays. The indirect ionizing radiation when absorbed, causes charged particals to be produced so it also causes direct ionizing radiation. The quantity of absorbed radiation and its secondary effects in the tissue determine

the relative biologic effectiveness (RBE) for certain types of radiation, (Boag 1971).

Gamma rays have been shown to be very effective in causing biologic damage. They have been used in many experiments to deplete or destroy the hematopoietic cell population.

These effects of radiation on the hematopoietic system have been known for a long time (Heineke 1903, 1905). Two compartments of cells can be discussed, a dividing immature group of cells that are radio-sensitive and a group of mature, fully differentiated, end stage cells that are radio-resistant.

The end stage cells are much more radio-resistant than the stem cells. It has been shown that radiation doses in the range of LD_{50} have little if any effect on the circulating erythrocytes (Patterson et al. 1962). However, the hematopietic stem cells responsible for erythropoiesis are affected. Rosenthal et al. (1951) showed that rats receiving a dose of 700R showed no recovery of erythropoiesis until 12 days after irradiation. In the macrophage-histiocyte system,

it was also shown that mature macrophages are extremely radio-restistant. Bloom and Bloom (1947) showed that a dose of 1000R of x-rays did not visably damage bone marrow macrophages. Dunlap (1942) discribed spleen and lymph node macrophages being plentiful in animals receiving lethal doses of irradiation. The stem cells are much different though. They are very radio-sensitive in most cases. McCullogh and Till (1962) studied the effect of Gamma radiation on the survival rate of colony-forming units in mouse bone marrow. They found that increasing doses of radiation caused a linear decrease in the surviving stem cells. At the LD_{50} for the mouse (between 600-700 Rads) only 2 to 3 stem cells per 1000 retain their ability to proliferate.

This effect on the stem cells can also be monitored in the peripheral blood. Loiselle et al. (1959) showed that rats given a dose of 400 Rads whole-body x-radiation had a reduction of peripheral white cells in the blood. The lymphocytes were the most sensitive, dropping to less than $1000/mm^3$ in just 2 days. The polymonphonuclear cells decreased to less than $200/mm^3$ in just 3 days. The platelets

took approximately 10 days to drop to less than 100,000/mm^3. Thus, there is a drastic change in the stem cell viability that can be monitured in the peripheral blood.

Ionizing radiation and it's effects on immature stem cell populations has been used by researchers to study various cells. Many have used rodents as a model and the LD_{50} as a radiation dose since this causes the required destruction of the stem cell pool but allows the animals to live long enough to finish the experiment.

The LD_{50} for the rat was determined in experiments by Moos et al. (1954) where they used Holtzman and Sprague-Dawley rats and x-rays to give and LD_{50} dose of approximately 600-750 Rads. Sinclair and Blackwell (1962) determined the LD_{50} for Sprague-Dawley rats to be 800 Rads, when they used Co^{60} Gamma radiation. Upton et al. (1955) called 730 Rads the LD_{50} for Gamma radiation in RF strain mice.

Thus, radiation has been used extensively to study various systems. It has also been used to study the origin, resorbing activity, population size and formation of osteoclasts. Walker (1975) used 600-900 Rads of whole-body

Gamma radiation to destroy the bone marrow in C57B/6J osteopetrotic mice. He then reinstated the stem cell population with a bone marrow transplant from normal littermates. The transplant cured these mice of osteopetrosis via the formation of normal osteoclasts. Ash et al. (1980) also used microphthalmic (mi/mi) osteopetrotic mice which they converted to radiation chimaeras by lethally irradiating with 600 Rads of whole-body x-radiation. The mice were then rescued and made chimaeras by infusing them with bone marrow from a beige (bg/bg) mouse. These mice have giant lysosomal granules in their osteoclasts which are easily identifiable with light microscopy. Loutit et al. (1981) used 600 Rads of whole-body radiation to examine the colony forming units and haematopoietic stem cells in osteoclastopoiesis. They used the mice with a double mutant to show that these are different populations of cells. Anderson et al. (1979) used 180-3780 Rads of whole-body radiation, given 45 Rads/day, to examine the skeletal changes, including alterations in marrow, growth plate and osteoclast populations in C3H mice. The authors found that

prolonged external iradiation caused no decrease in osteoblast function or numbers. However, the osteoclasts were reduced in number and bone remodeling was deficient. Scheven et al. (1985) used embryonic mouse metatarsal bones to examine the effects of 1000-2000 Rads of x-radiation on the formation and resorbing activity of osteoclasts in culture. They found that osteoclast numbers were reduced significantly by doses of only 250 Rads but that they could not be totally eliminated even with the very large doses.

Thus, it can be seen that whole-body irradiation is a very effective tool for examining osteoclasts and evaluating their origin and function. In long bone explants osteoclasts are fairly radio-sensitive although total stem cell destruction seems impossible for in vivo studies.

HETEROGENICITY OF OSTEOGENIC CELLS

Although the origin, precursors and fate of osteoclasts have remained obscure, the possibility that there may be a heterogenicity of osteoclasts has arisen. This heterogenicity exists for many cell types and is just now starting to be elucidated.

Young (1962), using growing rat tibiae and ^{3}H-thymidine autoradiography to look at cell cycles, showed that generation time for mesenchymal cells was shortest in the metaphysis, intermediate in the endosteum and longest in the periosteum. The author felt that this difference in generation time for long bone mesenchymal cells was due to differences in the G_1 phase of the cell cycle. This could also be due to differences in local factors affecting cell generations or possibly to slightly different cell types.

This idea also holds true for more differentiated cells. Ko et al. (1977) showed that PDM fibroblasts cultured in the presence of PGE_2 for 12-33 hours had a 42% reduction in cell proliferation and that DNA synthesis was inhibited. However, cells that had been previously cultured for 14 days with PGE_2

showed no reduction in DNA synthesis. His conclusions were that PGE_2 supresses the growth of some gingival fibroblasts allowing the non-PGE_2 sensitive fibroblasts to proliferate. Thus, the PDM may be made up of a heterogenous group of fibroblasts which will respond differently to similar stimuli. He further hypothesized that there exists an equilibrium within the PDM. There are cells that predominate during synthetic periods and those that predominate during degradative periods. Certain stimuli tip this equilibrium to favor either synthesis or degradation,

Davidovitch et al. (1976, 1977) have also found evidence for a heterogeneous population of osteoblasts. In their studies using cats undergoing orthodontic tooth movement, the levels of cyclic AMP and cyclic GMP in osteoblasts on the tension side of the tooth was examined. They found heavily stained osteoblasts situated next to non-staining osteoblasts. This would indicate either a different stage of cellular activity or possibly two or more different types of osteoblasts.

More support for the heterogenicity of osseous cells comes from authors such as Liu and Baylink (1984). They looked at osteoclasts on rat incisor alveolar bone, residing in the socket and on the endosteum. After 12 days of Ca^{++} depletion, they saw a 463% increase in osteoclasts on the endosteum. One the other hand, phosphate depletion, caused a 103% increase in the number of osteoclasts on the endosteum in the same time period. However, the osteoclast numbers on the socket wall remained relatively unchanged. Similar observations were also around the first molar. These differences were attributed to functional roles of osteoclasts at different sites: the osteoclasts around the tooth socket have a tooth support function and those located on the endosteal bone have a mineral homeostasis role. The authors felt this may be due to some local control mechanism or it could possibly be due to different subclasses of osteoclasts.

Also, Baron and Saffar (1977), using adult golden hamsters and studying the effects of calcitonin on serum Ca^{++} and PO_4^- levels and osteoclastic resorption of bone. They

looked at osteoclasts on socket walls and endosteum. Results showed that osteoclasts on the endosteum were unaffected by calcitonin levels after 30 days, whereas those on the alveolar socket wall were significantly reduced (-12.1%). There was also found to be a 34.3% increase in osteoid tissue on the socket wall due to reduced resorption. Again, this may indicate a different type of osteoclast being located on the alveolar socket wall which is more sensitive to calcitonin thereby preventing loss of socket wall integrity when there is a systemic need for calcium and/or phosphate.

Saffar and Baron (1977), also report a study where they used adult golden hamsters in an experimental periodontal disease model. One group was fed a normal diet and the other a hyperglycidic diet for 6 months. Results showed that different alveolar bone surfaces reacted in different proportions to this stimulus. They reported that the number of osteoclasts along the periosteum and endosteum were both increased significantly (0.001 and 0.010, respectively). However, osteoclasts were not affected along the alveolar socket wall. The authors felt that this apparent resistance

was due to an increased remodeling capability of the socket wall to inflammation. However, it could also be due to local factors being slightly different or possibly due to a different subpopulation of osteoclasts that react differently to similar stimuli. Again, this difference could be necessary for the socket to maintain it's integrity, even in the most adverse of conditions.

Another interesting paper from a related field was put forward by Butcher et al. (1980). They looked at the specificity of lymphocyte migration to specific organs mediated by organ-specific determinants on high endothelial venules. Using mouse lymphocytes isolated from Peyer's patches and lymph nodes, they found that these cells migrated to their respective organ of origin almost exclusively in tissue culture. The authors propose a lymphocyte surface receptor specific for organ-restricted endothelial cell determinants that mediate the antigen-independent organ specificity of lymphocyte migration. So what is being shown is that these cells all originate in bone marrow, however, there are different types which due to their specific surface

receptors, will only react with certain endothelial cells in venules that determine what organ they will reside in. This could also be true for cells of the macrophage-monocyte lineage. Possibly that is why monocytes (mature), pleural macrophages, peritoneal macrophages, Kupfer cells and others in this cell line can be induced to resorb bone but not in exacly the same manner osteoclasts do. Though they share a common precursor, they differentiate into cells which contain surface markers very specific for the organ in which they will ultimately reside.

STATEMENT OF THESIS

Current evidence seems to indicate that osteoclasts arise from a hematopoietic stem cell, located in the bone marrow. Osteoclast precursors migrate from the marrow to the skeletal tissues where they mature into the primary bone resorbing cell. Local factors may contribute to the formation of different subclasses of osteoclasts. The exact mechanisms of osteoclast differentiation, maturation and activation are still largely unknown.

This study was designed to examine osteoclast numbers and morphology during the local sequence of events resulting in osteoclastic bone resorption using an orthodontic tooth movement model. By eliminating most of the osteoclast precursors via whole-body Gamma irradiation, we wish to study, histologically, the fate of existing osteoclasts and their immediate precursors. It is hypothesized that without replenishment, the existing osteoclasts should gradually die out. Therefore, this model system should provide the opportunity to study the elimination phase of osteoclasts in the alveolar bone of rodents. This may allow further

elucidation of osteoclast fate, life span and the possibility of osteoclast subclasses as seen by differential responses.

METHODS AND MATERIALS

Thirty adult male Fischer 344 rats (Charles River Laboratories, Wilmington, MA.) were used in this experiment. Each animal was a retired breeder, between 9 to 12 months of age and weighed over 380 grams at the onset of the experiment. The animals were divided into two groups of fifteen animals each. Rats in the experimental group received 750 Roengtens (R) of whole body Gamma radiation. The other served as a control group and received no irradiation. Each animal was caged individually and was given both food and water ad Libitum throughout the experiment. Rats in both groups received the following treatment. Each animal had an orthodontic appliance placed from a maxillary molar to a maxillary incisor. All animals were observed twice daily and weighed once daily. These animals were then sacrificed at 6 days, 8 days, 10 days, 12 days and 14 days post-appliance placement. Three irradiated and three control animals were sacrificed on each date. The animals all had blood drawn just prior to sacrifice to be used for peripheral white blood cell (WBC) counts and

differential cell counts. After sacrifice the maxillae were removed and fixed in 10% buffered formalin (Fischer Scientific Co.) and then processed for histologic examination. Details on each step in the methods and materials follows.

I. ANESTHESIA: All animals received anesthesia prior to orthodontic appliance placement, irradiation and bleeding. Each animal was given ethyl ether (Fischer Scientific) via inhalation in a closed bell-jar, until unconscious and breathing easily. This rendered the animals immobile and easy to handle for further, more precise and profound anesthesia to be administered. Next, each animal received a mixture of Ketamine Hydrochloride (Ketaset, Bristol VPP) 100 mg/ml and Acepromazine Maleate (Promace, Techamerica) 10 mg/ml as an I.M. dose of 67.5 mg/kg. ketamine and .75 mg/kg acepromazine. A cocktail was made in the following manner (Kim 1987).

90% Ketaset and 10% PromAce cocktail
1) 9.0 ml. ketamine hydrochloride 100mg/ml.
2) 1.0 ml acepromazine malate 10 mg/ml.
1.0 ml contains 90.0 mg ketamine and
1.0 mg. acepromazine.

This rendered the animals unconscious, more easy to manipulate and free of pain for approximately 2-3 hours.

II. FORCE DELIVERY SYSTEM: The orthodontic force delivery system used was that developed by Heller and Nanda (1979) and Williams (1984). It consisted of an F-33 0.008 x 0.030 inch closed coil spring (Elgiloy, Closed coil spring, Rocky Mountain Orthodontics) attached to a maxillary molar and incisor. A 7.0 mm. length of spring was attached to a maxillary incisor via a stainless steel 0.010 inch ligature wire passed through a hole drilled through the tooth with a 1/4 round bur in a highspeed dental air turbine at the free gingival margin. At the posterior end a stainless steel ligature, of the same diameter, was passed between the maxillary first and second molars and secured by being cinched tight around the first molar at the gingival margin. Each spring was then activated 2.0 mm. which resulted in a force delivery of 50 grams, measured via a Stress and Tension gauge, (Dentaurum Federwooge, 10-100gm., Dial Type).

III. IRRADIATION: Each animal in the experimental group received 750R of whole body Gamma radiation, equivalent to the LD_{50} for the rat, immediately before orthodontic appliance placement. This dose was delivered via a medical Co^{60} source

used in the Radiation Oncology Department at Cleveland Metropolitan General Hospital. The dose was calculated and delivered by a radiation technician at the hospital. The animals were anesthesized and then placed in a plexiglass cage designed to hold six animals simultaneously, each in their own individual compartment. The dose was delivered at 230.4 Rad/min at a distance of 58cm over a 3.26 minute interval. After irradiation the animals were removed and allowed to recover in their own individual cages.

IV. BLEEDING OF THE ANIMALS AND PERIPHERAL WHITE BLOOD CELL COUNTS: After anesthesia, the distal 4mm of the tail was removed from each rat with a surgical blade. Blood was collected from the proximal segment for the determination of peripheral white blood cell (WBC) count and a differential cell count. The tails were allowed to bleed and the sixth full drop of blood was collected in a white count pipet and aspirator (A.O. Scientific Instruments). The blood was then diluted with Turk's diluting fluid.

Turk's diluting fluid - from (A.O. Hemacytometer Handbook)

a. Glacial Acetic Acid 3.0 ml.
b. Aqueous Gentian Violet 1% 1.0 ml.
c. Distilled H_2O up to 100 ml.

After agitation for 60 seconds the solution was placed on a Neubauer bright-line hemacytomer with coverglass (A.O. Scientific Instruments). Using a Leitz light microscope and the 10X objective the four outside corner squares and the center square were used for counting the WBC's, and in the calculation of the number in the peripheral blood. The following formula was used:

> Number of WBC's counted x Correction for volume x Correction for dilution = Number of WBC's/mm^3 of peripheral blood.

For the differential cell count, 4 slides were made by placing a drop of blood on one end and lightly smearing it across the slide with a coverslip. These slides were then stained with Wright's Stain (Fisher Scientific Co.) and coverslipped with Permount (Fisher Scientific Co.).

V. SACRIFICE: Each animal was sacrificed via ethyl ether (Fisher Scientific) inhalation overdose in a closed-bell jar. Sacrifice occurred 6, 8, 10, 12 and 14 days after appliance placement for both control and experimental groups.

VI. FIXATION, DECALCIFICATION, EMBEDDING AND SECTIONING: After sacrifice, the entire maxilla and the right femur were dissected out and placed into a 10% buffered formalin

solution (Fisher Scientific Co.). Specimens were left in this solution for 24 hours in a 4.0 °C cold room. The samples were then washed in a 1x phosphate buffered saline (PBS) solution for another 24 hours. Each sample was then placed in a 0.5M EDTA (Fisher Scientific) pH. 7.2 solution to decalcify for 14 days. The solution was changed daily and all samples were constantly agitated via a rotary mixer, at room temperature. After decalcification samples were again washed with PBS solution for 24 hours.

1. Phosphate-buffered Saline solution (10x)
   a. 800.0 ml. distilled H_2O
   b. 84.0 g NaCl 1.46 M.
   c. 2.7 g NaH_2PO_4 H_2O 0.02 M.
   d. 10.66 g Na_2HPO_4 anhydrous 0.075M.
   e. pH. to 7.4
   f. bring up to 1000.0 ml. with distilled H_2O

2. Ethylenediamine Tetra Acetic Acid 0.5M Solution
   a. Dissolve into 800.0 ml. distilled H_2O
   b. 190.1 g EDTA
   c. 6.055 g Tris
   d. pH. to 7.2 with concentrated HCl
   e. bring up to 1000.0 ml. with distilled H_2O

Next, each incisor and surrounding alveolar bone and a portion of the mid-shaft of the femur bone were dissected out and dehydrated in graded alcohol (ETOH, U.S. Industrial Chemicals Co.) solutions, placed in xylene and then embedded in paraffin. The following schedule was used:

a.	10% buffered formalin	24 hrs.
b.	1 X PBS pH. 7.4	24 hrs.
c.	0.5 M. EDTA, Tris buffered pH. 7.2	14-21 days
d.	1 X PBS ph. 7.4	24 hrs.
e.	10% buffered formalin	24 hrs.
f.	95% ethanol	1 hr.
g.	95% ethanol	1 hr.
h.	95% ethanol	1 hr.
i.	100% ethanol	1 hr.
j.	100% ethanol	1 hr.
k.	100% ethanol	1 hr.
l.	Xylene	1 hr.
m.	Xylene	1 hr.
n.	Paraffin	1 hr.
o.	Paraffin	2 hr.+

Samples were then oriented and sectioned at 6 um on a microtome (American Optical). Due to the size of the samples and the need to use mild decalcifying agents, there were areas that did not totally decalcify. Strong decalcifying agents cause a loss of basophilic staining in the prepared specimens, each doubling in acidity causes a 10 fold decrease in stainablility. Therefore, in these situations it was necessary to soak each block in fabric softener containing an aqueous dispersion of a cationic fabric softening agent. This caused the surface of the specimen to decalcify more completely and permitted more ease in sectioning. This procedure needed to be repeated several times during the cutting of each sample. The cut sections were collected and floated on a 54 °C waterbath with ethyl alcohol added to help

in flattening and spreading the samples. The sections were collected on clean microscope slides (American Scientific Products) coated with egg albumin (Harleco, Meyer, Albumin Fixative). These slides were then placed on a 60 °C hotplate overnight. The specimens were then ready for routine histologic staining. Slides were stained with Hematoxylin and Eosin in the following manner:

a.	Xylene	5 mins.
b.	Xylene	5 mins.
c.	100% ETOH	1 min.
d.	100% ETOH	1 min.
e.	100% ETOH	1 min.
f.	95% ETOH	1 min.
g.	distilled H_2O	1 min.
h.	distilled H_2O	1 min.
i.	Hematoxylin - nuclear stain	4 mins.
j.	Tap H_2O	rinse
k.	Tap H_2O	rinse
l.	Scott's Tap H_2O blueing agent	3 mins.
m.	Running Tap H_2O remove excess Scotts	1 min.
n.	95% ETOH - to dehydrate	1 min.
o.	Eosin - cytoplasmic stain	2.5 mins.
p.	95% ETOH	rinse
q.	100% ETOH	rinse
r.	100% ETOH	rinse
s.	100% ETOH	rinse
t.	100% ETOH	rinse
u.	Xylene	1 min.
v.	Xylene	1 min.

Once stained the specimens were mounted in Permount (Fisher Scientific Co.) and coverslipped for microscopic examination. All specimens were examined light microscopically using a Leitz microscope and 1.6X, 10.0X and 40.0X objectives.

Osteoclasts were identified as cells that were multinucleated and residing in Howship's lacunae. No attempt was made to identify single nucleated bone cells nor multi-nucleated cells away from the bone surface.

RESULTS

I. ANIMAL SURVIVAL: The animals survived the experiment very well. There was very little morbidity associated with the Gamma irradiation. Most of tHe animals showed some changes in their normal activities. The animals were slightly more lethargic for the first 48 hours after irradiation and it took several days for the animals to resume normal eating patterns. This was also true for the non-irradiated animals, however. The only noticeable differences were that the non-irradiated animals resumed normal eating activity and mobility sooner than the irradiated animals. Also, some of the irradiated animals showed diarrhea for several days following irradiation and a few displayed some bleeding and bruising around the eyes (an indication of capillary permability) during the course of the experiment.

II. FORCE DELIVERY SYSTEM: The force delivery system appeared to work well and predictably. Initially it was difficult to place on the animal, but with practice and persistence it became much easier. There did appear to be

some qualitative effect on tooth eruption in the irradiated animals when compared to the non-irradiated group. The difference was the time it took the wire passing through the incisor at the level of the free gingival margin to reach the incisal edge. For the non-irradiated animals this took approximately 14 - 16 days. For the irradiated animals this never occurred. In most instances, at 14 days, the ligature was just over 3/4 of the way up the tooth. These data were not accurately kept, however, and these results are more empirical in nature.

III. ANAESTHESIA: The anesthesia of the animals worked very well. All were easy to handle and free of any discernable pain throughout the experiment. This is an excellent method for working with rats, being easy to administer and maintaining a very wide margin of safety.

IV. WEIGHT CHANGE RESULTS: The weight loss demonstrated by the control and experimental animals resulted in an expected curve for both groups (Graph 1). In the irradiated animals, there was a drop in weight of approximately 21.3 grams during the initial phases of the experiment where the animals were

anesthesized. This was followed by much more rapid weight loss after orthodontic appliance placement and 750R whole body Gamma irradiation. This phase of weight loss lasted approximately four days, and the animals averaged a weight loss of 18.2 grams at two days and 46.3 grams at four days post-appliance placement and irradiation. Next, there was a phase during which the drop in weight leveled off with only minor daily fluctuations throughout the remainder of the experiment. The control, non-irradiated animals, which only received the orthodontic appliance, also showed a minor weight loss during the initial phase of the experiment. This loss was similar to that seen in the irradiated animals. They also exhibited a rapid weight loss for two days after appliance placement which resulted in an average weight drop of 23.5 grams and at four days the average drop was only 24.1 grams. After this two day period, the weights tended to stabilize and showed only minor daily fluctuations.

V. RESULTS OF THE PERIPHERAL WHITE BLOOD CELL COUNT: Results of the peripheral white blood cell counts were as follows: At 6 days post-irradiation, there was an average of 190 WBC/mm^3 of blood. By 8 days post-irradiation, the number of WBC had dropped slightly to 127 WBC/mm^3 of peripheral blood. Ten days after irradiation, the cell counts had started to recover somewhat and were at 280 WBC/mm^3 in the peripheral blood. At 12 days, the number had more than doubled from the 10 day total, up to 787 WBC/mm^3 and at 14 days, they had increased to 1,160 WBC/mm^3 of blood. In the control, non-irradiated animals, the average peripheral WBC count was 5,212 WBC/mm^3. These data are summarized in Table 1.

IV. RESULTS OF THE DIFFERENTIAL CELL COUNTS: Results for the differential counts were inconclusive. On the 4 slides prepared from each of the irradiated animals and cover-slipped, enough cells could not be identified to give the 100 needed to perform the count. This was true for all of the irradiated animals, even those surviving 14 days after irradiation.

VII. EFFECTS OF IRRADIATION ON BONE MARROW: The bone marrow in rat long bones is made up of branching vascular sinues lying in a reticular stroma which supports hematopoiesis. Observation of the histologic sections prepared from non-irradiated rats are essentially similar to that described by Weiss (1976). The hematopoietic space is a spongework of reticular cells which surround sinuses within which hematopoietic cells reside. The most prevelant cell type, the erythoblast, is clustered into islets, and megakaryocytes lie just outside sinuses. These two cell types and the fat cells are the easiest to identify. The mononuclear leukocytes and the probable stem cells are clustered around the arterial vessels. Fat cells are located proximal to

vascular sinuses and are thought to be reticular cells which accumulate fat (Weiss 1976) (Fig. 1).

Major cell types that can be identified in these sections include megakaryocytes and fat cells (Fig. 2) The osteocytes appear viable and the marrow cells are packed in and around the trabeculae and fat cells (Fig. 3). There appear to be areas of clustered mature red blood cells, possibly within sinuses (Fig. 4). Other cells that can be identified are eosinophilic myelocytes, neutrophilic metamyelocytes, normoblasts, erythoblasts and possibly basophillic myelocytes. However, even in these 6 um sections, the nuclei overlap and cellular definitions and outlines are difficult to discern (Fig. 5). Most of these cells are involved in erythroycyte production. These cells retain their nuclei until the final stage of late normoblast, which is just before the nucleus becomes pyknotic and is lost from the cell and the mature erythrocyte enters the blood stream. There are approximately 600 red blood cells for every one white blood cell. The rest of the marrow is dedicated to the development and maturation of lymphocytes,

granulocytes, monocytes and platelets.

When compared to the non-irradiated controls, the irradiated animals showed radically different morphology in the shafts of their long bones. These histologic results coincide well with the decreased peripheral white blood cell counts.

At three days post-irradiation, the rat long bone shaft bone marrow show the signs of advanced destruction (Fig. 6). Most of the cellular components of the bone marrow are gone. What remains are cells undergoing degeneration (Fig. 7) These remaining cells show pyknotic nuclei and their cell boundries are becomming indistinct (Fig. 8). There are scattered RBC's, and there are either fat cells or stromal compartmentalization. The megakaryocytes, the stromal element cells, the fat cells and the endothelial cells associated with the blood vessels appear to be relatively unaffected by the radiation at this time. These are fully differentiated end stage cells that are known to be radio-resistant compared to undifferentiated mesenchymal cells. Thus, at three days post-irradiation in the experiment it

appears that the stem cells have been either completely or very greatly depleted. Also, at three days post-irradiation, the osteocytes and bone lining cells appear viable and healthy (Fig. 9). Once again, these are fully differentiated cells and are expected to be radio-resistant.

At 6 days, the bone marrow is completely decimated (Fig. 10). The marrow cavities have undergone what appears to be a fatty degeneration. Cellular morphology is non-existant. Discernable nuclei are not present and any structure that can be seen appears to be associated with the residual stromal elements of the marrow (Fig. 11). However, the endothelial cells associated with the sinusoids and blood vessels are still present. Also, the osteocytes and bone lining cells are still identifiable (Figs. 12 & 13).

By 8 days, in the irradiated animals, the marrow still remains destroyed (Fig. 14). However, more nuclei can be seen and red blood cell production appears to be recooperating to a limited degree (Fig. 15). Most of the nuclei that can be seen are located around blood vessels running through the marrow cavities (Fig. 16). These are

most likely due to a certain amount of recent stem cell proliferation and indicate an initial rejuvination of the bone marrow. Again in these animals, the osteocytes and bone lining cells are identifiable (Fig. 17).

Ten days after irradiation, there appears to be very little difference from the 8 day samples. For the most part, the bone marrow is completely destroyed (Fig. 18). There are some nuclei present, but most are very immature looking and are most likely involved in red blood cell production (Fig. 19). There are vast areas of vacuolation (Fig. 20), which may just be the stromal remnants. However, these also appear to be fat cells, although no nuclei can be demonstrated. These could possibly representing the "spongework" described by Weiss (1976). Again, the osteocytes and bone lining cells are present (Fig. 21).

At 12 days post-irradiation, the appearance of the bone marrow still has not changed much from the eight and ten day results (Fig. 22). There does appear to be a slight increase in the number of nuclei and cells (Fig. 23). The cells that are present contain small single nuclei. They are small

cells that appear very immature (Fig. 24). Agian, at this time the osteocytes and the bone lining cells are present and appear healthy (Fig. 25).

By 14 days post-irradiation, the rat bone marrow is definitely showing signs of rejuvination (Fig. 26). The marrow is still only a fraction of it's normal cellularity, but many of the elements appear to be returning (Fig. 27). As in the previous sacrifice times, most of the marrow space is still made up of the stromal elements surrounding either vacuolar spaces or fat cells (Fig. 28). Also, as in previous sections, the osteocytes, lining cells and endothelial cells all appear normal and viable (Fig. 29).

Osteoclasts could not be identified in any of the sections examined from the irradiated animals. Osteoclasts could be seen only occasionally in the non-irradiated animal sections. However, in the sections of long bone examined, the marrow contents had a tendency to pull away from the bone surface during decalcification and sectioning, which made identification of osteoclasts difficult. This was especially true of the irradiated animals when the bone marrow was

nearly completely destroyed. Separation of the bone marrow cells from the bone could be due to the lipids being removed during the processing of the samples, making them friable. However, even with the separation problem, enough cell-to-bone contact would remain in most samples to allow partial examination for osteoclasts. As stated, osteoclasts could not be identified in any of the irradiated bone marrow sections and only rarely in the non-irradiated bone marrow sections.

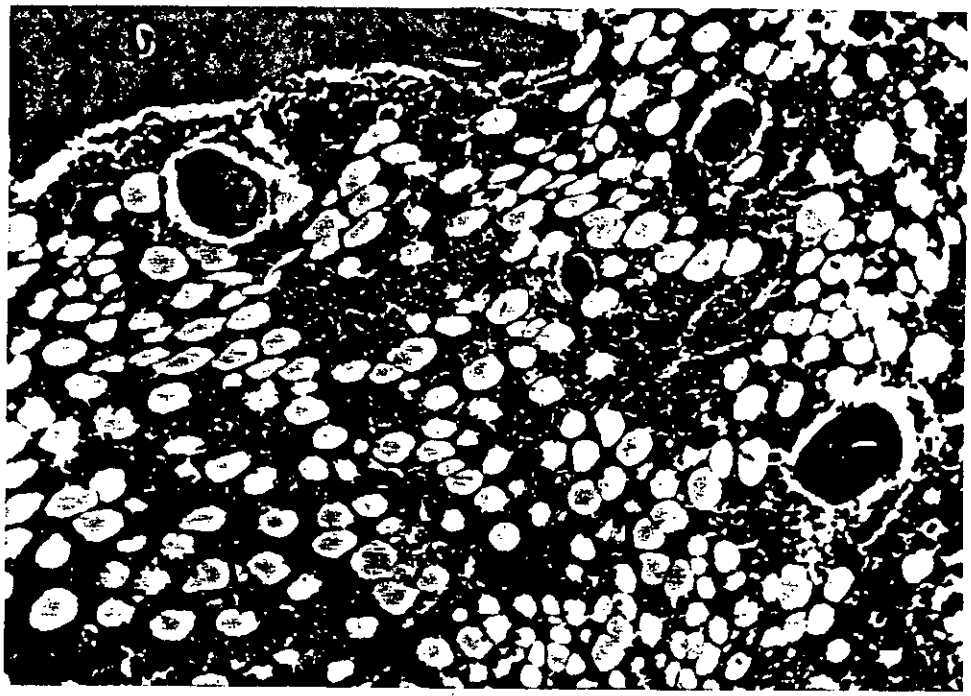
All of the sample times selected show drastic changes in the cellularity of the marrow. By three days the stem cell pool appears to be severely compromised. This lasts for the entire duration of the experiment, making a single dose of the whole-body Gamma radiation effective for at least 11 days in supressing hematopoiesis.





Fig. 1: Overview, rat long bone shaft, non-irradiated femur (16X).

Fig. 2: Marrow, rat long bone shaft, non-irradiated femur megakaryocytes, adipose cells and erythrocytes (400X).



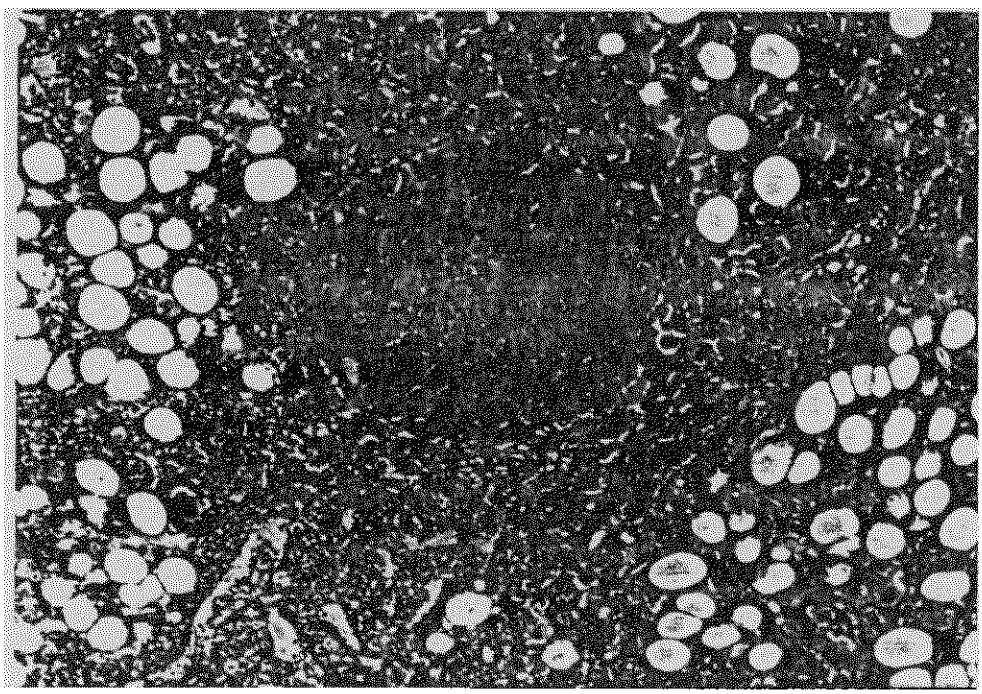
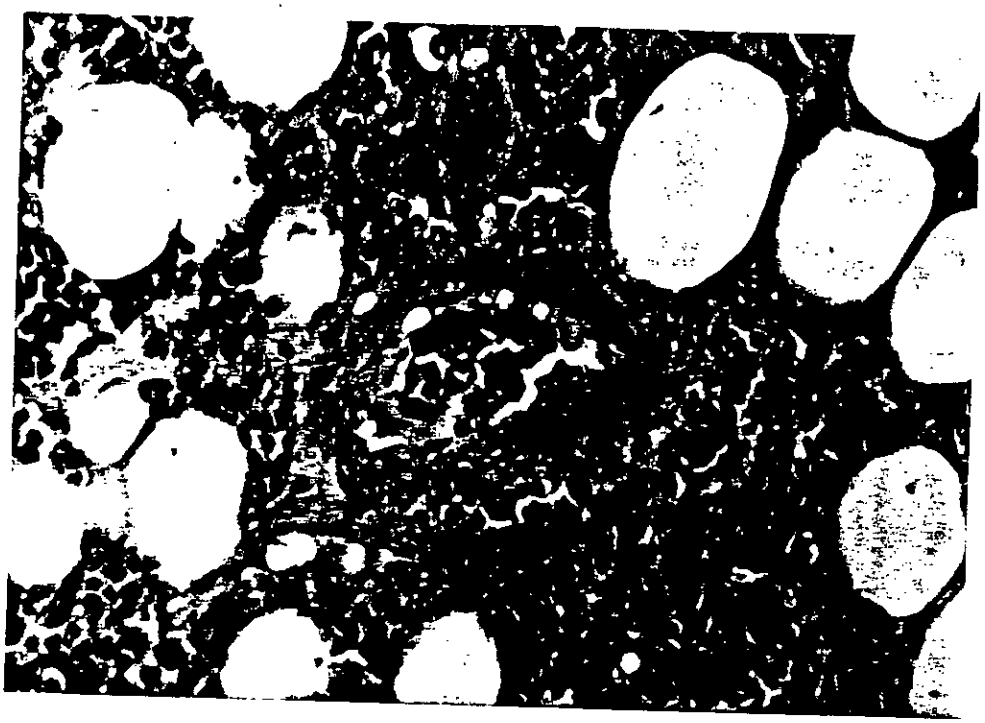


Fig. 3: Marrow space, rat long bone shaft, non-irradiated femur, boney trabeculae with osteocytes (100X).

Fig. 4: Marrow space, rat long bone shaft, non-irradiated femur, congested vascular channels with erythrocytes (100X).



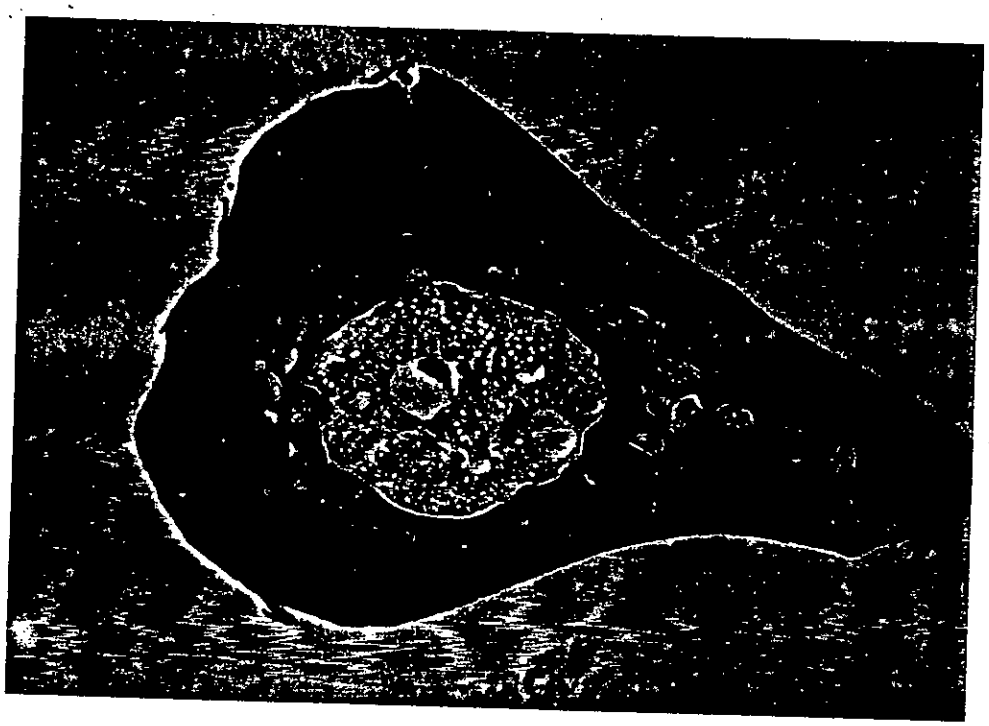
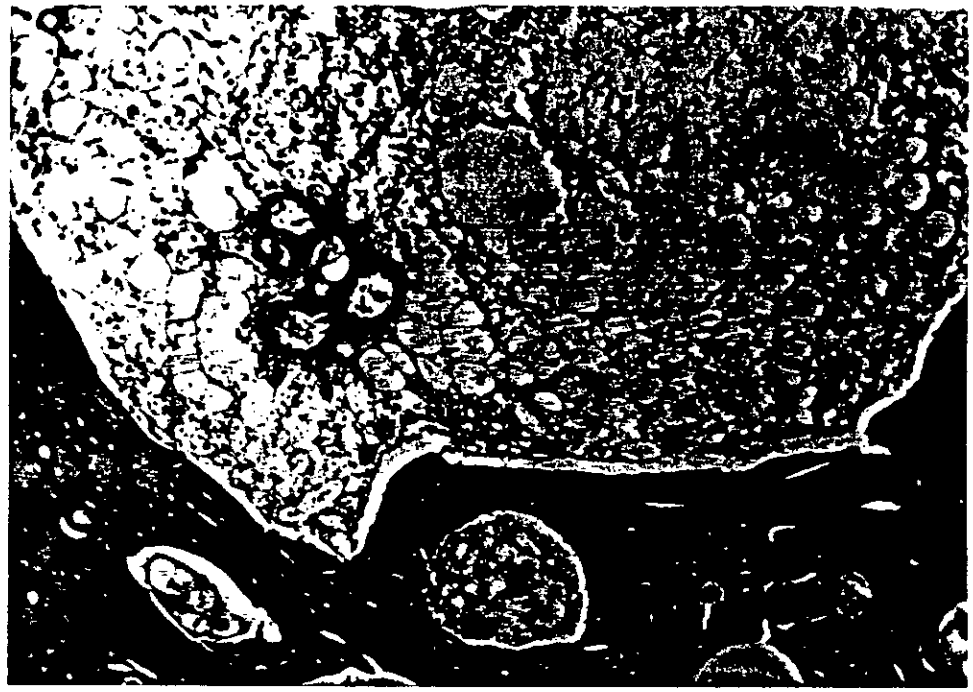


Fig. 5: Marrow Space, rat long bone shaft, non-irradiated femur, vascular channel containing erythrocytes and other marrow elements (400X).

Fig. 6: Overview, rat long bone shaft, 3 day irradiated femur (16X).



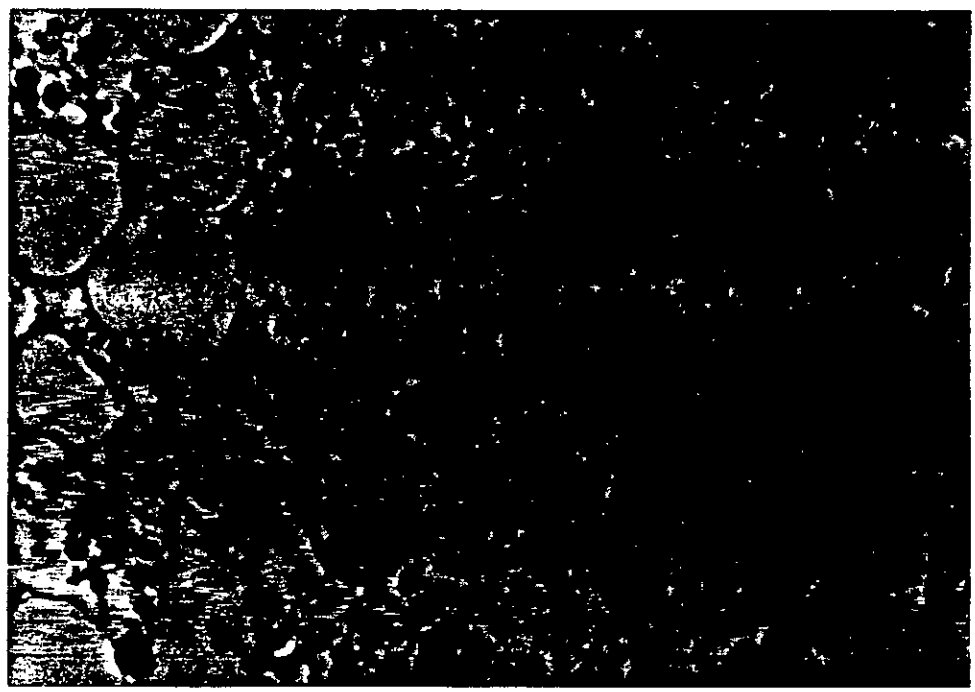
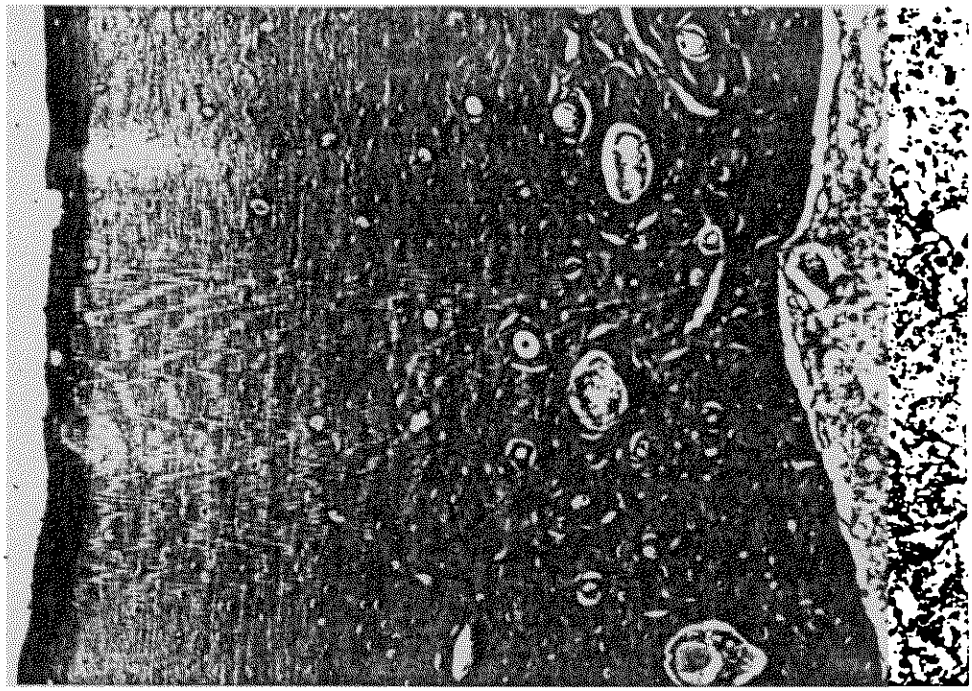


Fig. 7: Marrow space, rat long bone shaft, 3 day irradiated femur, generalized destruction of marrow elements (100X).

Fig. 8: Marrow, rat long bone shaft, 3 day irradiated femur, scarcity of cells, most show pyknosis (400X).



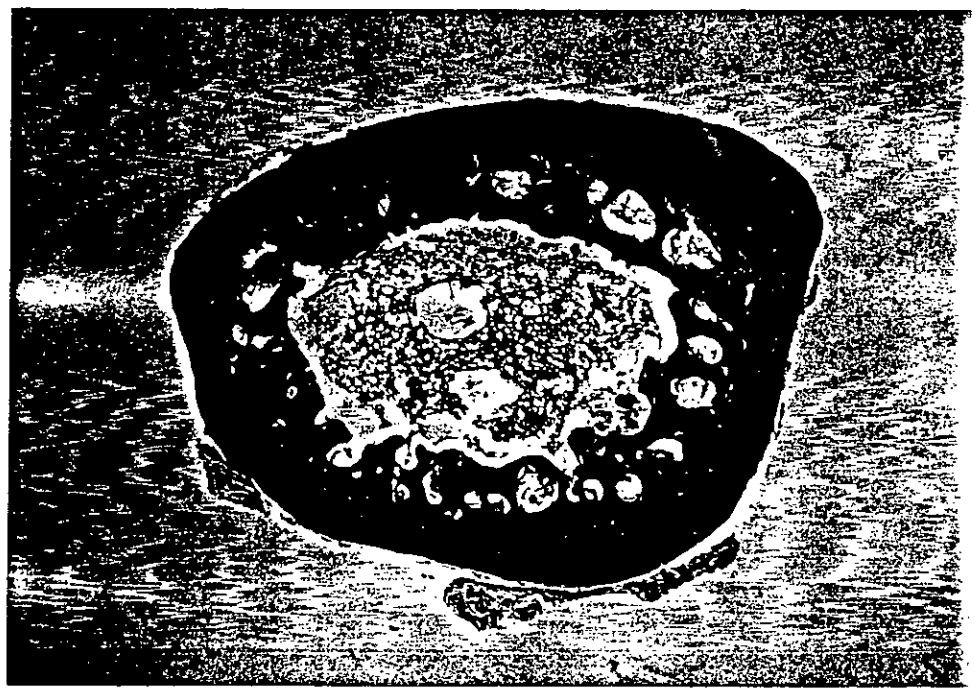
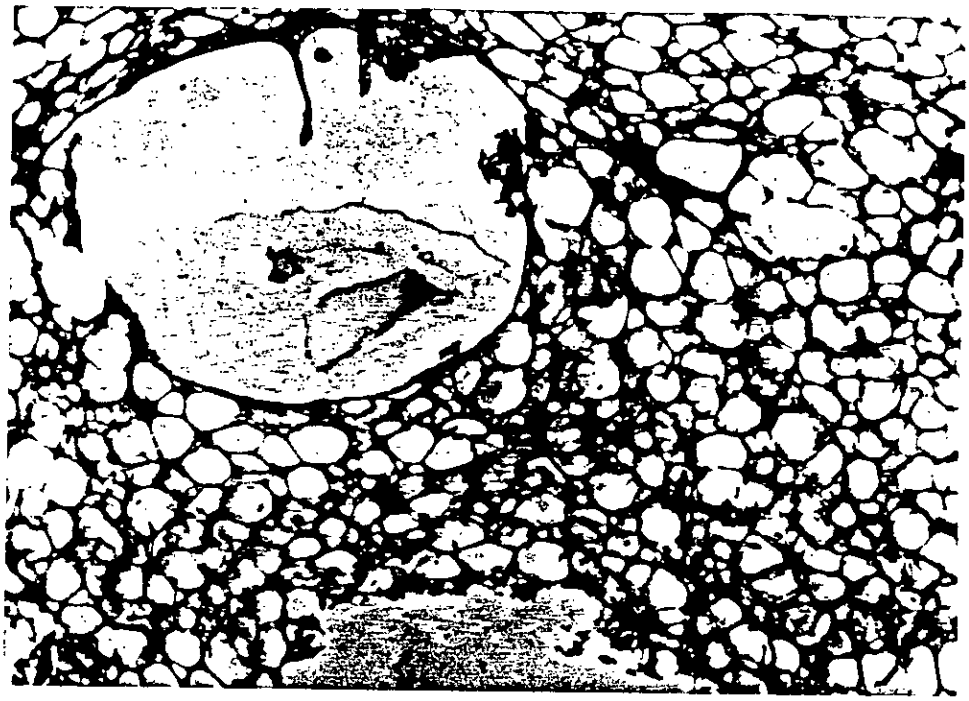


Fig. 9: Marrow and bone, rat long bone shaft, 3 day irradiated femur, osteocytes in lacunae (100X).

Fig. 10: Overview, rat long bone shaft, 6 day irradiated femur (160X).



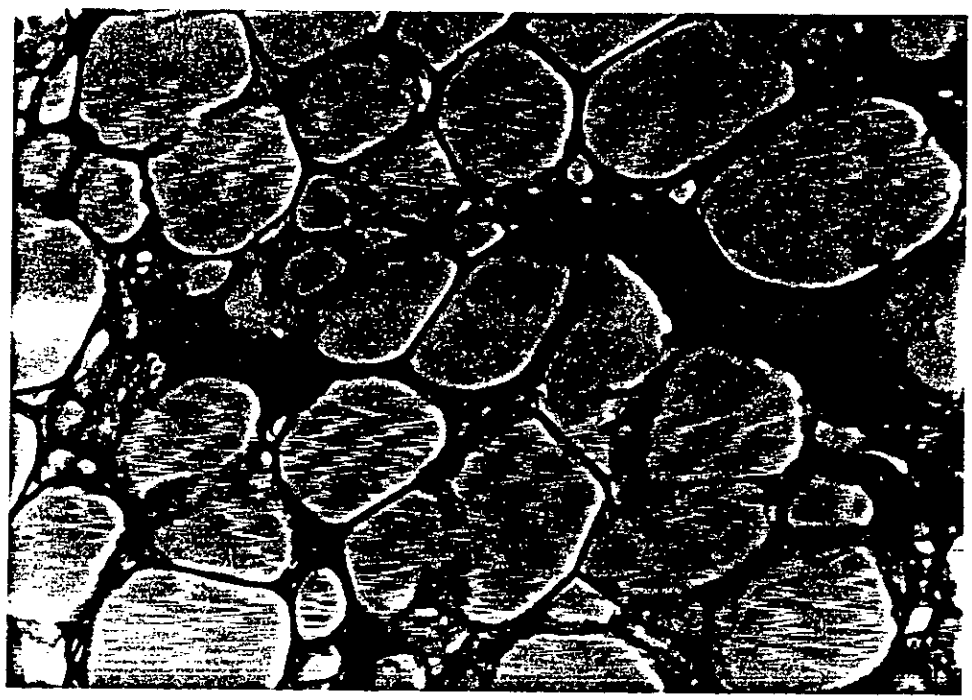


Fig. 11: Marrow space, rat long bone shaft, 6 day irradiated femur, remaining stromal elements devoid of cells (100X).

Fig. 12: Marrow, rat long bone shaft, 6 day irradiated femur, bone trabeculae and destroyed marrow (400X).



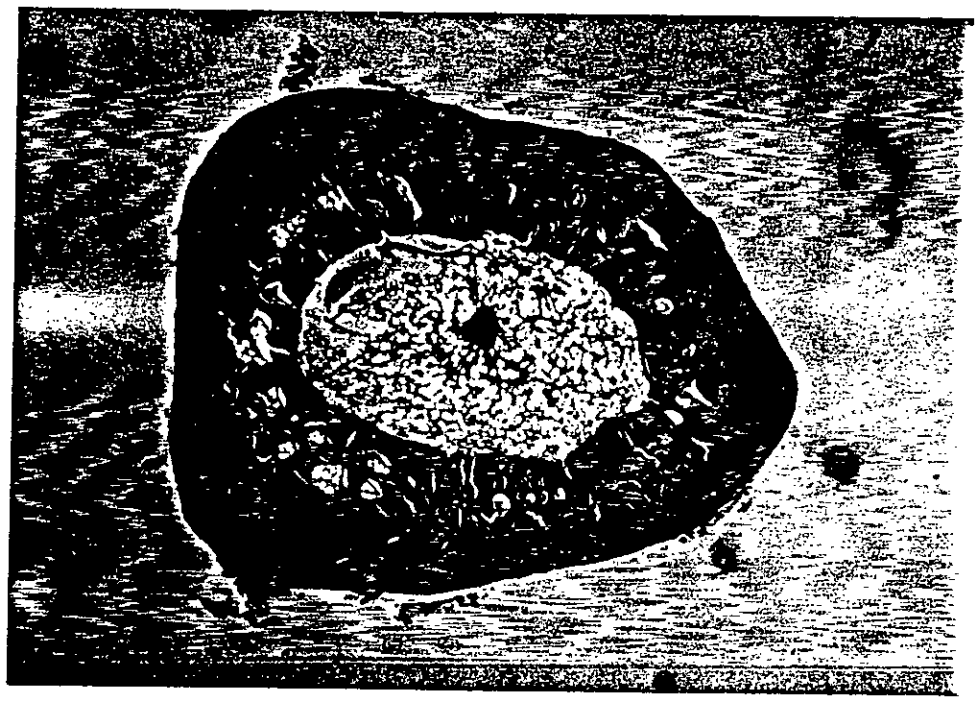


Fig. 13: Shaft bone, rat long bone shaft, 6 day irradiated femur, shaft bone with osteocytes (100X).

Fig. 14: Overview, rat long bone shaft, 8 day irradiated femur (16X).

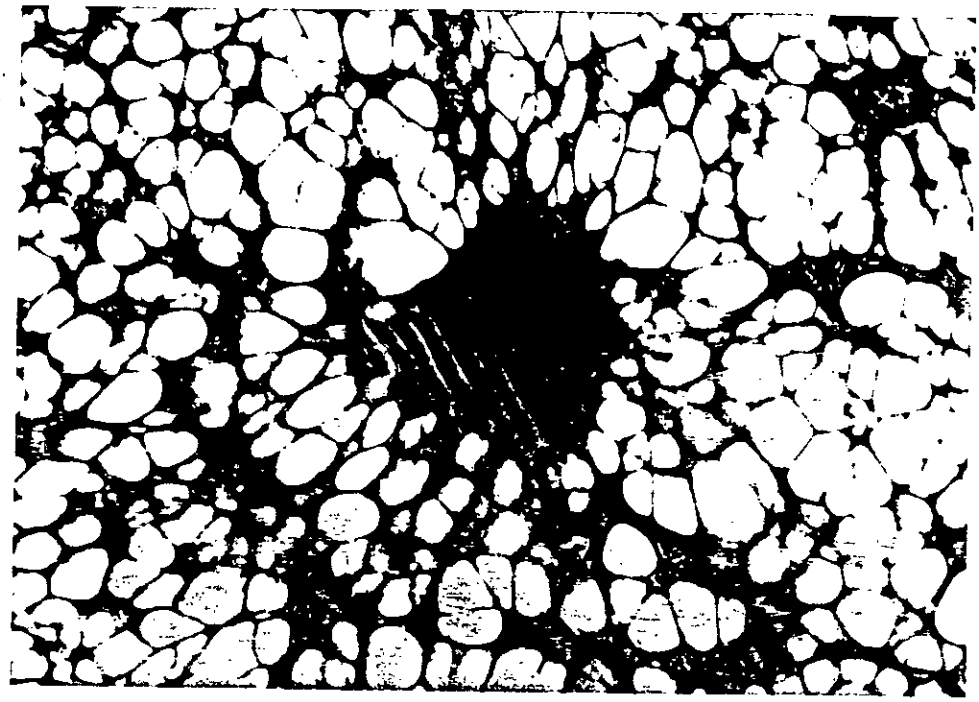




Fig. 15: Marrow space, rat long bone shaft, 8 day irradiated femur, continued generalized destruction, but some cellularity returning (100X).

Fig. 16: Marrow space, rat long bone shaft, 8 day irradiated femur, some return of stem cell population and erythrocyte production (400X).



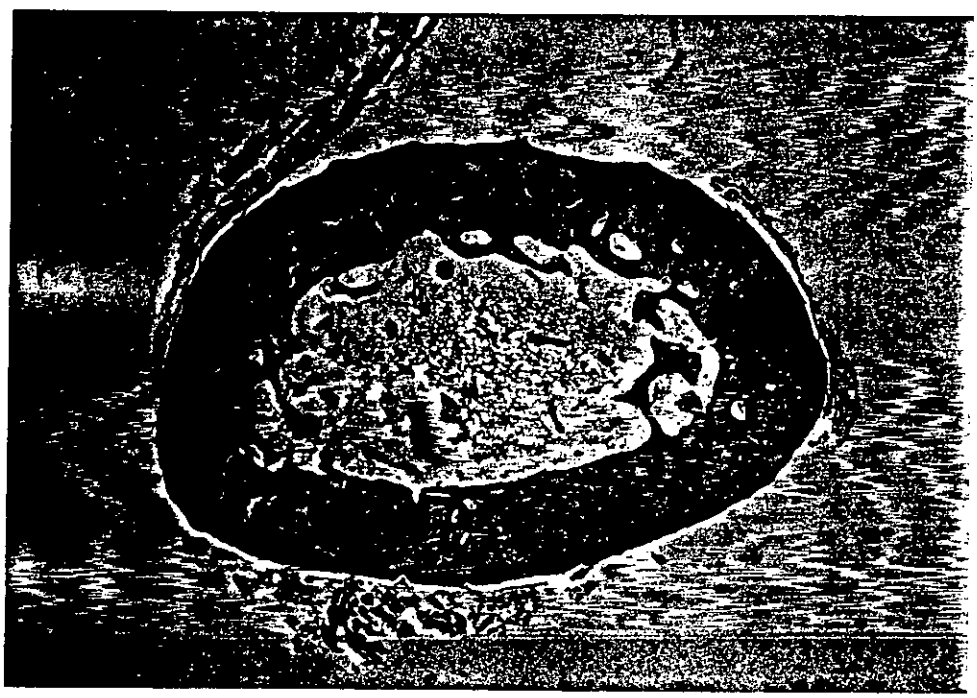


Fig. 17: Shaft bone, rat long bone shaft, 8 day irradiated femur, shaft bone with osteocytes (100X).

Fig. 18: Overview, rat long bone shaft, 10 day irradiated femur (16X).

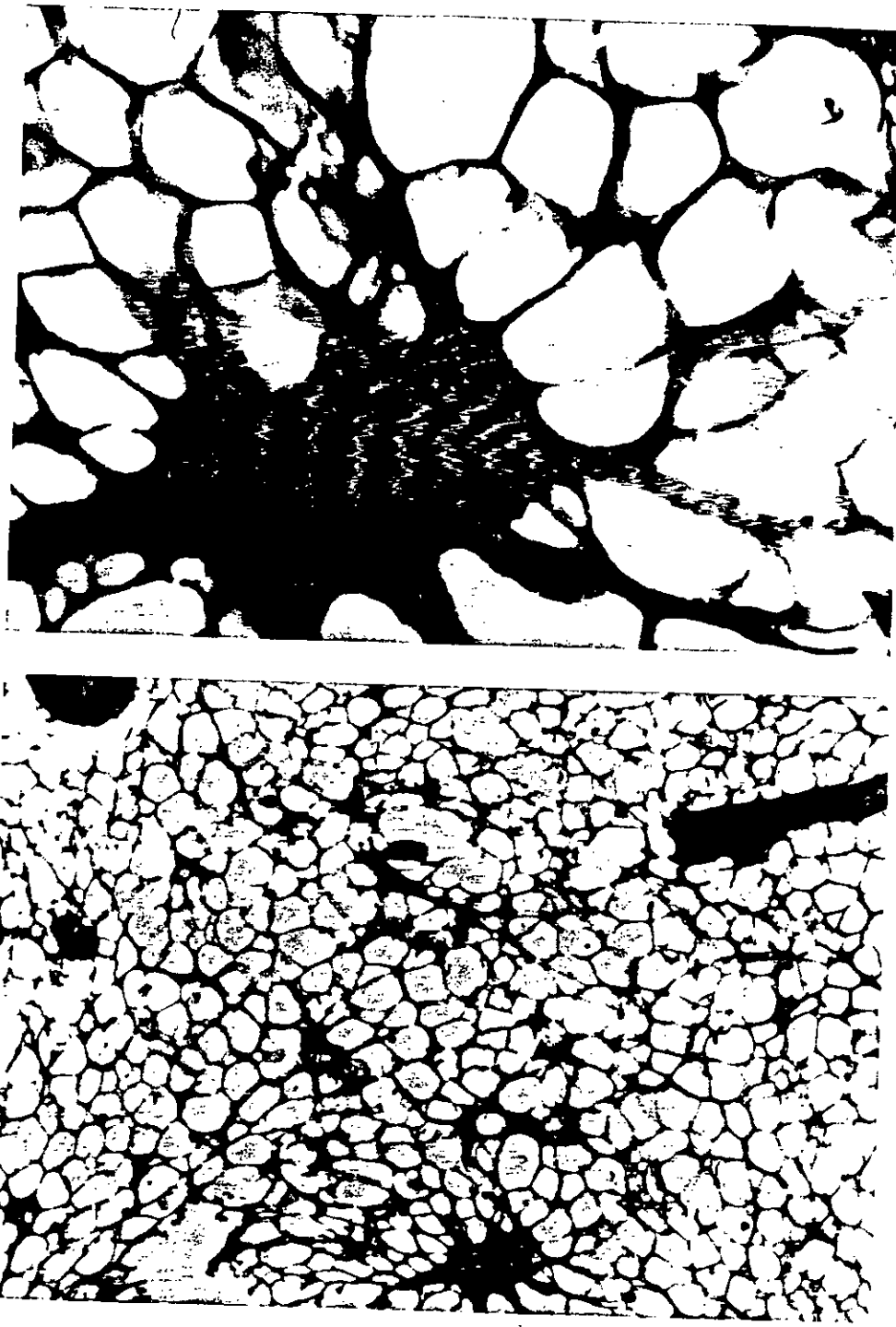
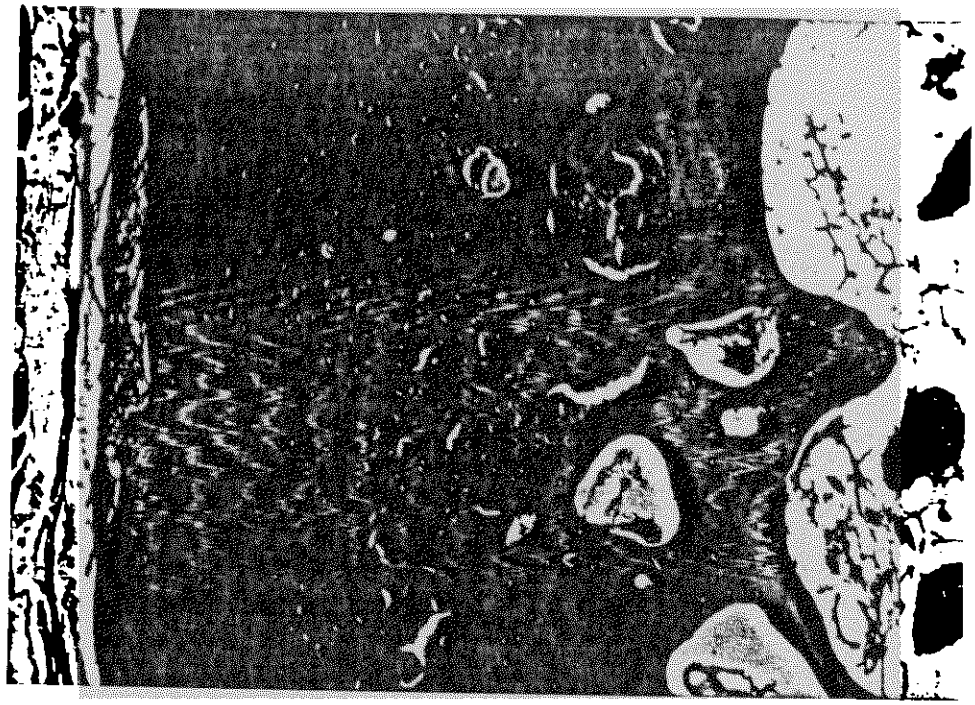


Fig. 19: Marrow, rat long bone shaft, 10 day irradiated femur, some cellular return (400X).

Fig. 20: Marrow space, rat long bone shaft, 10 day irradiated femur, continued generalized destruction of cellular elements (100X).



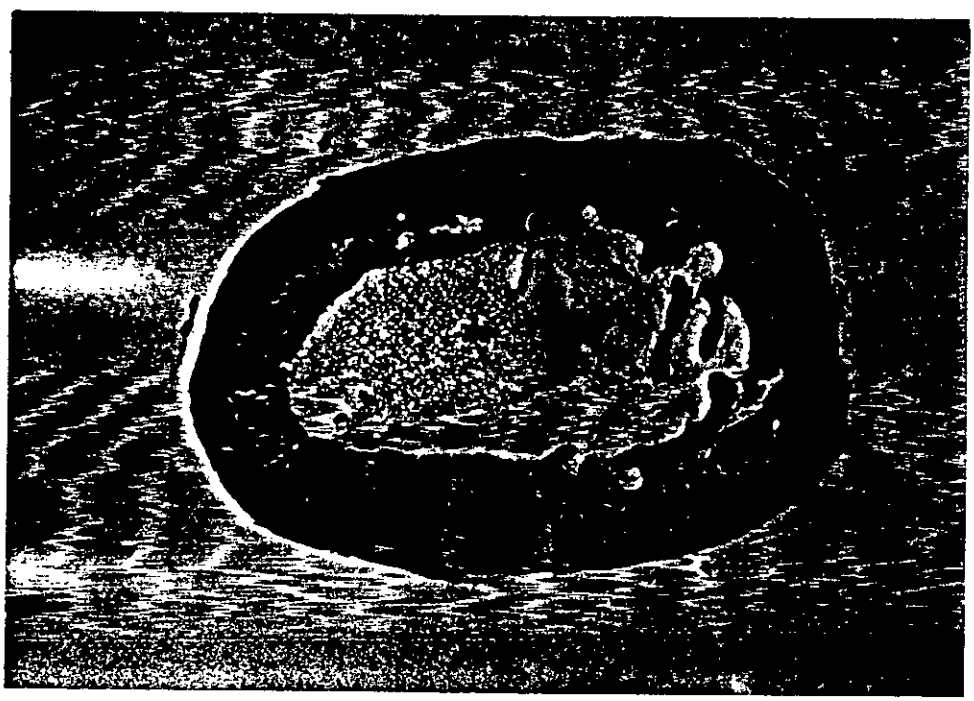


Fig. 21: Shaft bone, rat long bone shaft, 10 day irradiated femur, shaft bone with osteocytes (100X).

Fig. 22: Overview, rat long bone shaft, 12 day irradiated femur (16X).

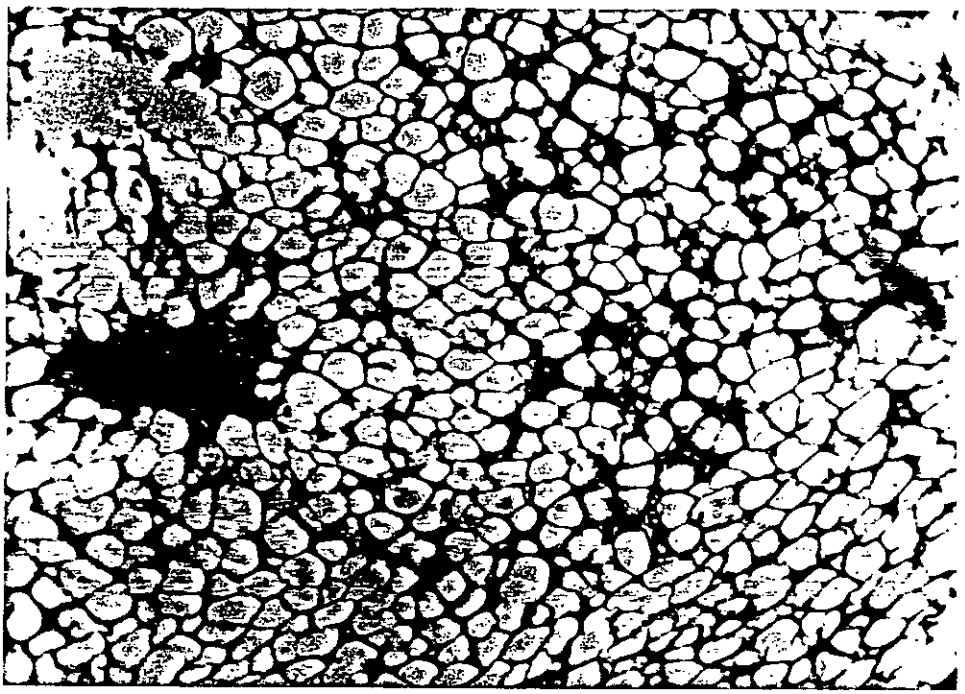




Fig. 23: Marrow space, rat long bone shaft, 12 day irradiated femur, continued generalized destruction of marrow some cellularity returning (100X).

Fig. 24: Marrow, rat long bone shaft, 12 day irradiated femur, returning cellular elememts (400X).

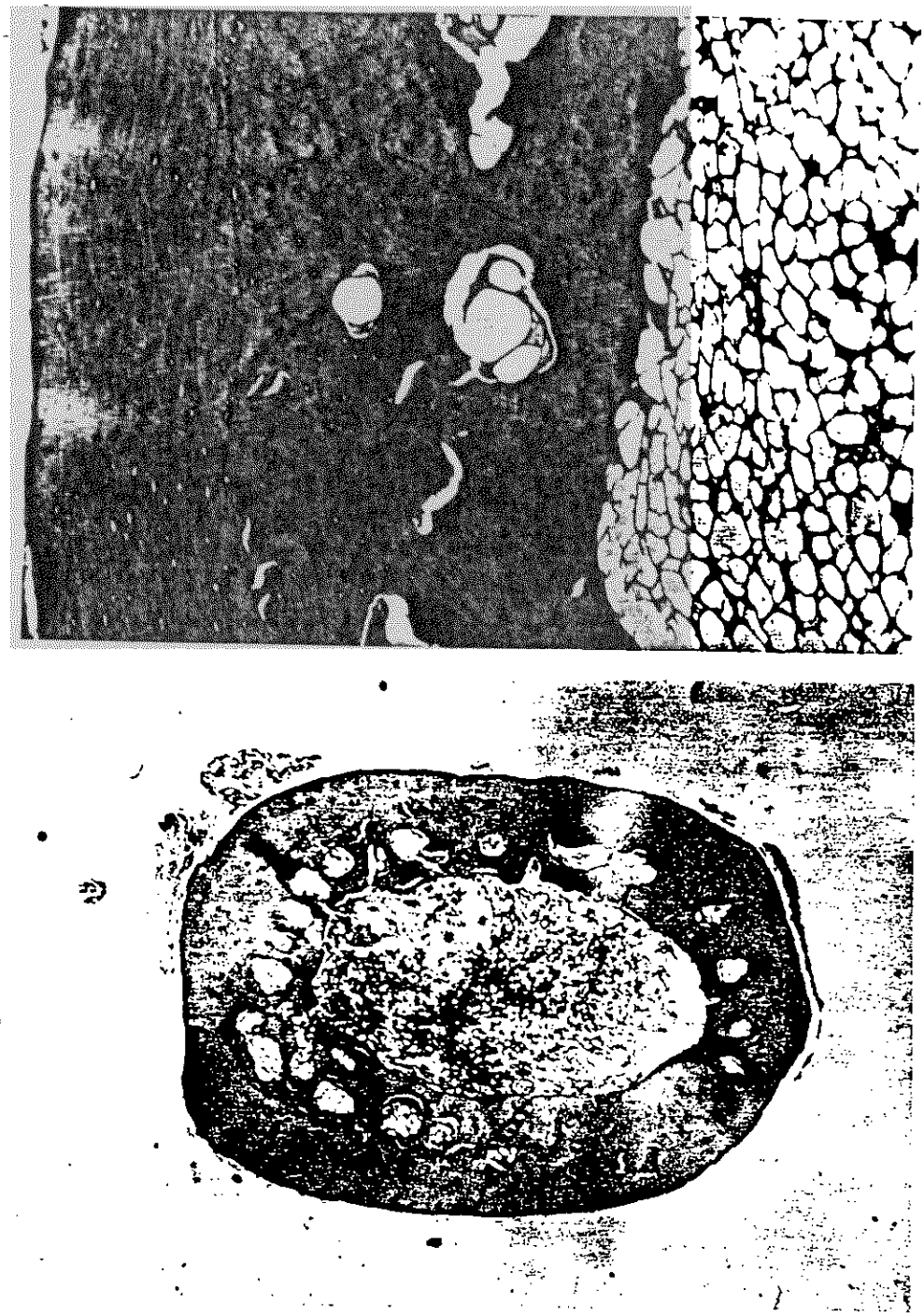
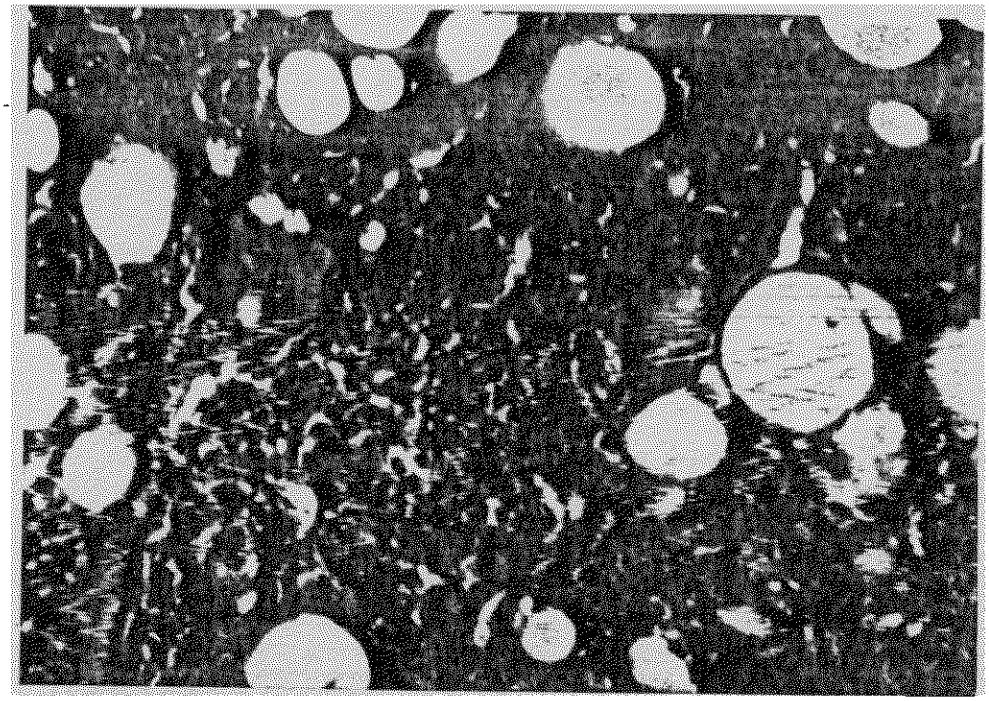


Fig. 25: Shaft bone, rat long bone shaft, 12 day irradiated femur, bone with osteocytes (100X).

Fig. 26: Overview, rat long bone shaft, 14 day irradiated femur (16X).



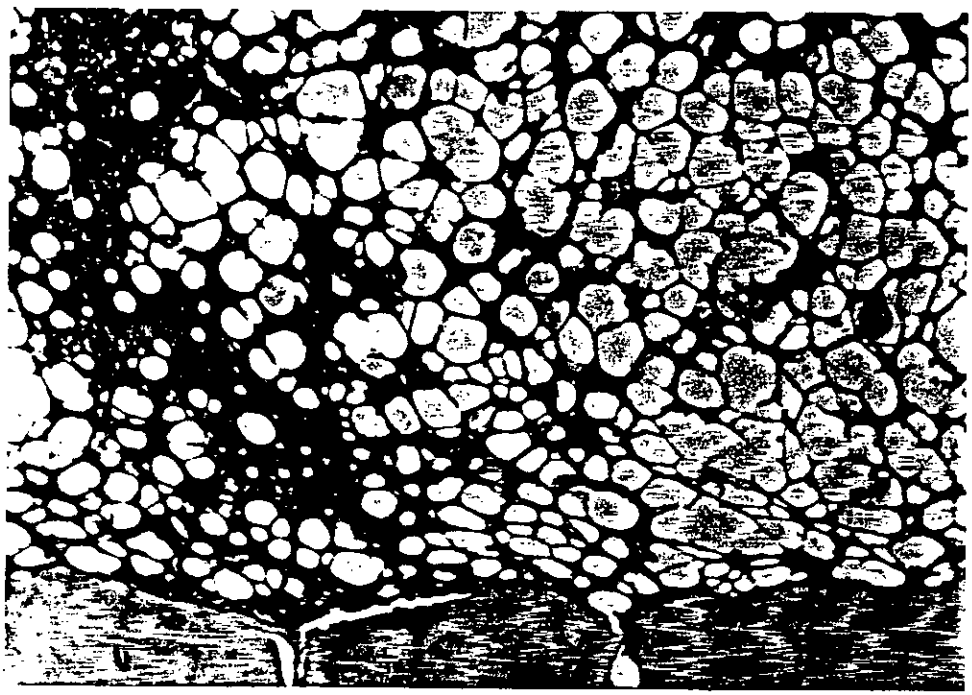


Fig. 27: Marrow, rat long bone shaft, 14 day irradiated femur reorganization of the marrow components in localized areas (400X).

Fig. 28: Marrow space, rat long bone shaft, 14 day irradiated femur bone marrow shows approximately 1/4 of it's normal cellularity (100X).

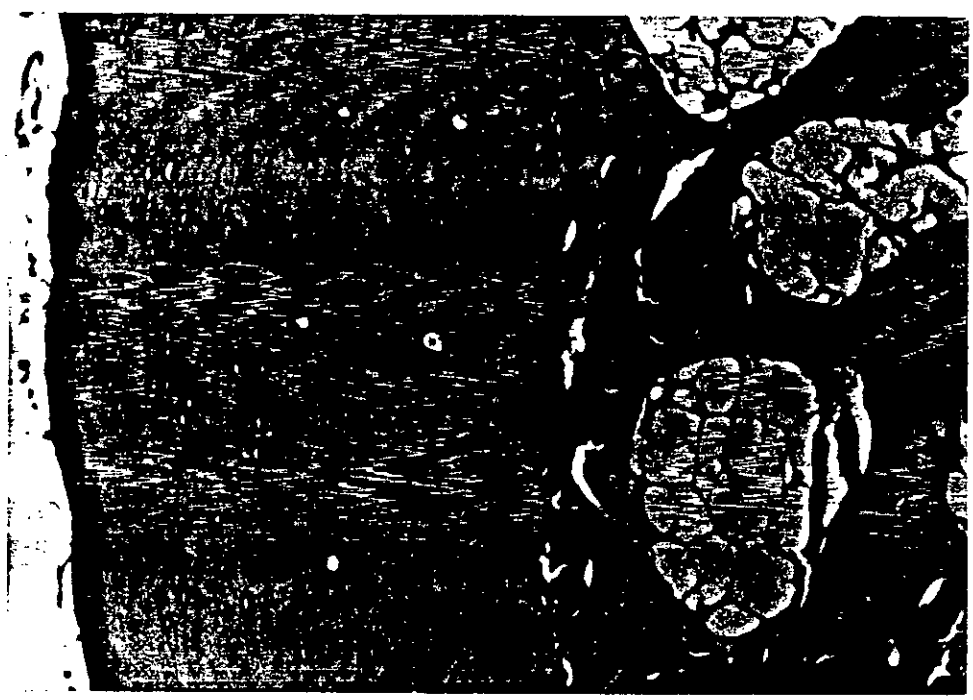


Fig. 29: Shaft bone, rat long bone shaft, 14 day irradiated femur, bone with osteocytes (100X).

VIII. HISTOLOGIC CHANGES OF ALVEOLAR BONE INCIDENT TO AN ORTHODONTIC FORCE:

A. NORMAL HISTOLOGY OF ALVEOLAR BONE

The architecture of the maxillary incisor and alveolar process of a non-irradiated, non-orthodontically treated rat is depicted in Figure 30. As an overview, there is a large curving tooth, the enamel being removed during decalcification. Within the tooth (incisor) the pulp chamber is seen running the length of the structure. Next to the tooth is a periodontal membrane (PDM), containing collagen fibers arranged in bundles. Running in the periodontal membrane space are nerves and patent blood vesseles. In a few sections, "Rests of Malassez's" could be identified, also within the PDM. The PDM is anchored in the alveolar bone via Sharpey's fibers. The alveolar bone, lingual to the tooth, can be divided vertically into three distinct areas. A crestal area which includes the crest tip down 2.5 - 3.8 mm, following the curvature of the root surface. This area shows solid bone with very few vascular channels. The bone facing the PDM is smooth showing no osteoclastic activity (Fig. 31). The second area is the midroot portion of the alveolar bone

and extends from the bottom of the crestal area down to the nasal sinus. In this area the bone is filled with vascular spaces and sinusoids. Again, the bone surface facing the PDM is relatively smooth. The only deviation in this smooth curve is where the blood vessels travel into the PDM from the bone (Fig. 32) The third and final area is the root portion and extends from the base of the mid-root area of the apex of the root. This area was extremely variable, but basically showed a smooth curvature following the root surface.

B. HISTOLOGIC OBSERVATIONS OF ALVEOLAR BONE SUBJECTED TO ORTHODONTIC TOOTH MOVEMENT WITH OR WITHOUT IRRADIATION

The histologic results for the 6 day group of animals which received only an orthodontic appliance were as follows. Marked resorption is observable at the crest of the alveolar bone lingual to the incisor (Fig. 33). Osteoclasts could clearly be demonstrated in this area (Fig. 34). The PDM in this area appears compressed and most cellular and fiber definition has disappeared. There appears to be a zone of reduced cellularity, possibly an area of hyalinization. The blood vessels which could be seen in the PDM of control animals are completely compressed and in some cases

extravasated RBC's can be demomstrated. The deeper portions of the crestal bone appear normal, viable osteocytes remaining in their lacunae. The mid-root areas showed more drastic changes. Areas of both undermining (Fig. 35) and direct resorption (Fig. 36) can be identified. Osteoclasts can clearly be demonstrated and the alveolar surface takes on a more ragged apperance. The osteoclasts can be seen near blood vessels (Fig. 37). The root portions showed very little, if any, variation from the non-treated control incisors.

The 6 day animals which received both irradiation and an orthodontic appliance showed very similar results to the non-irradiated animals. These specimens also show an area where cellularity is apparently absent in the crestal PDM (Fig. 38). They do not show as much crest tip resorption as the non-irradiated group, but osteoclasts could be demonstrated in this area. However, in the mid-root area, resorption appears to have been actively taking place (Fig. 39). Undermining resorption appears to be predominant at this point, but some direct resorption can be seen.

Osteoclasts (Fig. 40) can easily be demonstrated in all sections of each animal examined. These osteoclasts (Fig. 41), can be seen near the vascular channels and vessels located in the bone. Also, the amount of osteoclastic activity seems to be slightly decreased. However, this could be due to animal variation. The root portion again showed very little, if any, variation form the non-irradiated, non-moved incisors.

At 8 days post-orthodontic appliance placement, the non-irradiated animals continued to show resorptive changes. There were resorption lacunae at the crest tip with osteoclasts being apparent. Also, at this point it appears that the PDM vascular channels are becomming patent again. The area of reduced cellularity is still present (Fig. 42) and there appears to be undermining resorption taking place. Osteoclasts are easily demonstrated in both the crestal region and the mid-root portions of the alveolar socket. In the mid-root area, resorption (Fig. 43) is very apparent, showing both frontal and undermining resorption taking place. The osteoclasts are large, multinucleated and closely

associated with resorption lacunae (Fig. 44). As in the non-treated specimens the alveolar bone appears to be healthy and osteocytes can be seen throughout.

The irradiated animals, by the 8th day, also showed a progression in the resorptive process. The crestal area shows resorption where osteoclasts can be demonstrated in Howship's lacunae (Fig. 47). The area of apparent hyalinization (Fig. 45) is present and some undermining resorption is occuring at the crest tip (Fig. 46). In the PDM of the crest area, the blood vessels are patent in some animals and still occluded in others. In the animals where they are occluded, the resorptive process appears to be lagging compared to the animals where they are already patent.

The mid-root areas in the 8 day irradiated animals showed vast areas of resorption, both frontal and undermining (Fig. 48). Many vascular channels can be demonstrated that are patent, and the osteoclasts can be seen around these vessels (Fig. 49). The osteoclasts are large and multinucleated and can be seen in resorption lacunae (Fig.

50). Despite the large dose of whole body irradiation, there is no detectable change in both the number and morphology of the osteoclasts. Also in these animals, the osteocytes are present in their lacunae and appear as in the non-irradiated animals.

At 10 days, the alveolar bone lingual to the non-irradiated orthodontically moved teeth show continued osteoclastic crestal resorption (Fig. 51). The area of reduced cellularity is still present, but is becomming progressively smaller (Fig. 52). The vascular channels in the PDM, all appear to be patent. There are areas of frontal and undermining resorption.

The mid-root portions all show extensive areas of resorption. Numerous osteoclasts can be seen in Howships lacunae (Fig. 53). They are large, multinucleated, and can be seen in great numbers, both in the areas of frontal and undermining resorption (Fig. 54).

Bone resorption continues to be detectable in animals 10 days after irradiation. The crest tip shows vast resorptive changes (Fig. 55). The alveolar bone margin is

pitted with resorption lacunae, and osteoclasts are obvious (Fig. 56). There is an area of reduced cellularity however, at this time the PDM vascular channels all appear to be patent. There are also areas deeper in the bone, away from the tooth, were vascular spaces are apparent.

The mid-root areas of the alveolar bone show great resorptive changes (Fig. 57). There are large areas where osteoclasts have removed great quantities of bone. Osteoclasts are obvious in both frontal and undermining positions (Figs. 58 & 59). This appears to be the point of greatest resorption in the irradiated animals and the non-irradiated animals. The irradiated specimens, in this region, look almost exactly like their non-irradiated counterparts. Here, in both groups of rats, the number of osteoclasts present and the amount of resorption seems to be similar. The bone is undergoing massive remodeling in both groups.

Animals 12 days after orthodontic tooth movement without irradiation in the alveolar crest area continue to show resorptive changes (Fig. 60). Again, the crest tip is

undergoing resorptive changes and this now carries down the face of the socket wall further than any previous time point. There is still an area of apparent hyalization that is much smaller and has almost been completely replaced with normal PDM structure (Fig. 61).

The mid-root areas still show large quantities of bone resorption taking place (Fig. 62). Resorption is still both frontal and undermining. Osteoclasts are very obvious and appear to have been active in bone resorption (Fig. 63). The alveolar bone appears normal with osteocytes present in all lacunae.

In the 12 day post-irradiated animals, the crestal areas show osteoclastic activity as in the non-irradiated animals (Fig. 64). A hyalinizaed area can be seen, although again this is becoming smaller and smaller. The PDM is becoming more cellular and fiber orientation again appears to be more normalized.

The mid-root areas continue to show obvious areas of undermining and frontal resorption (Fig. 65). Large areas of previous osteoclastic activity with obvious osteoclasts

can be seen (Fig. 66). The alveolar bone surface shows many resorption lacunae filled with osteoclasts (Fig. 67). The bone appears to be normal and corresponds closely with the non-irradiated animals.

By day 14, the resorption in the crestal areas gives no indication of cessation in the non-irradiated animals (Figs. 68 & 69). At this point in time, the PDM is very cellular and the fiber orientation is almost normal.

The mid-root portion of the non-irradiated animals continues to show large areas of bone resorption with many multinucleated osteoclasts (Figs. 70 & 71). The resorption continues to show both frontal and undermining areas (Fig. 72).

One novel aspect of the 14 day animals is that at this time, tooth resorption becomes evident on the root surface in crestal areas (Fig. 73).

In the 14 day irradiated animals, bone resorption continues as in the non-irradiated animals. The crestal areas continue to show resorption and osteoclasts can easily be demonstrated (Figs. 74 & 75). The hyalinized area is

still present, but is greatly diminshed. Most of the PDM is again very cellular and fiber orientation can be seen to be differentiating. As in the 14 day non-irradiated animals, there again appears to be the unique finding of ondontoclastic activity in the crestal area (Fig. 74).

The mid-root areas continue to show massive areas of bone resorption, both frontal and undermining (Fig. 76). The alveolar socket wall is scalloped with resorption lacunae, filled with osteoclasts (Fig. 77). Again, many of the resorptive areas can be seen near vascular channels (Fig. 78). The osteoclasts, are still of the multinucleated variety, and reside in Howship's lacunae (Fig. 79).

In all animals, and at all time periods, the apex portion of the root appeared to be very similar. There were no osteoclasts that could be demonstrated and the cells, their morphology, and their alignments all appear to be the same, whether in the non-irradiated or the irradiated animals.

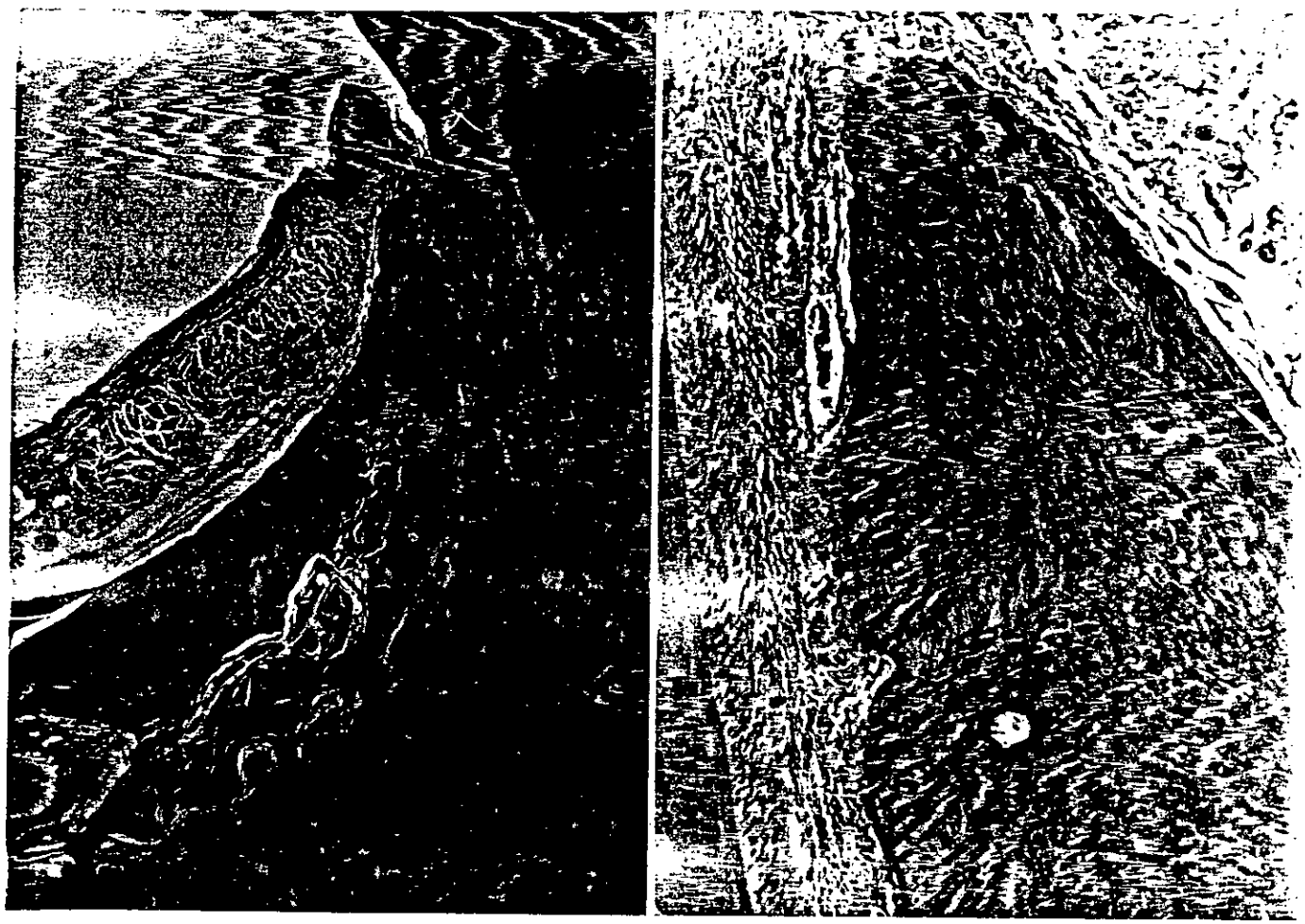


Fig. 30: Overview, non-irradiated, non-orthodontically treated incisor area (16X).

Fig. 31: Alveolar crest, non-irradiated, non-orthodotically treated incisor, patent vascular channels and highly cellular periodontal membrane (100X).

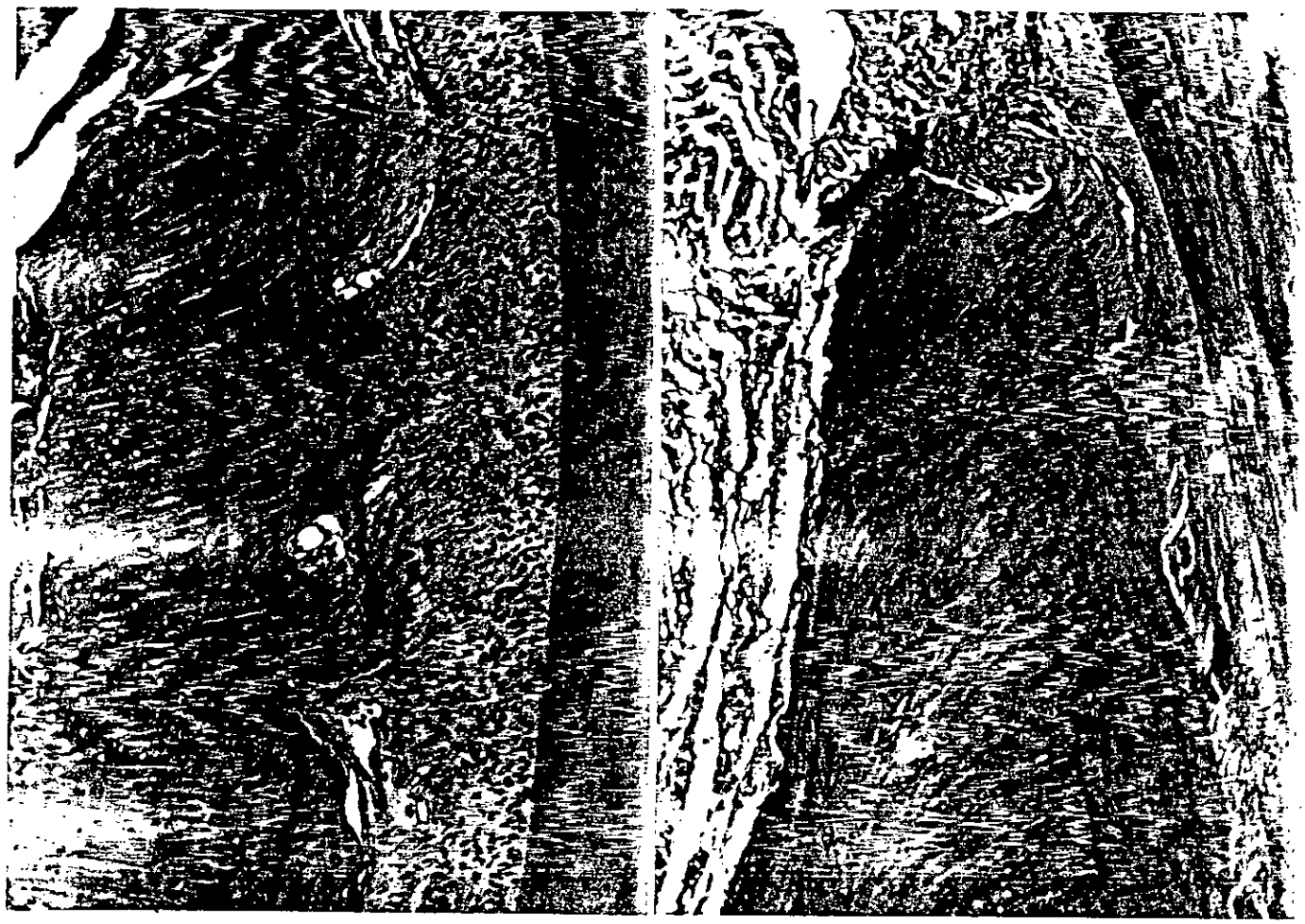


Fig. 32: Mid-root area, non-irradiated, non-orthodontically treated incisor, vascular channels entering the PDM (100X).

Fig. 33: Alveolar crest, six day, non-irradiated, non-orthodotically treated incisor, PDM showing a lack of cellularity (100X).

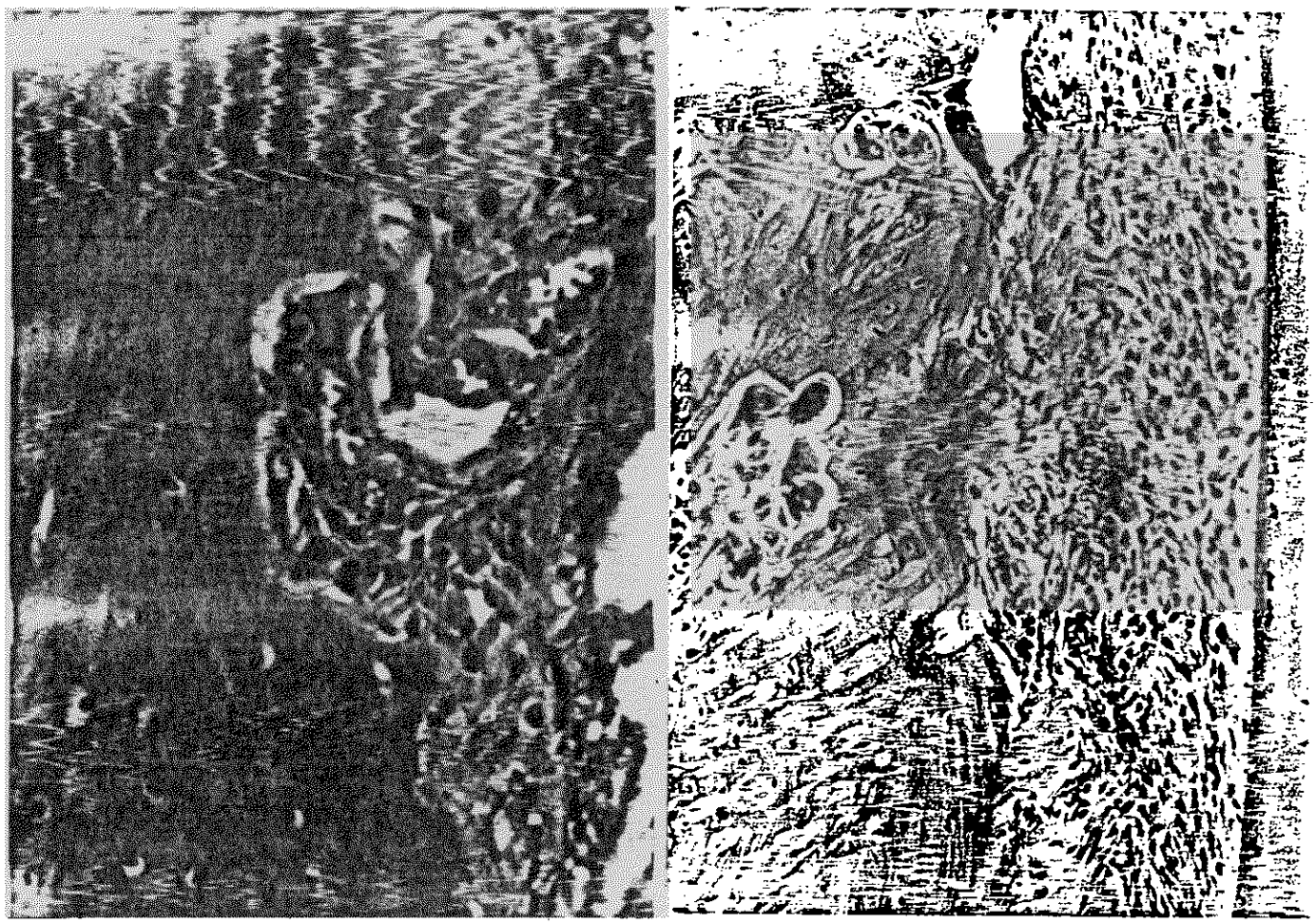


Fig. 34: Alveolar crest tip, 6 day, non-irradiated, orthodontically treated incisor, osteoclastic activity near blood vessels (400X).

Fig. 35: Mid-root area, 6 day, non-irradiated, orthodontically treated incisor, osteoclasts involved in frontal and undermining resorption (160X).

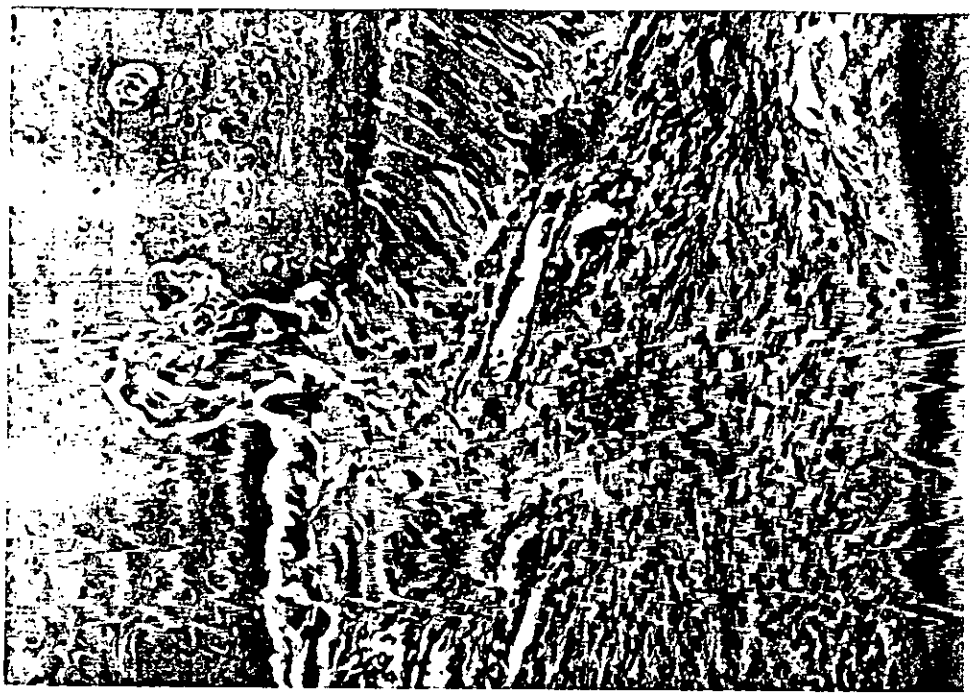




Fig. 36: Mid-root area, 6 day, non-irradiated, orthodontically treated incisor, osteoclastes with blood vessels (160X).

Fig. 37: Mid-root osteoclasts, 6 day, non-irradiated, orthodontically treated incisor, osteoclasts involved in frontal and undermining resorption (400X).

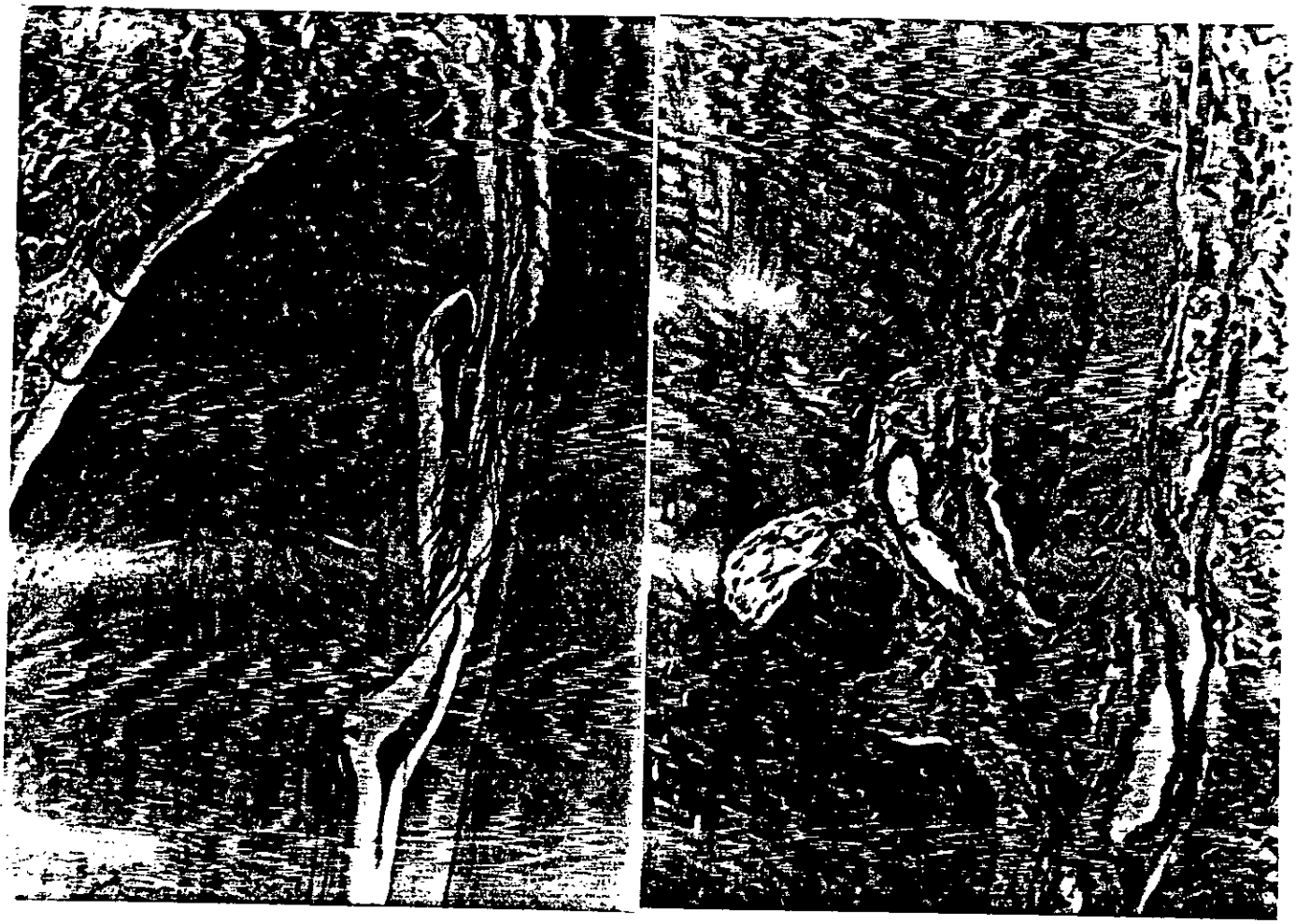
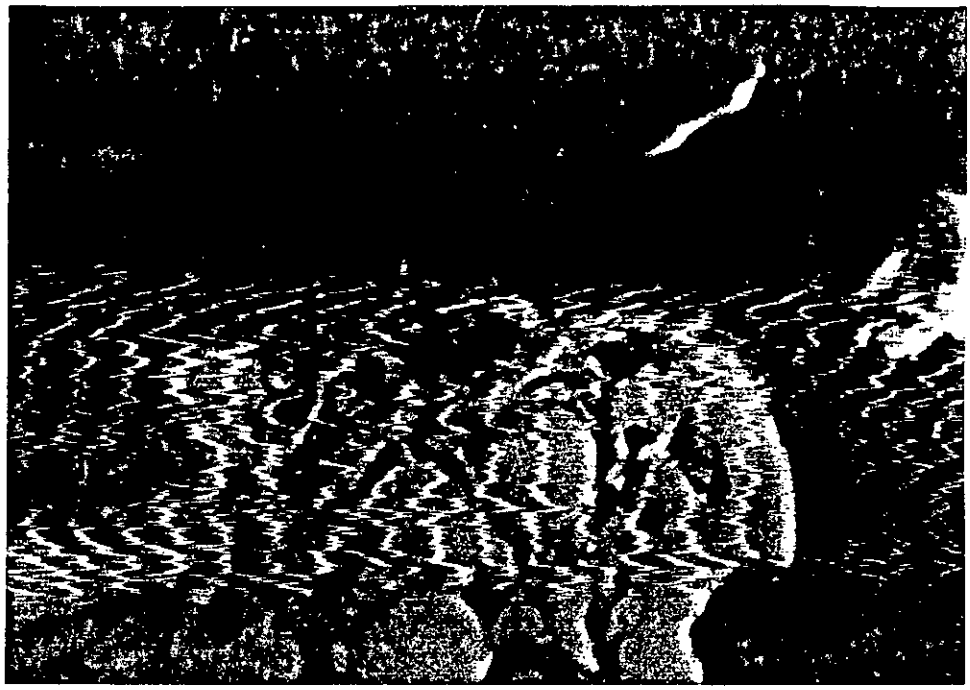


Fig. 38: Alveolar crest, 6 day, irradiated, orthodontically treated incisor, an area of reduced cellularity with a loss of vascularity (100X).

Fig. 39: Mid-root area, 6 day, irradiated, orthodontically treated incisor, blood vessels and osteoclastic activity (160X).



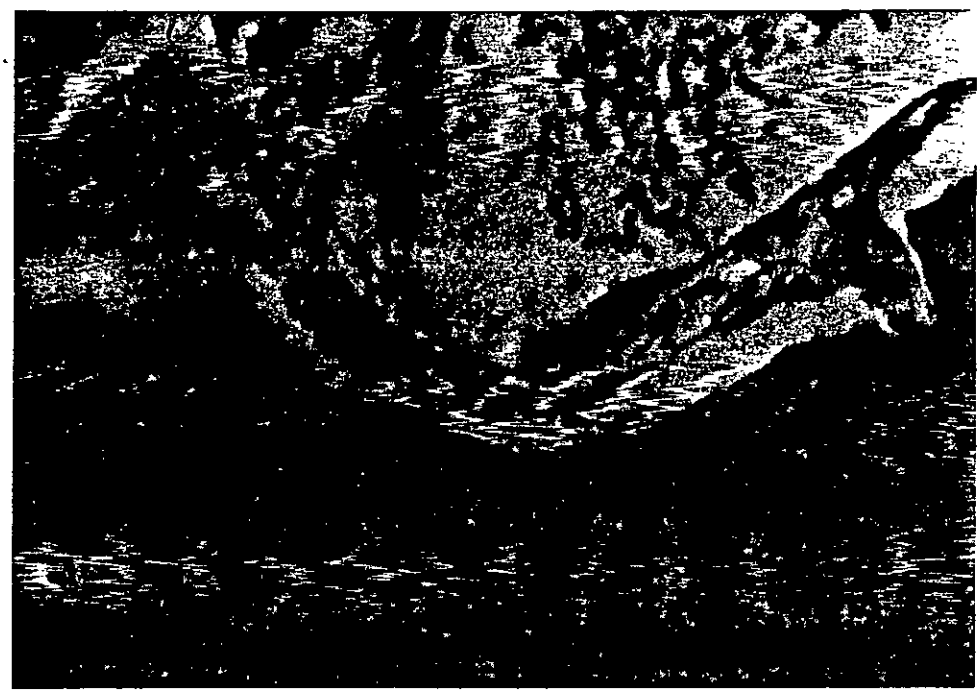


Fig. 40: Mid-root osteoclast, 6 day, irradiated, orthodontically treated incisor (400X).

Fig. 41: Mid-root osteoclast, 6 day, irradiated, orthodontically treated incisor (400X).

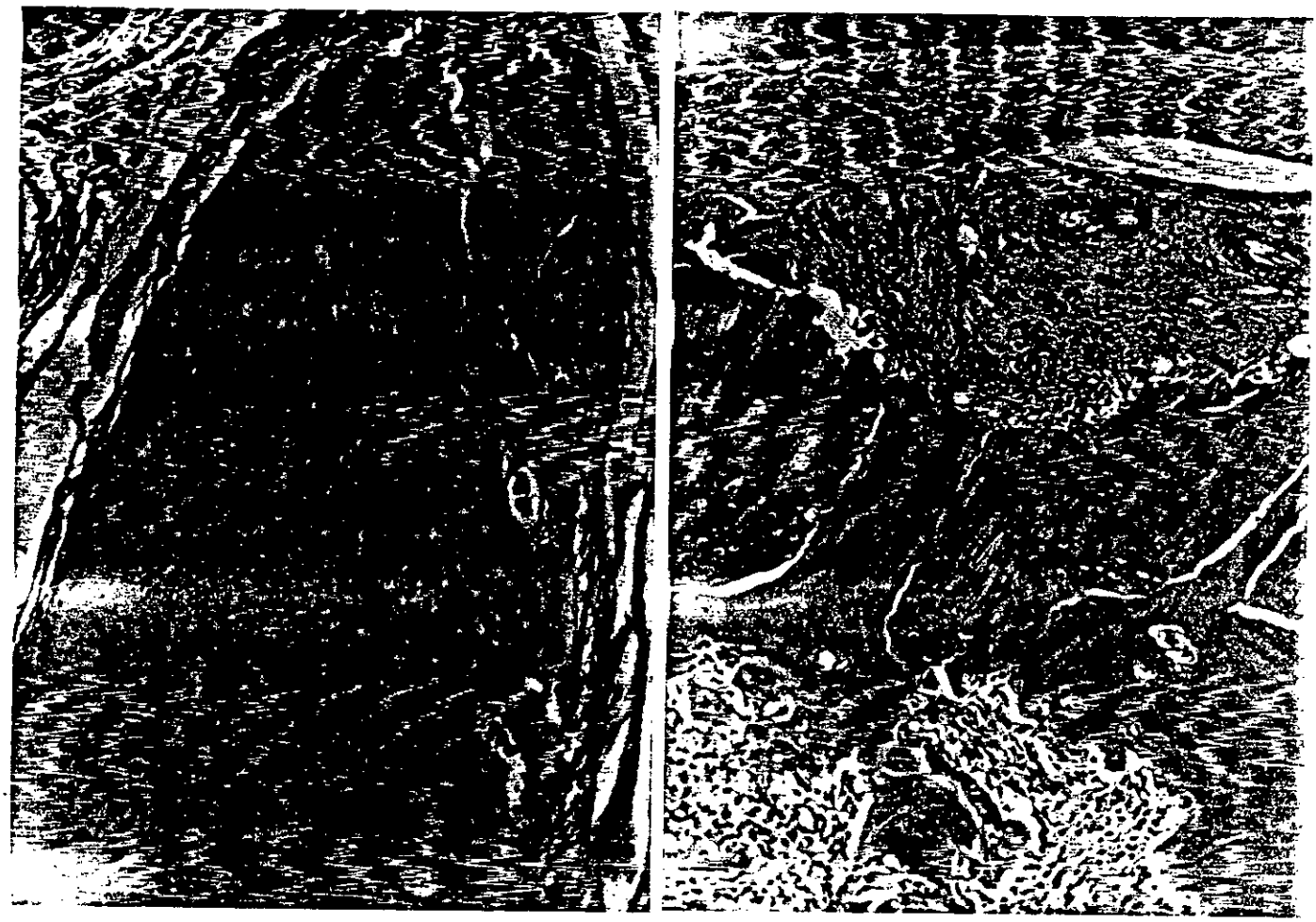


Fig. 42: Alveolar crest, 8 day, non-irradiated, orthodontically treated incisor, osteoclastic activity, reduced cellularity of the PDM (100X).

Fig. 43: Mid-root area, 8 day, non-irradiated, orthodontically treated incisor, osteoclasts in both frontal and undermining positions (100X).

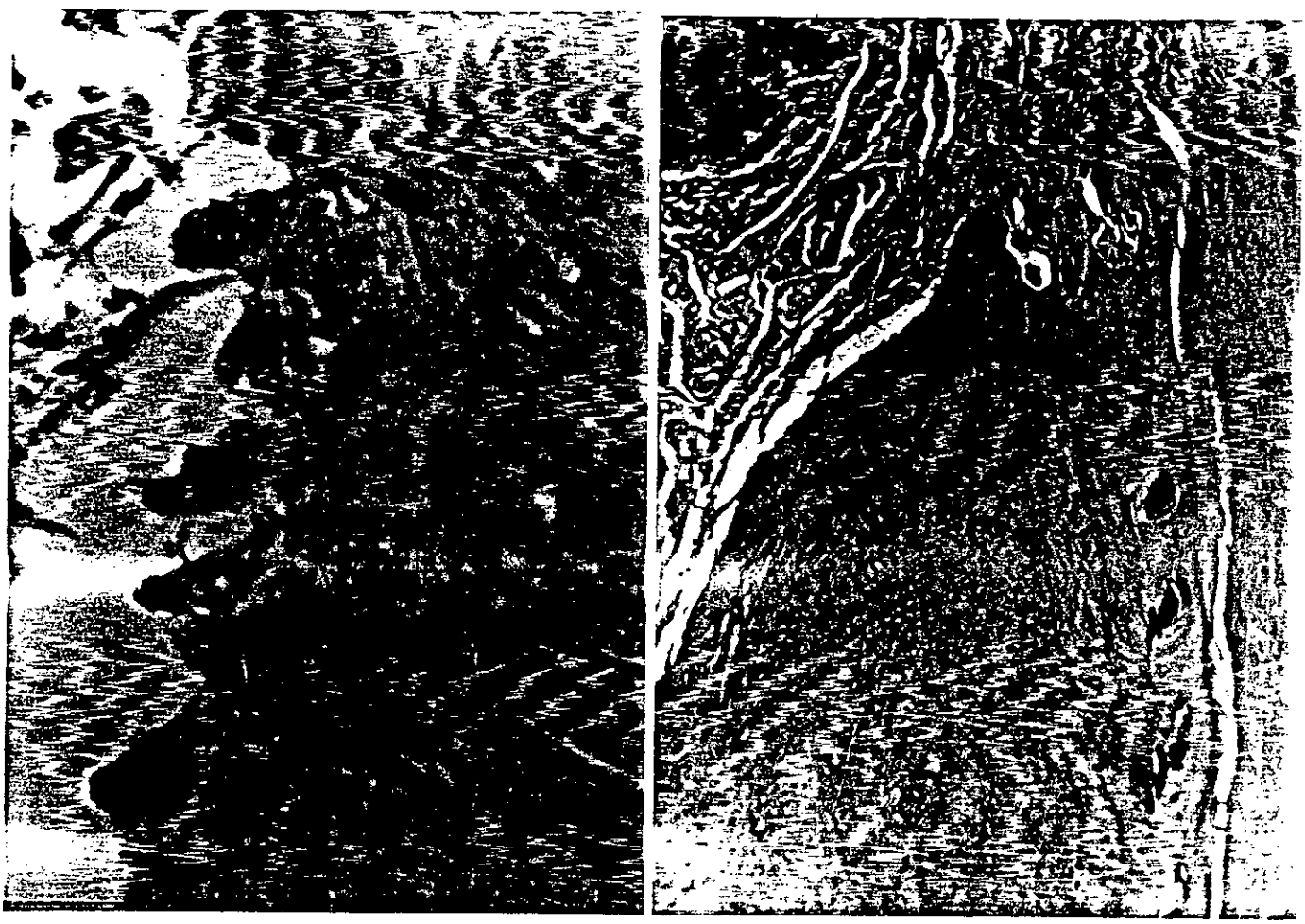
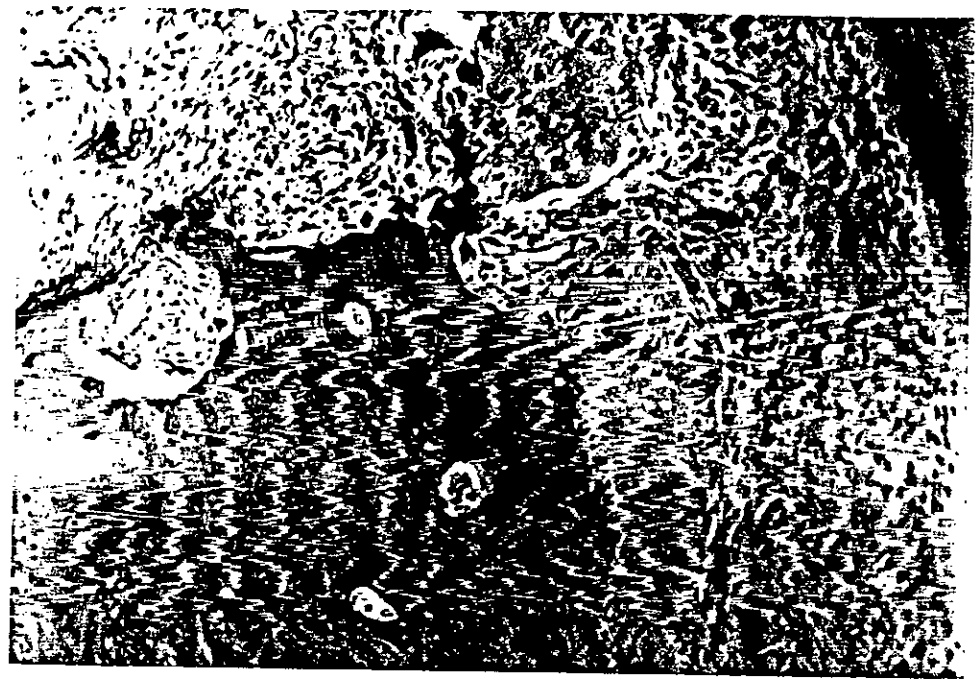


Fig. 44: Mid-root osteoclasts, 8 day, non-irradiated, orthodontically treated incisor (400X).

Fig. 45: Alveolar crest, 8 day, irradiated, orthodontically treated incisor, reduced PDM cellularity and osteoclastic activity are apparent (100X).



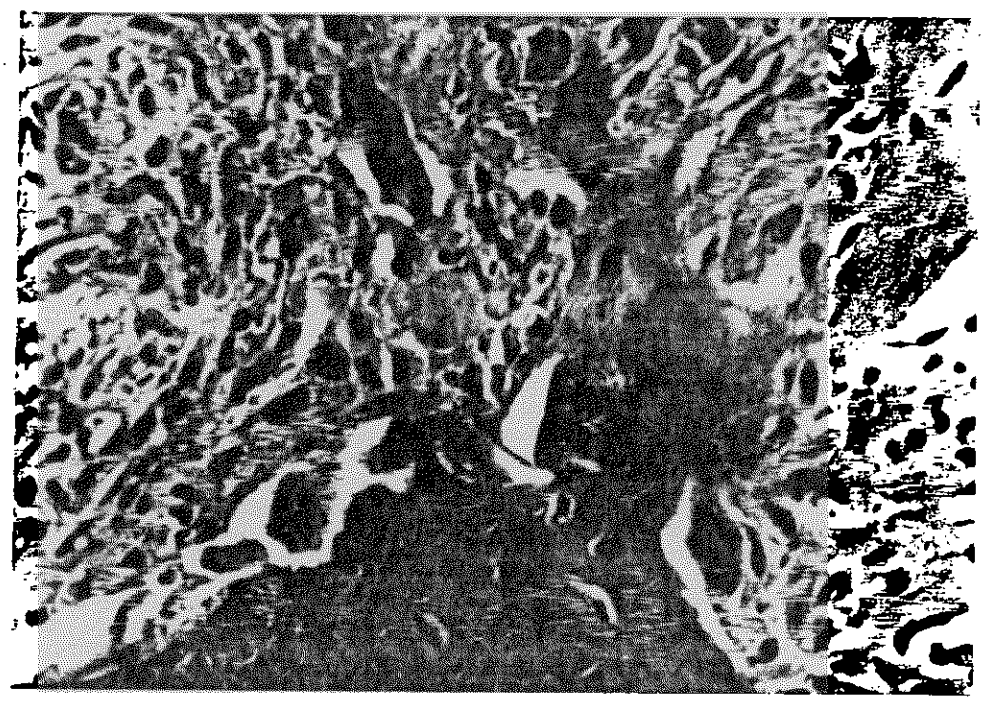


Fig. 46: Alveolar crest, 8 day, irradiated, orthodontically treated incisor, osteoclatic activity is apparent (100X).

Fig. 47: Crest tip, 8 day, irradiated, orthodontically treated incisor, multinucleated osteoclasts (400X).

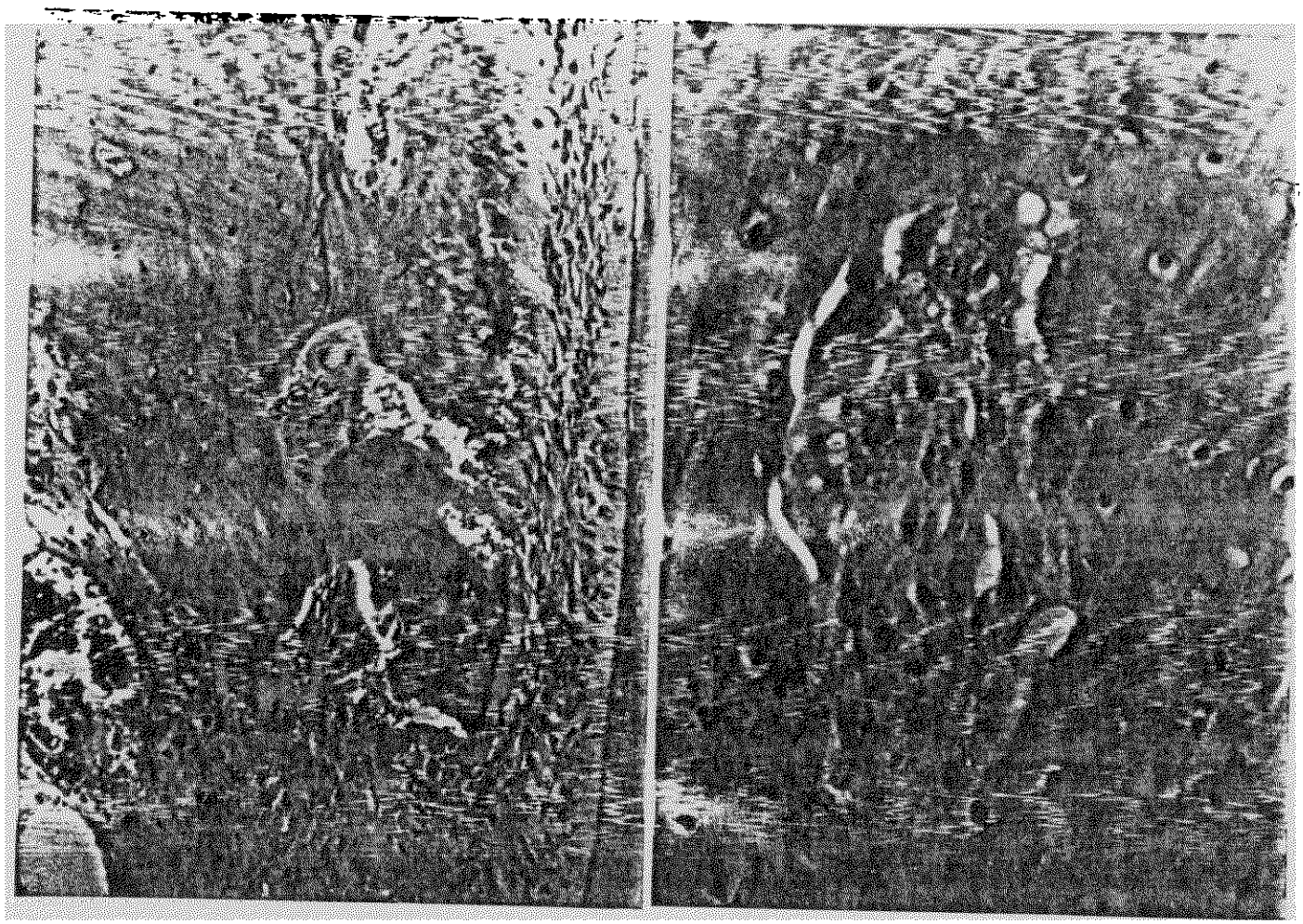


Fig. 48: Mid-root area, 8 day, irradiated, orthodontically treated incisor, osteoclasts and blood vessels (100X).

Fig. 49: Mid-root area osteoclasts, 8 day, irradiated, orthodontically treated incisor (400X).

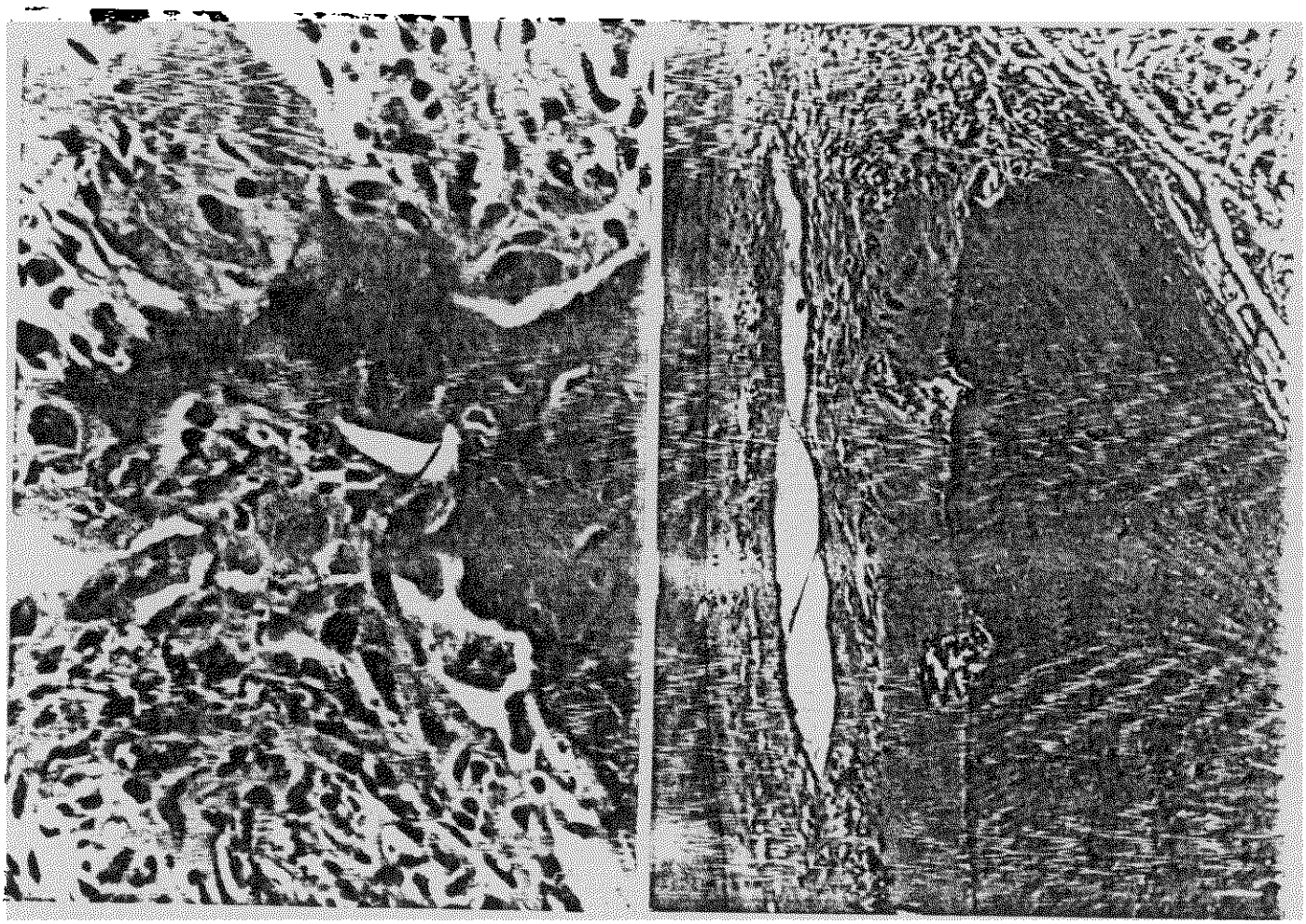
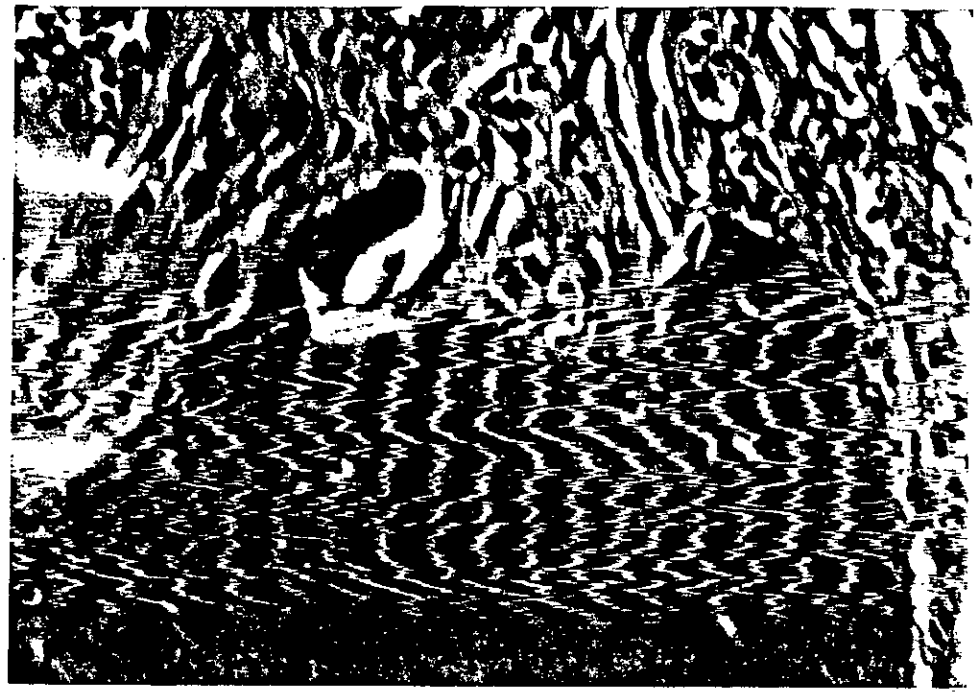


Fig. 50: Mid-root area osteoclasts, 8 day, irradiated, orthodontically treated incisor (400X).

Fig. 51: Alveolar crest, 10 day, non-irradiated, orthodontically treated incisor, reduced PDM cellularity and areas of crestal resorption (100X).



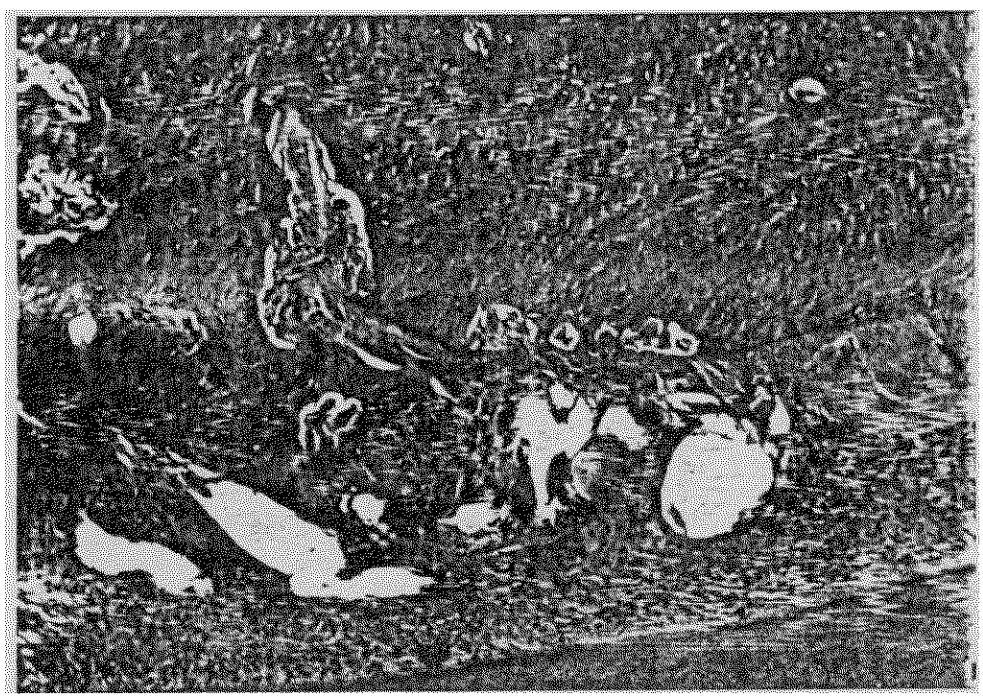


Fig. 52: Crest tip, 10 day, non-irradiated, orthodontically treated incisor, osteoclast in lacunae (400X).

Fig. 53: Mid-root area resorption, 10 day, non-irradiated orthodontically treated incisor (100X).

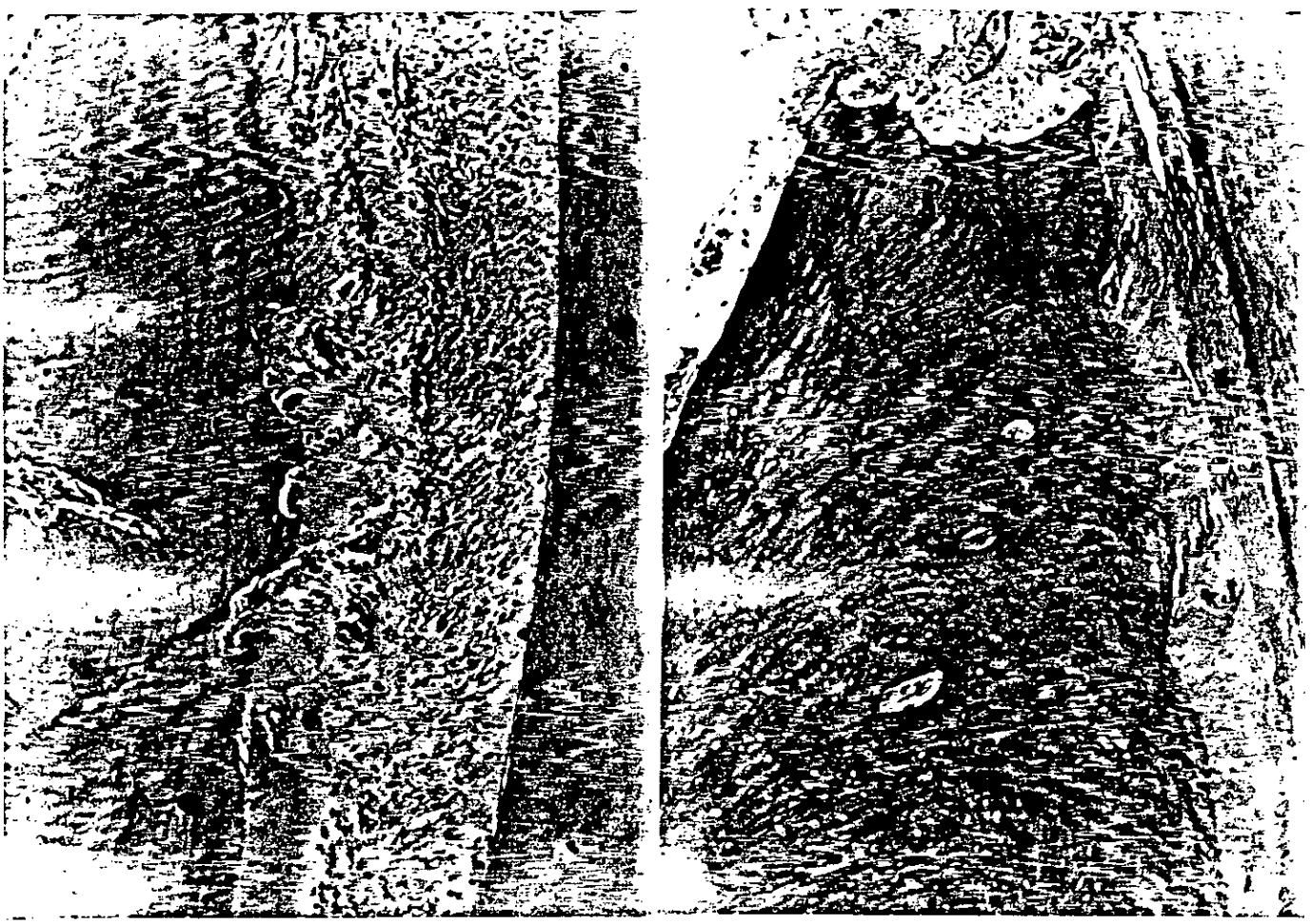


Fig. 54: Mid-root area resorption, 10 day, non-irradiated orthodontically treated incisor (100X).

Fig. 55: Alveolar crest, 10 day, irradiated, orthodontically treated incisor, reduced PDM cellularity and crestal resorption (100X).



Fig. 56: Alveolar wall resorption, 10 day, irradiated, orthodontically treated incisor (100X).

Fig. 57: Mid-root area resorption, 10 day, irradiated, orthodontically treated incisor (100X).

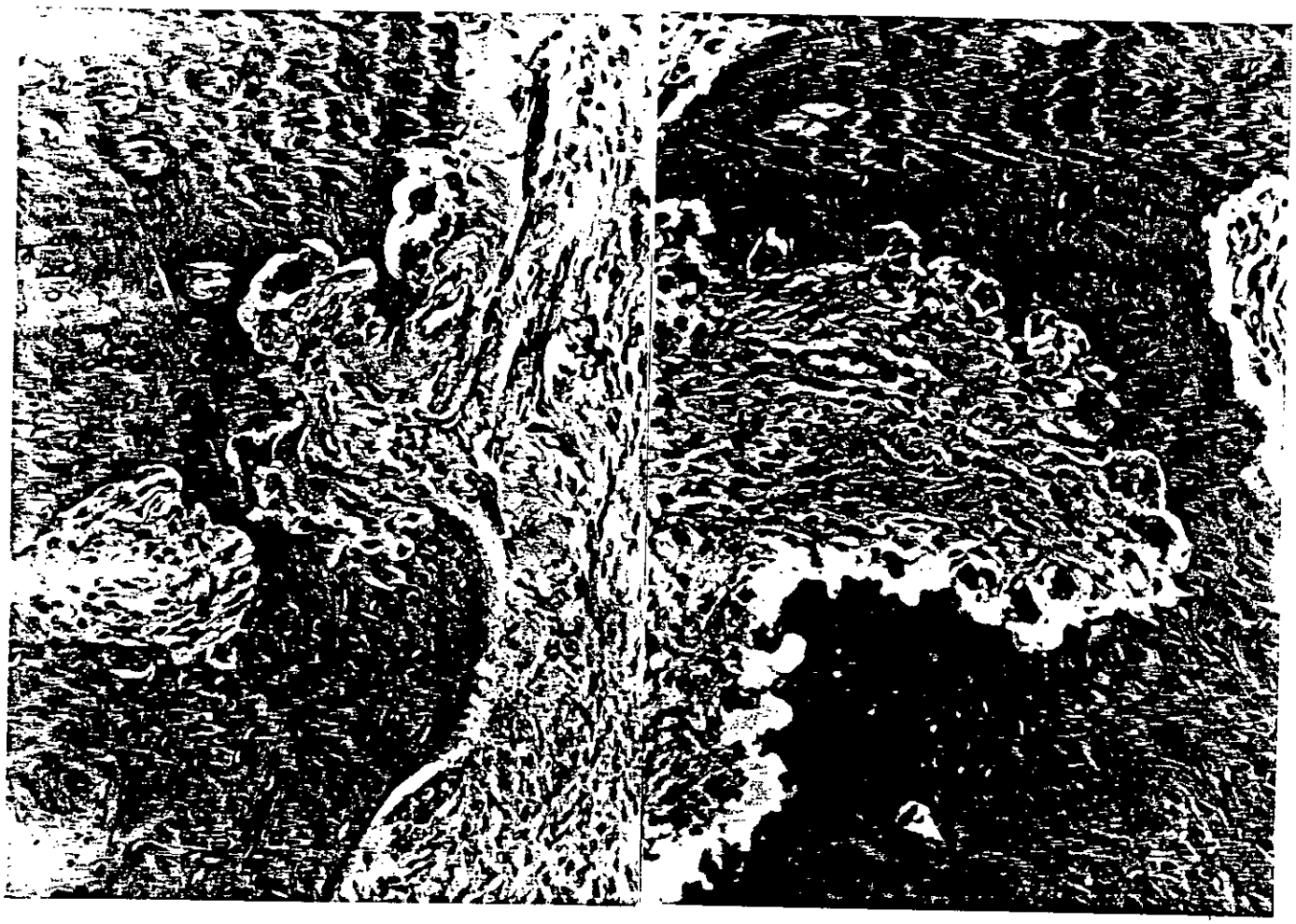


Fig. 58: Mid-root area resorption, 10 day, irradiated, orthodontically treated incisors, areas of frontal and undermining resorption are apparent (160X).

Fig. 59: Mid-root area osteoclasts, 10 day, irradiated, orthodontically treated incisors (160X).

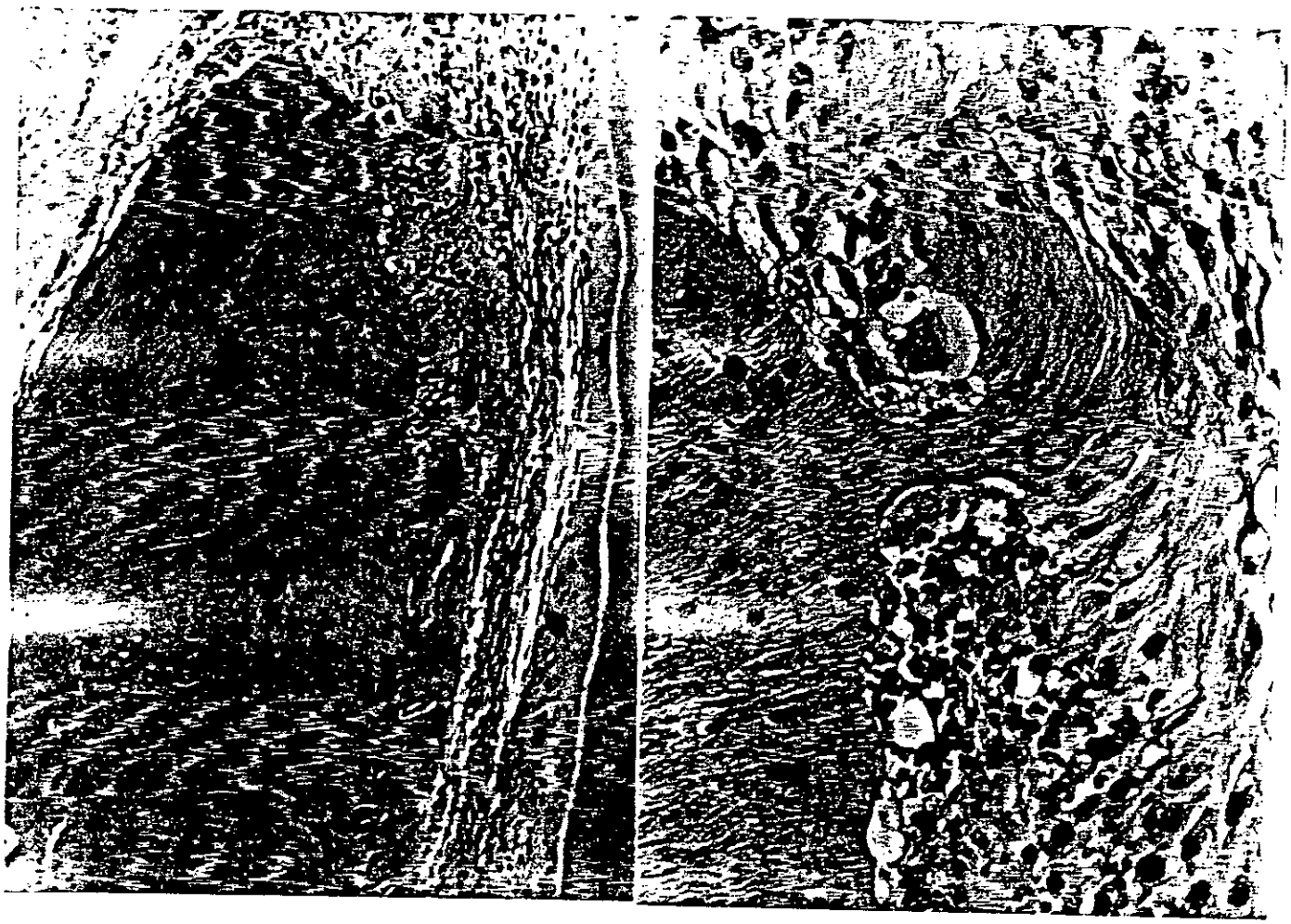


Fig. 60: Alveolar crest, 12 day, non-irradiated, orthodontically treated incisor, returning PDM cellularity with crestal resorption (100X).

Fig. 61: Crestal resorption, 12 day, non-irradiated, orthodontically treated incisor (400X).

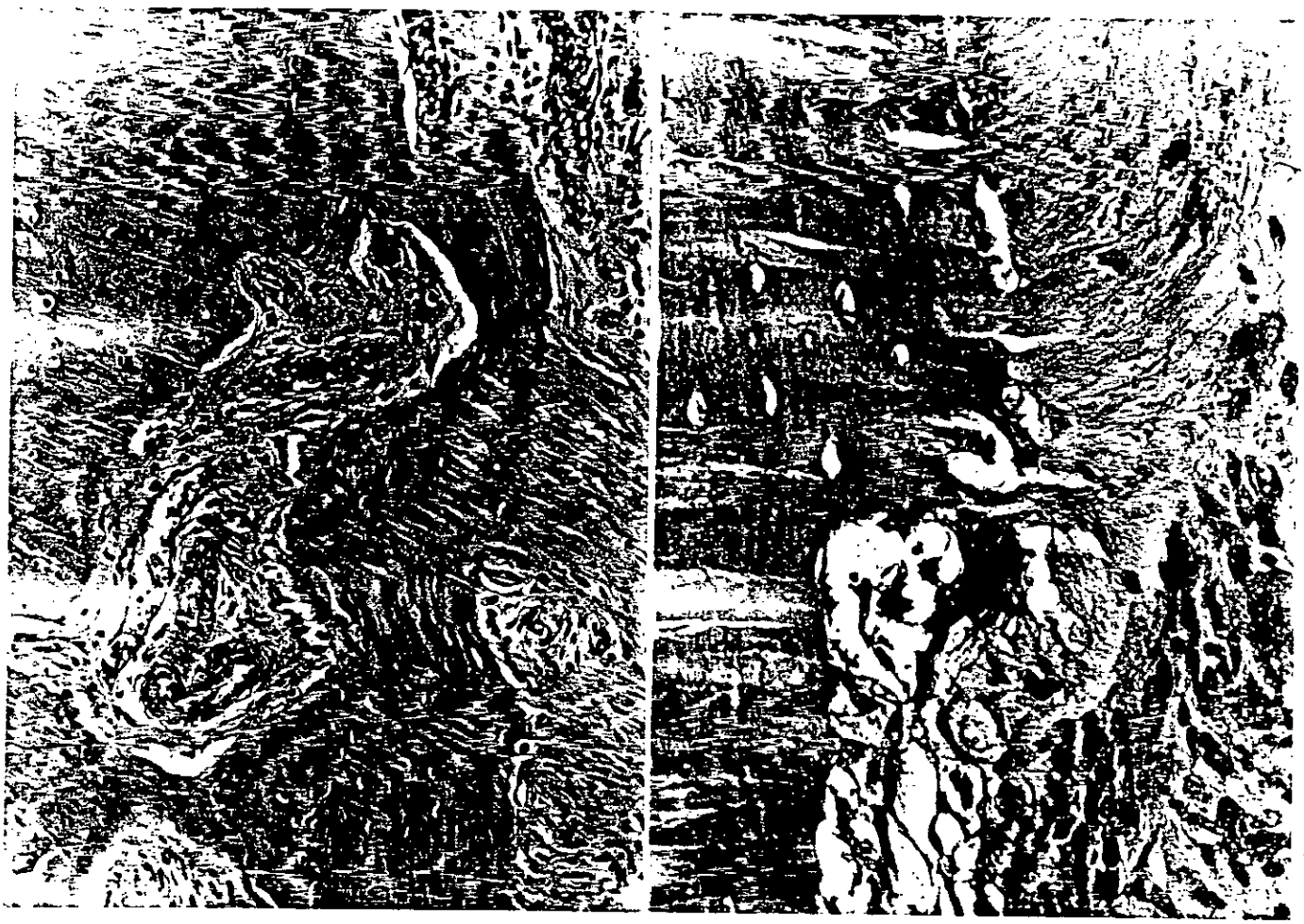


Fig. 62: Mid-root area resorption, 12 day, non-irradiated, orthodontically treated incisor (160X).

Fig. 63: Mid-root area resorption, 12 day, non-irradiated, orthodontically treated incisor multinucleated osteoclast (400X).

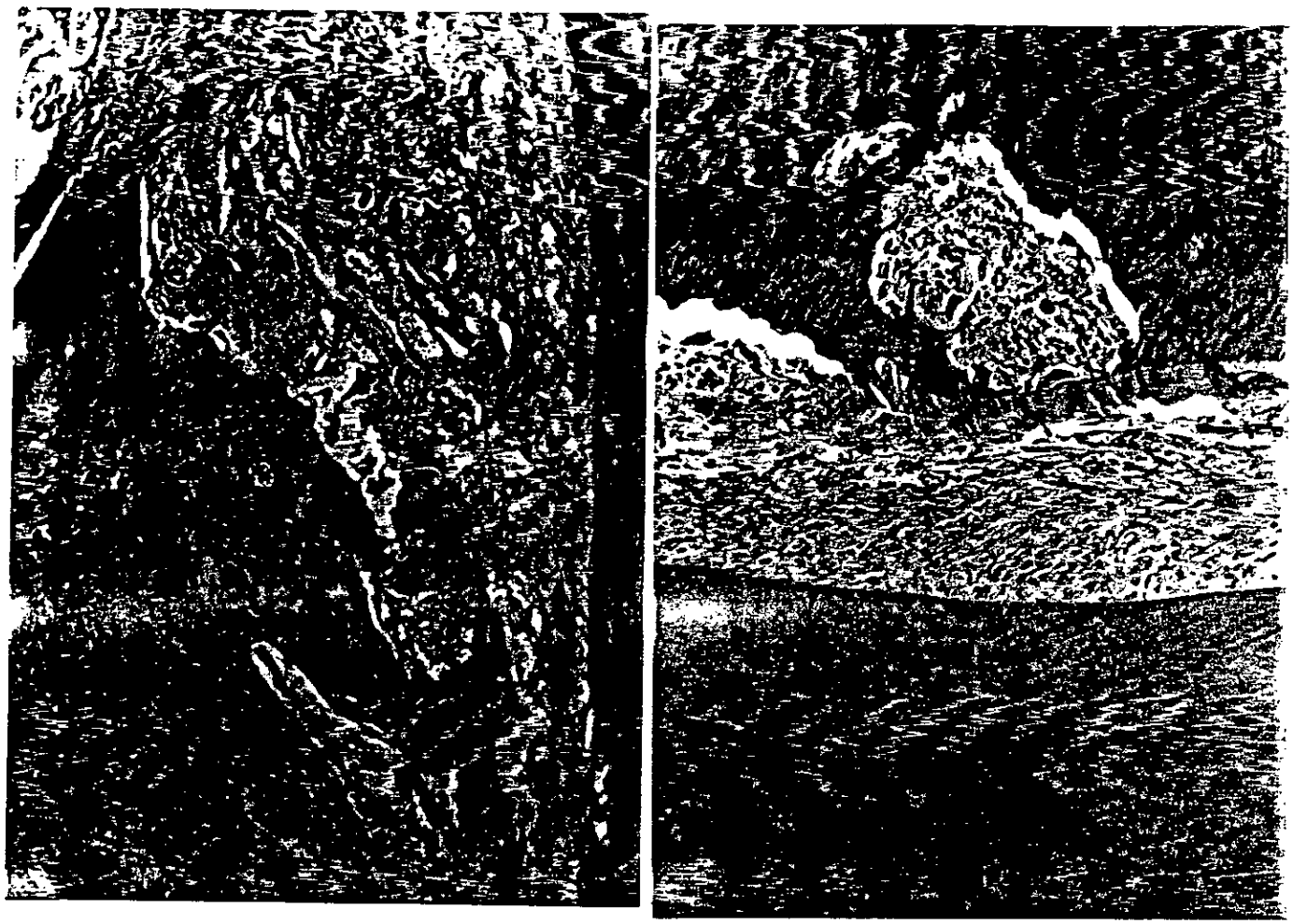


Fig. 64: Alveolar crest, 12 day, irradiated, orthodontically treated incisor, crestal resorption and reduced PDM cellularity persists (100X).

Fig. 65: Mid-root area resorption, 12 day, irradiated, orthodontically treated incisor, frontal and undermining resorption (100X).

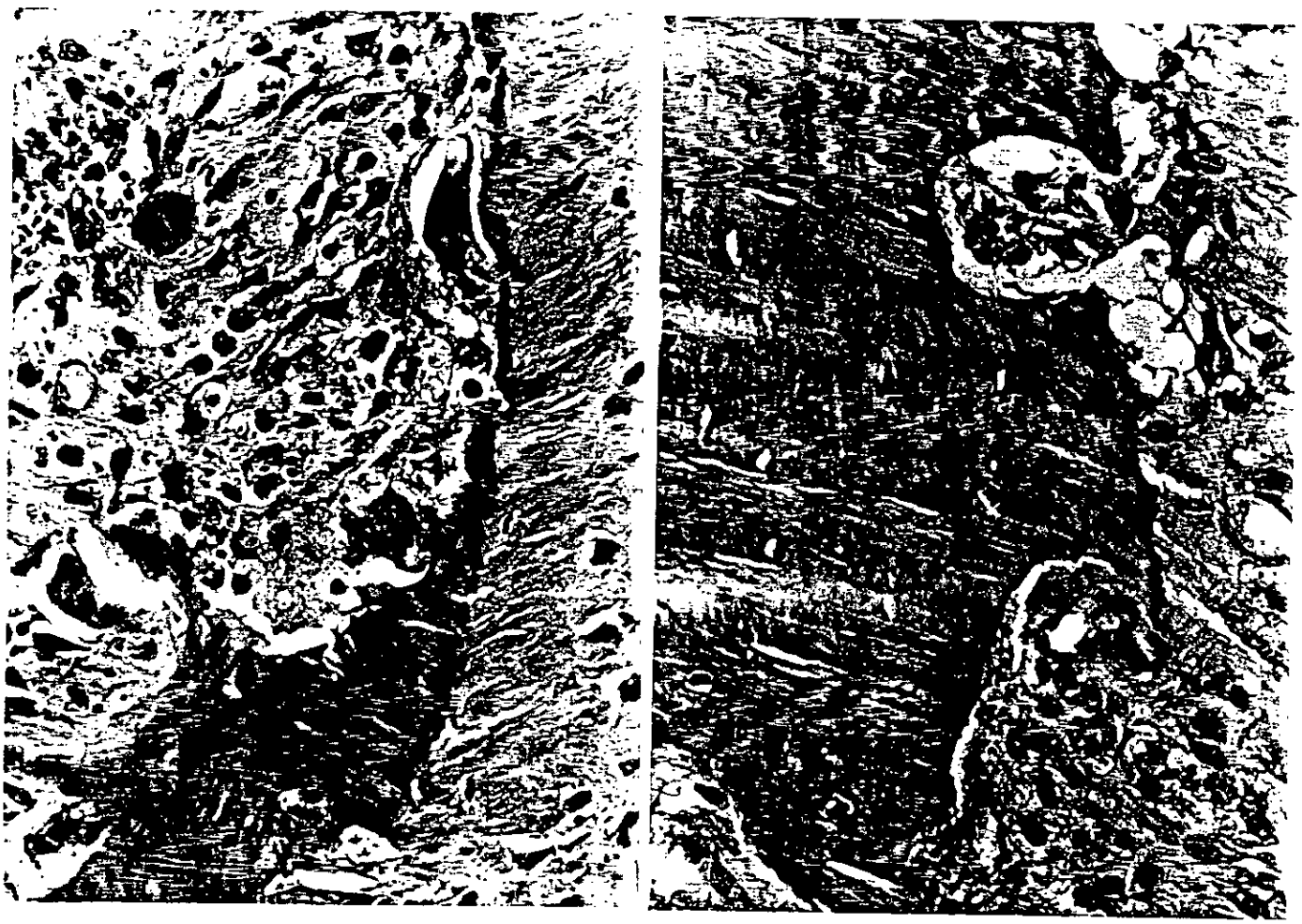


Fig. 66: Mid-root area osteoclasts, 12 day, irradiated, orthodontically treated incisor (400X).

Fig. 67: Mid-root area resorption, 12 day, irradiated, orthodontically treated incisor (400X).

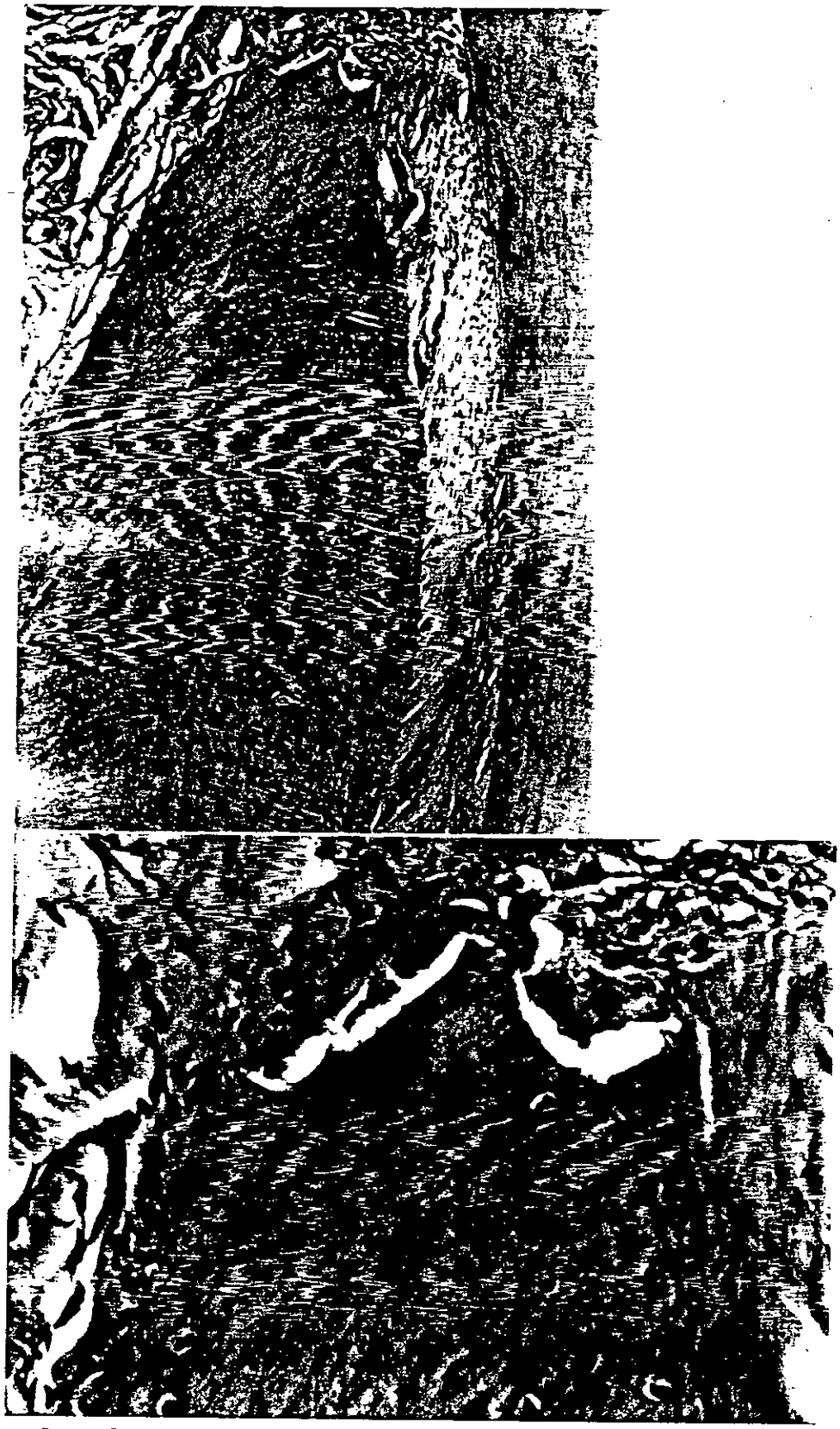


Fig. 68: Alveolar crest, 14 day, non-irradiated, orthodontically treated incisor, crestal resorption (100X).

Fig. 69: Crestal osteoclasts, 14 day, non-irradiated, orthodontically treated incisor (400X).

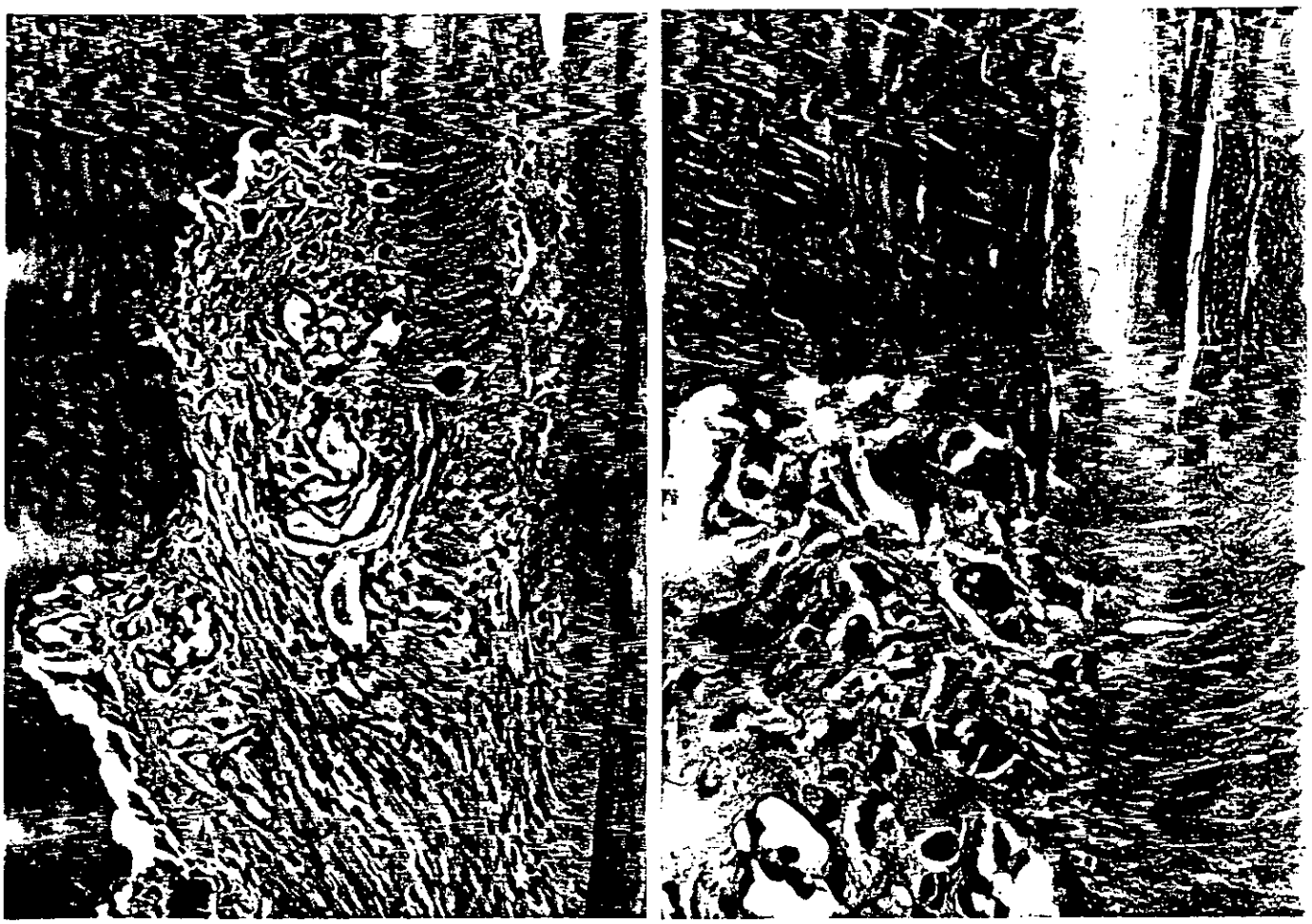


Fig. 70: Mid-root area resorption, 14 day, non-irradiated, orthodontically treated incisor (160X).

Fig. 71: Mid-root osteoclasts, 14 day, non-irradiated, orthodontically treated incisor (400X).



Fig. 72: Mid-root area osteoclast, 14 day, non-irradiated, orthodontically treated incisor (400X).

Fig. 73: Crest area root resorption, 14 day, non-irradiated, orthodontically treated incisor (400X).

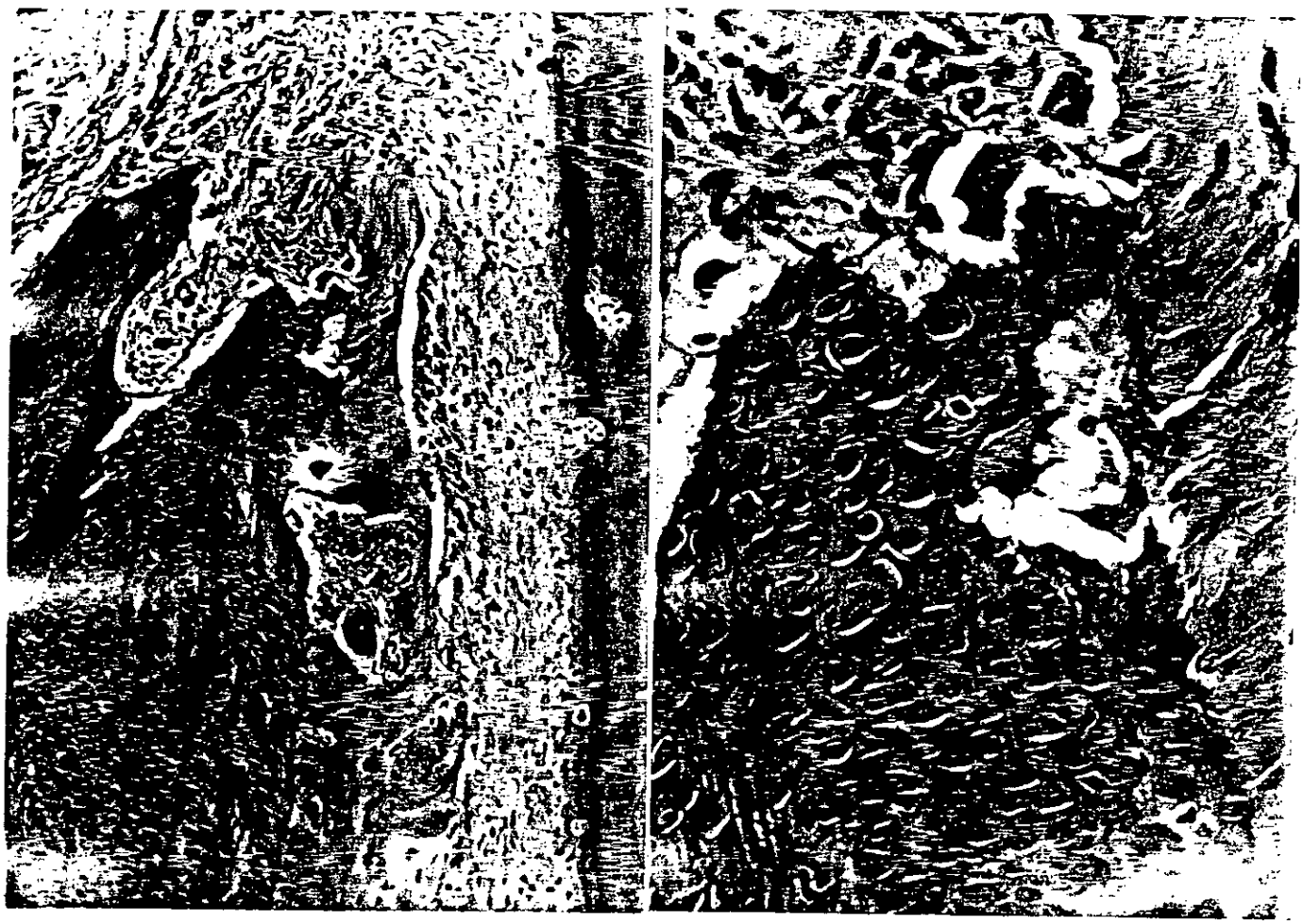


Fig. 74: Alveolar crest, 14 day, irradiated, orthodontically treated incisor, crestal resorption and areas of root resorption (100X).

Fig. 75: Crestal osteoclasts, 14 day, irradiated, orthodontically treated incisor (400X).

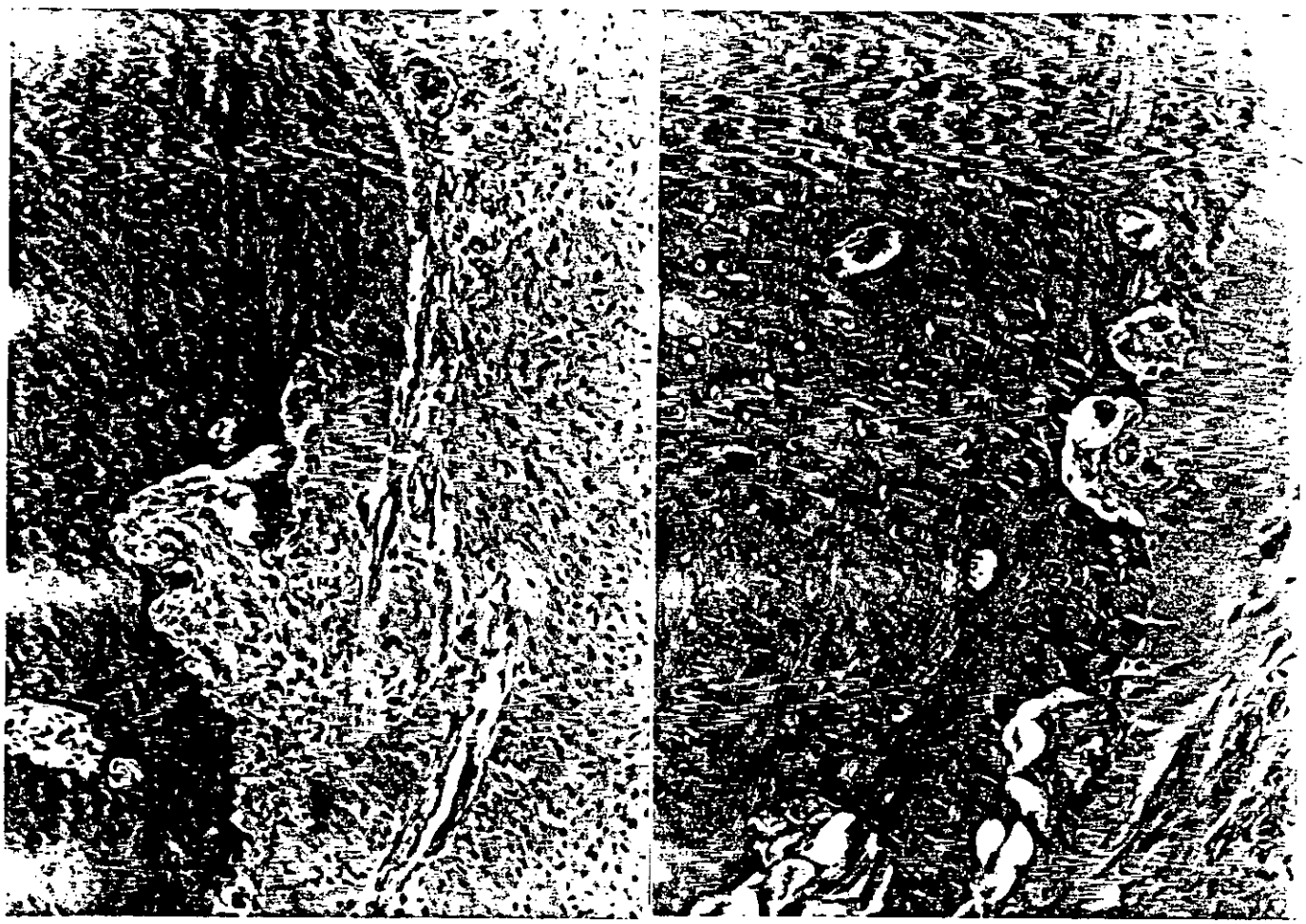


Fig. 76: Mid-root area resorption, 14 day, irradiated, orthodontically treated incisor (100X).

Fig. 77: Mid-root area resorption, 14 day, irradiated, orthodontically treated incisor (160X).

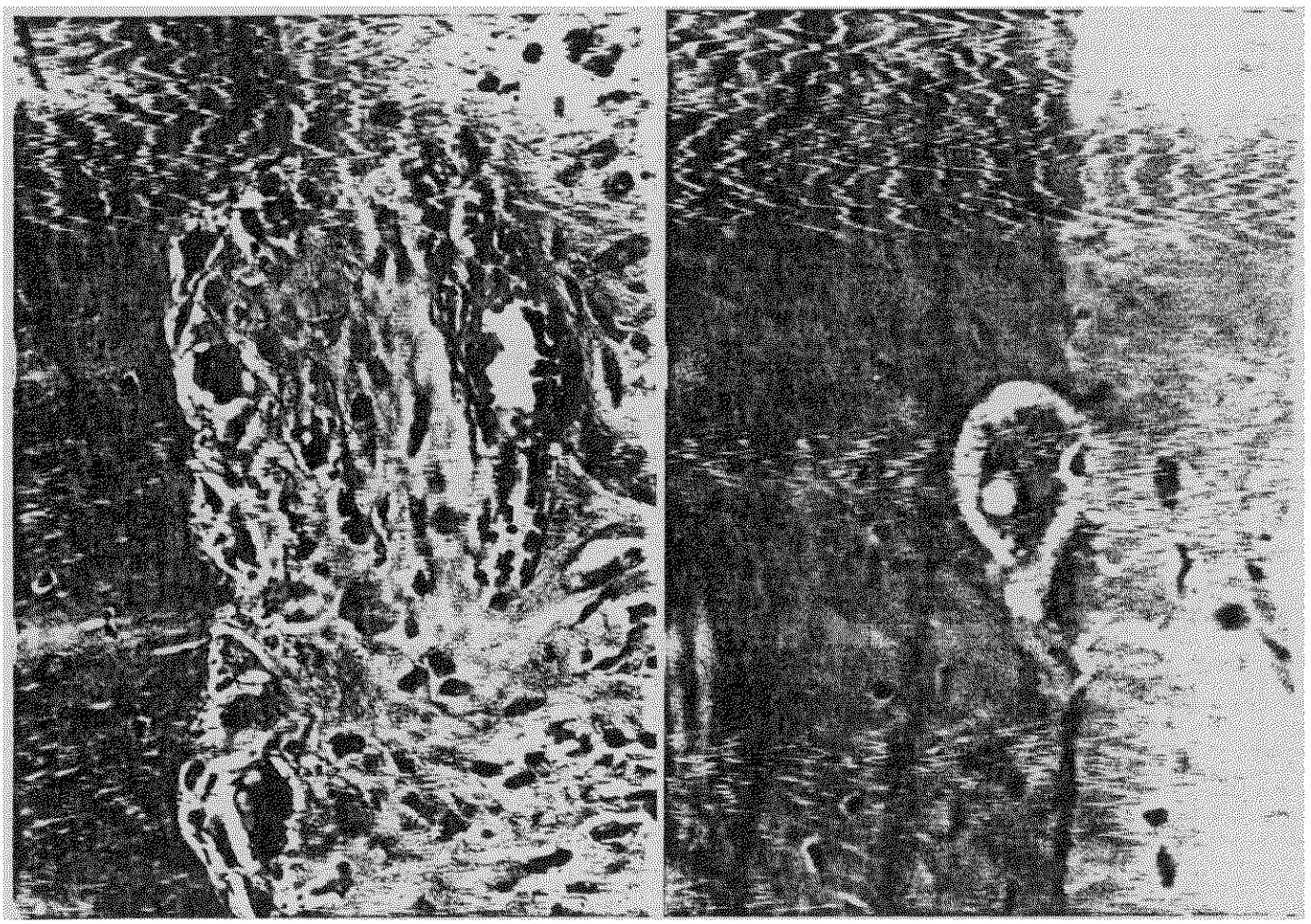


Fig. 78: Mid-root osteoclasts, 14 day, irradiated, orthodontically treated incisor, osteoclasts and blood vessels (400X).

Fig. 79: Mid-root area osteoclast, 14 day, irradiated, orthodontically treated incisor (400X).

DISCUSSION

The animal chosen was suitable as a model for this type of experiment. The Fischer 344 rats are easy to handle, mild in temperament, and are a hearty breed of animal. They are syngenetic which would allow future studies involving parabiosis or cell depletion followed by replacement, etc. These were adult animals and although bone remodeling is supposed to continue throughout a rats life, these animals appeared to show little or none. The fact that all the animals were male also made them useful since there were not the hormonal changes during estrous seen in female animals. WEIGHT CHANGES: The weight loss data from the experimental, irradiated versus the control, non-irradiated animals was as expected. There were similar weight losses due to similar procedures and an increased weight loss in the irradiated animals due to the 750R Gamma radiation. However, the weight loss period due to irradiation was short, only two days in duration, and all the animals tolerated this very well. These data compare well with those reported by Chapman and Jerome (1956) where they used white Swiss mice and gave them

a dose of 775R whole body X-irradiation. They showed a 4.6% loss in weight by two days and a 10.5% loss by four days post irradiation. After this they showed a leveling off for the next 11 days. In this experiment, there was an initial drop in weight of 4.6% at two days and 11.6% at four days post-Gamma irradiation.

The results of the differential cell counts drastically differ between the irradiated and non-irradiated rats. The numbers of white blood cells in the peripheral blood of irradiated animals dropped so drastically that at the early stages of the experiment and throughout its course an accurate WBC differential cell count was not obtainable. These counts are done by staining the smear with Wrights stain and then making a scanning pattern on the slide and recording the first 100 WBC's seen and typing them. However, even with four smear slides per animal, 100 cells could not be counted during the experimental period. A rough idea of cell types present in the irradiated animals could possibly be implied from the peripheral WBC counts where most of the cells seen were polymorphonuclear granulocytes (PMN) and the

rest made up of lymphocytes. Not a single monocyte was seen in the irradiated animals and only very rarely in the non-irradiated controls. The non-irradiated controls conversely showed a predominance of lymphocytes over PMNs. These data would correspond well with that presented in Clinical Biochemical and Hematological Refernce Values in Nomal Experimental Animal (Mitruka and Rawnsley 1977), in which PMNs were reported as 11.5 - 45.6% of the leukocytes in normal Fisher 344 rats and lymphocytes amounts to 43.0 - 79.5% of the WBCs. In this variety of rat the monocytes make up 0.00 - 2.00% of the WBC component in normal animals. Also in data presented in the Manual on Radiation Hematology (Blackett 1971), it was reported that in rats receiving a radiation dose equivalents to the LD_{50}, the lymphocyte count drops to less than 25% of the initial level and this lymphopenia continues for more than 4 weeks. The greatest decrease in lymphocyte numbers occurs between 3 and 8 days. This would explain the reversal in lymphocyte-PMN dominance between the non-irradiated and irradiated animals. The data from the peripheral WBC count compared well with the reported

numbers in the literature. According to Bond 1971, when pigs received 600 R irradiation, there was an initial "shoulder" and then a rapid fall reaching a minimum at 9 days post-irradiation and then slowly recovering over the next 20 days but never recovering to more than 20% of the normal levels (Manual on Radiation Hematology). This also reflects well with the reported data on stem cell survival where mice received a dose of 650 R Gamma radiation and the survival rate was less than 0.2 percent.

These data are radically different from the control, non-irradiated animals which had an average peripheral WBC count of 5212 WBC/mm^3, in this experiment. This also compares well with the data reported in Clinical Biochemical and Hemotological Reference Values in Normal Experimental Animals (Mitruka and Rawnsley 1977), where a range of 5,350 - 11,200 WBC/mm^3 in the peripheral blood of Fisher 344 inbred rats was reported. Thus, in the irradiated rats, it appears that there is a quick depletion of the WBC's in the peripheral blood that reached a maximum depression at 8 days and was followed by a slow, gradual increase until the end

of this experiment. At the end of the experimental period the WBC's had recovered to 22.2 percent of the control values.

The histologic results of the current experiment are somewhat unexpected. They show that a 750R dose of whole-body Gamma radiation does not inhibit osteoclast formation and function in an alveolar process subjected to orthodontic forces. Thus, if the radiation destroys the bone marrow as evident in this experiment and other studies and if osteoclast stem cells originate in the bone marrow, then the expected would be a sharp decrease in viable osteoclasts after irradiation. This was not the case in this study. A difference between the non-irradiated, orthodontically moved incisors and the irradiated, orthodontically moved incisors could not be shown. Throughout the experiment and at each time point sampled, the numbers and morphology of osteoclasts all appeared very similar, if not identical. However, it was easily seen that 750 Rads of whole-body Gamma radiation was extremely effective in destroying the bone marrow and it's cellular component. This raises several questions as to the

life span of alveolar osteoclasts, their origin and whether or not different populations of osteoclasts exist.

Another factor which appears to indicate continued function of the osteoclasts and their cellular development from their stem cell source, is that in all cases the teeth continued to erupt and to be moved lingually by the orthodontic appliance. This requires the presence of functioning osteoclasts as shown by Marks (1981) where by using osteopetrotic rats her showed that osteoclasts are required for the eruption of teeth. However, in the current experiment, the incisors in the non-irradiated, orthodontically treated animals continued to erupt and required between 14 - 16 days for the anterior wire attachement, and at 14 days the attachment was only approximately three-fourths of the way to incisal edge. This could indicate an inhibitory affect of irradiation on tooth eruption. Since the osteoclasts were not affected by irradiation, the logical explanation may indicate the involvement of other cell types in tooth eruption which are affected by irradiation. What factor is affected could not

be determined by the scope of this experiment.

The next question that must be asked is why or how could these results possibly come about? There are many possible answers.

First, osteoclasts may be derived from a macrophage-histiocyte precussor, which are much more radio-resistant than other leukocytes. This difference in radio-sensitivity has been shown to occur by the International Atomic Energy Agency (Kozinets 1971). The Agency found that lymphocytes are extremely radio-sensitive, "one of the most radio-sensitive cells in the organism." They showed a 50% decrease in lymphocytes with a whole-body radiation dose of only 150R, and when given at the LD_{50} the survival rate was only 25%. They also showed that the macrophage-histiocyte sytem is much more radio-resistant. Bloom and Bloom (1947) showed that even with x-ray doses up to 1000R, the bone marrow macrophages were not visibly damaged. Also, Shouse et al. (1931) reported that nearly all types of cells are severely damaged by lethal doses of irradiation with the exception of macrophages. Other studies have shown that they also

maintain their functional capabilities after large doses of irradiation. Benaceraf et al. (1959), showed that rats given 850R could still clear carbon particals and Escherichia coli from their bloodstreams as rapidly as non-irradiated animals. On the other hand, in more recent studies by Scheven et al. (1985), they show that osteoclasts, or more likely their precursors, are very sensitive to irradiation. A dose of 250 Rads was sufficient to significantly reduce the number of osteoclasts developing in metatarsal bone explants in culture. This effect is attributed to the radiation interfering with the proliferation phase of osteoclast production before it becomes a mononuclear postmitotic preosteoclast. They also showed that doses of 500 Rads can inhibit the resorbing activity of mature osteoclasts. It must be kept in mind though, that these results come from bones in explant prior to osteoclast formation and only osteoclasts in leg bones were studied.

The second possible explanation would be that osteoclasts have a life span longer than the 14 days of this study. Many studies, in the recent literature, have

concluded that osteoclasts have no definite life span. Probably the more pertinent fact is that most of these authors feel that there is a specific "transit time" that can be determined for the life span of specific nuclei within the osteoclast (Young 1962; Jaworski et al. 1981; Loutit and Townsend 1981, 1982; and Zallone et al. 1982), approximately 10 days.

Thus, it appears that if these estimates of "transit time" are accurate, they would conflict with the results of this current study. This study, though not quantitative, gave no indications for a reduction in the number of osteoclast nuclei/cell. There were also no indications for a reduction in the number of osteoclasts. The current results may not contradict the possibility of a "transit time" for osteoclast nuclei but they would put this time at something longer than 14 days.

The third possibility is that osteoclastopoiesis recovers from irradiation more rapidly than the "transit time" for the osteoclast nuclei. This would seem unlikely, though, since the marrow at 14 days post-irradiation, and

peripheral WBC counts are not even up to 25% of their normal complement.

Another possible explanation would be that there is a local pool of osteoclast precursors that are in the alveolar bone, insensitive to irradiation, and ready to differentiate into osteoclasts when activated. Some evidence for this exists in te literature. Kember (1960) showed that it takes three weeks for an autoradiographic label to appear in osteoclasts after infusion. Gothlin and Ericson (1972) and Fischman and Hay (1962) also had similar results where labeled osteoclasts could not be identified until 10 - 21 days after bone fracture on limb amputation respectively. However, in all cases there were other non-labeled osteoclasts in the sections. More recent evidence in favor of th existence of a local pool of cells ready to become osteoclasts comes form Scheven et al. (1984, 1985a and b, 1986a). In a series of experiments they showed that osteoclast progenitors are in the periosteum at a very early developmental stage. These progenitor cells are still dividing and are highly vulnerable to irradiation. These

cells become mononuclear preosteoclasts which are radio-resistant and reside in the tissue until they are needed. This possiblity also, could not be refuted by the current study. If anything, this study would tend to support the occurence of a local preosteoclasts pool since the marrow stem cells were apparently destroyed and there was no noticable reduction in osteoclast numbers. The only argument to discredit a local pool would be that we used relatively old animals that had limited potential for growth and developmental change. The existence of a large pool of osteoclast precursors in the incisor areas of these rats is less likely. Since these rats are virtually complete in their growth and development, the incisors would need osteoclasts only for physiologic tooth eruption. However, the number of precursors present in the tooth socket may still be sufficient for significant bone resorptive activities.

A final, and I feel most likely, explanation would be that there are different types of osteoclasts for different physiologic functions at different bone sites. This is a

relatively new area of thought for bone cell biologists, but one that seems to be gaining support for many types of cells that were once thought to be homogeneous populations.

In the current experiment, the differential response of osteoclasts to whole-body irradiation at least suggests that there is a possibility of multiple subclasses of osteoclasts. This theory is supported by the fact that in this experiment 750 Rads of whole-body Gamma radiation caused a transient destruction of the bone marrow, resulting in a peripheral WBC count of less than 23% of the control level 14 days post-irradiation, and there was no apparent change in number or morphology of alveolar bone osteoclasts. Also, no osteoclasts could be demonstrated in the long bone shafts of the irradiated animals. Combined with the fact that in many other studies, osteoclast numbers in long bones were reduced by irradiation (Anderson et al. 1979, Scheven et al. 1985, Gungor et al. 1982). This indicates a basic difference in the osteoclasts located in long bone periosteum and endosteum and those found in the alveolar socket wall of incisors undergoing orthodontic tooth movement. It is interesting to

note that Parodi et al. (1973) showed an increase in alveolar bone resorption in rats treated with whole-body irradiation and orthodontic tooth movement. These authors used 200 gram Wistar rats which received 900 Rads of whole-body x-irradiation and an orthodontic appliance 48 hours later. The orthodontic appliance was designed to move the maxillary first molar bucally. The animals were sacrificed 1, 2 and 4 days post-appliance placement. Making 6 days post-irradiation the length of this study. They found that irradiation increased bone resorption, without an orthodontic appliance and that an orthodontic appliance increased resorption, without radiation. Together, irradiation and an orthodontic appliance caused the greatest amount of bone resorption. In the current study, an increase in osteoclast numbers could not be detected yet, there was most certainly no decrease in osteoclast numbers.

Another point to note would be that in this experiment, there was an apparent proximity relationship between osteoclastic activity and vascular channels. Thus, if there are specific determinents on the osteoclast precursor cell

surface which are recognized by endothelial cells in the venules, they would allow osteoclasts precursors to leave the bloodstream only if they had these determinants. This could help explain their proximity to the vascular channels.

There is also such a perponderance of data which shows that there are subclasses of various cells, which give each cell type the ability to respond differently to similar stimuli, that the notion of subclass of osteoclasts would only make sense. These data are true for many of the macrophage-monocyte cell lineage. Coonrod and Rehn (1982) and Zlotnik et al. (1982) have shown that pleural peritoneal and pleural alveolar macrophages have differences in their cell surface characteristics which give them different functional properties. Also, Rumpold et al. (1982) have shown that alveolar, peritoneal and splenic macrophages can be identified by specific monoclonal antibodies even though they are all macrophages and all have phagocytic capabilities. They also have some minute differences which can be detected selectively by monoclonal antibodies. Yet, other authors, Papadimitriou et al. (1980) and deBakker and

Daems (1981), have shown that resident peritoneal macrophages differ from induced peritoneal macrophages. They are different in their cytochemical, biochemical, cytomorphological and functional properties.

This also holds true for monocytes. Falk et al. (1982) showed that some blood monocytes, but not all, are responsive to chemo-attractant proteins. This responsiveness correlates with specific cell surface receptors located on these responsive cells.

These data, which suggest a possible site specific subclass of cells, would support the theory that there are different subclasses of osteoclasts. Taken in conjunction with the evidence of Liu and Baylink (1984), Barron and Saffar (1977) and Saffer and Barron (1977), which show a varing response of osteoclasts at different sites to similar stimuli, it seems highly likely that the osteoclasts located in the alveolar socket wall of the rat incisor are different from osteoclasts located elsewhere in the rat.

This would help explain some of the variability seen when orthodontic forces are applied. Possibly, in some

animals, the stem cells responsible for these induced osteoclasts were damaged more than in others by the irradiation. It may also explain why similar levels of force can induce so many osteoclasts in one individual yet not nearly as many in another.

It would also help to explain how oteoclasts could be involved in so many various activities, such as calcium and phosphate homeostasis and structural skeletal maintenence. This would require at least two subpopulations of osteoclasts. One, involved in ion homeostasis would be responsive to systemic factors which would allow more calcium and/or phosphate to be liberated and made available for use. These would most likely be situated in the skeleton at various points and would be constantly renewed so that there was always enough to maintain this balance. The second, would be involved in maintainence of structural skeletal integrity. This would be an inducable cell that would not normally be scattered throughout the skeleton. It would require a stimulus, ie) micro fracture, overt fracture, pressure, etc. to stimulate a stem cell or a possible local

pool to proliferate and differentiate into cells which would not be involved with mineral homeostasis, but would be there to repair changes in the skeleton. This may be why it takes time for an orthodontic stimulus like this to elucidate a response while a hormonal stimulus gets immediate results.

SUMMARY AND CONCLUSIONS

Orthodontists have been moving teeth for many years without understanding how these movements are actually taking place. At present, the variety of cell types associated with tooth movement have been named, but they are just now being defined as to their origin, fate and function.

The present study was undertaken to try to elucidate just one of these cells, the osteoclast. An attempt was made to severly compromise the osteoclast stem cell population in the bone marrow by giving each animal a LD_{50} dose of whole-body Gamma radiation. The objective was to gain knowledge as to the origins and fate and variations of the osteoclast.

The experiment required the use of 30 Fischer 344 rats divided into two groups of 15 each. One group received a whole-body Gamma radiation dose of 750 R, and the other received no irradiation. Both groups had an orthodontic spring appliance placed in the maxillary arch between a molar and an incisor and it was activated to give 50 grams of force. Animals were sacrificed at 6, 8, 10, 12 and 14 days post-irradiation and appliance placement or just appliance

placement (controls). Just prior to sacrifice, blood was drawn for a peripheral white blood cell count. After sacrifices, the maxillae were surgically removed along with the right femur and prepared for histologic examination.

The weight change data showed that during the experiment, all animals tolerated the procedures very well. The irradiated animals lost slightly more weight and the weight loss lasted for two more days than that seen in the control non-irradiated animals. All tolerated the orthodontic appliance well and resumed normal activity within 72 hours of appliance placement. The peripheral white blood cell counts correlated very well with the existing data in the literature. There was an initial steep drop in the number of cell/mm^3, until 8 days post-irradiation. After this, the numbers started to recover but even by the end of the experiment, they had recovered to less than 23% of the non-irradiated control levels.

The results of the long bone shaft histology correlated very well with the peripheral WBC counts. At three days, the marrow was severely compromised and by six days there

appeared to be near total destruction. This reached its greatest depletion at 8 days and then slowly recovered until the end of the experiment, never reaching control levels again.

Results of the histology from the alveolar bone on the lingual aspect of the maxillary incisor did not correlate well with the other results. The expected reduction in osteoclasts in the irradiated animals never occured. At all time points sampled the non-irradiated orthodontically treated incisors looked very much, if not exacly, like irradiated, orthodontically treated incisors. These results contradict many of the current concepts of osteoclast origin and fate. The experiment does not support a hematogeneous origin for osteoclast precursors since the marrow was nearly completely destroyed, yet it had no effect on relative numbers of osteoclasts or continuing tooth movement or erruption. The findings may imply a monocyte/macrophage precursor for osteoclasts since these are very radiation resistant cell lines. These results support a "life span" for osteoclasts that is longer than 14 days,

and also shows that the current "transit time" data for nuclei in the osteoclast could be somewhat longer than 14 days. Finally, the data would suggest a theory that osteoclasts are a heterogeneous group of cells. Thus, different subclasses of osteoclasts would carry out mineral/ion homeostasis functions and the structural skeletal maintenance functions.

The next question that needs to be asked is: "Where do we go from here?" There are many possible changes in the current experimental protocol which could yield additional information. The first and foremost would be to continue the experiment for a longer period of time. This would require an initial dose of Gamma radiation followed by a maintainence dose, possibly every day or week, to keep the peripheral leukocyte numbers down. This could also involve the removal of the spleen, to remove another possible source of stem cells, prior to the experiment. This would give us a much better idea of the life span of an osteoclast. It would also require that the orthodontic appliance be repositioned and reactivated more frequently. In the present experiment, only

16 days were required for anterior attachment to be lost due to incisor eruption.

Another change would be to use a stain specific for osteoclasts. This would involve the use of a tartrate-resistant acid phospatase marker stain. It would not label the early precursors of osteoclasts which are most likely in the alveolar process (Scheven et al. 1986a), but it would enable the identification of mononuclear osteoclasts that were not addressed in the current study. To use this specific stain, it would require the use of frozen, non-decalcified sections. In the current experiment, several attempts were made to use stains specific for osteoclasts, however, it did not work. This is most likely due to the decalcification of the specimens, even though very mild methods were used. Most, if not all, the studies to date have used non-decalcified frozen sections. (Mostafa et al. 1982, Minkin et al. 1982, Van de Wijwgaert et al. 1986.) Using an osteoclast specific stain would enable counting of cells, more specific information to be obtained and allow for direct numerical comparison to be made.

To create a clinical fracture in a leg long bone simultaneously with orthodontic tooth movement to further elucidate the presence of different osteoclast subclasses is the final change which would greatly enhance the outcome of a continuation of this experiment. This would allow a direct comparison between periosteal, endosteal and alveolar osteoclasts. It would also allow for comparison of osteoclasts all involved in the maintainence of the skeletal integrity. This is important in that another experiment could be devised to check the effects of whole-body gamma-irradiation on osteoclast numbers when the animal is stimulated by parathyroid hormone. Therefore, a direct comparison of osteoclasts involved with mineral and ion homeostasis can be made with those responsible for structural skeletal maintainence.

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