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**THE HISTOGENESIS OF THE OSTEOCLAST**

**by**

**JOHN J. MAGON**

**A Thesis Submitted to the Faculty of the Graduate School  
of Loyola University in Partial Fulfillment of  
the Requirements for the Degree of  
Master of Science**

**JUNE**

**1963**

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## LIFE

John J. Magon was born in Chicago, Illinois on July 16, 1932. His elementary education was received at St. Michael the Archangel Elementary School (Chicago), and his secondary education was received at Hirsch and Bowen High Schools (Chicago).

From September 1949 to January 1952 he attended Wilson Jr. College of the Chicago School System. During the period from February 1952 until August 1952 he found employment with the steel industry in Chicago. In August 1952 he entered the active naval service as a naval reserve member.

During active duty he received training as a dental technician. Later he was assigned to teaching duties at the U. S. Naval Dental Technician School, Great Lakes, Illinois, during which period he attended instructor training school at Great Lakes, Illinois.

After separation from active duty in June 1954 he began his professional education at the Chicago College of Dental Surgeons, Loyola University in September of the same year. His participation in a graduate educational program had begun in September of 1958 leading to a Master of Science Degree in Oral Anatomy.



## CHAPTER 1

### Introduction

Thru the introduction of new materials and techniques, new avenues of investigation are made available.

This study was undertaken in order to utilize the autoradiographic technique and apply it for the purpose of detecting radioactively labeled cells.

Radioactive tritiated thymidine will be picked up during the desoxyribosenucleic synthesis phase (DNA synthesis phase) of mitotic division.

Slight controversy exists as to how the osteoclast forms, namely by multiple division or from a fusion of many cells. In particular it is believed that osteoclasts are formed by transmutation of the osteoblasts.

This paper will introduce to the reader the opinion that the osteoclasts may be derived from undifferentiated cells.



## CHAPTER II

### REVIEW OF THE LITERATURE

#### 1. The Parathyroid Glands and the Hormone

The parathyroid glands in man are very closely adapted to the posterior surface of the thyroid gland. Usually the glands are present as a pair of two but their number and location may vary. Abberant glands are seen in about one of every ten glands usually then in such case in the mediastinum, occasionally within the thyroid, and rarely behind the esophagus. The weight of the four glands is approximately 118 milligrams with the average size about 5x5x3 millimeters. The glands are seen to be reddish or yellowish brown in color each containing distinct stalks containing blood vessels and nerves. The glands are surrounded each by an ill defined fibrous capsule, a rich capillary plexus, a few non-medullated nerve fibers and lymphatics.

The glycogen-containing chief cells, which constitute most of the gland before puberty, measure six to eight microns in diameter and are the source of the parathyroid hormone. The non-glycogen containing oxyphil cells appear after puberty, vary in size about eleven to fourteen microns, and are not identified with any function.

The parathyroid gland in the rat lies closely applied to the antero-lateral surface of the thyroid gland. Hoskins and Chandler (1925) state that the appearance of accessory parathyroid glands in the rat is rather rare.



Selye (1949) describes the preparation of the hormone for clinical

use:

" The usual method of preparation consists in extracting cattle parathyroids with aqueous hydrochloric acid and precipitating the hormone with trinitrophenol. The precipitate is subsequently extracted with acid acetone and alcohol; the active material is reprecipitated by the addition of water and is then ready for clinical use. Better yields are obtained, however, if the original extract is precipitated with  $(\text{NH}_4)_2\text{SO}_4$  at pH 6.0 and then absorbed to benzoic acid.

The hormone has never been isolated, but it is almost certainly a simple protien."

With regard to the nature of the parathyroid hormone McLean, F.C.

and Urist, M.R., (1961) refer to Rasmussen and Craig. McLean and Urist write:

" Rasmussen and Craig have isolated and characterized a single protien with a molecular weight of approximately 9,500, which is homogenous by countercurrent distribution, paper and column chromatography, and ultracentrifugation; contains no cystine; and possesses a single end-terminal amino acid alanine. The amino acid composition of the pure bovine hormone is known. The present evidence indicates that this hormone consists of a single polypeptide chain, which can be partially hydrolyzed without complete loss of biologic activity. The pure preparation has both calcium mobilizing and phosphaturic activity; there is now no support for the view that the parathyroid glands elaborate two hormones."

By bioassay one U. S. P. unit of parathormone is defined as 1/100th of the amount necessary to raise the serum calcium of normal dogs by 1 mg.% within 16 to 18 hours after subcutaneous injection. Williams (1955) compares the U. S. P. unit as equal to one fifth of the original Collip Unit which is the unit of measure still employed in Europe.

The only effective mode of administration for the drug is the parental route in as the hormone is apparently destroyed by the digestive enzymes.



Sensitization and resistance has been noted to the parathyroid hormone by several authors. Selye (1949), points out that short term treatment not only fails to bring about the expected bone resorption but produces an inversion of the effect by osteoblastic bone formation. He states that there is no direct evidence of any true antohormone formation against parathyroid hormone or of any absolute acquired insensitivity since the organism continues to respond although in an absolutely reversed manner. Weinmann and Schour (1945) agree with the explanation offered by Selye that the injection of parathyroid hormone of bovine origin might cause the formation of an antibody in the rat. They also note that Shelley, Asher and Jackson agree to the presence of this sensitizing effect of parathormone.

There is no information available to explain the fate of the parathyroid hormone in the body. Selye (1949) states that although the liver is a very important site for the detoxification of the steroid hormones it appears to have no effect on the metabolism of the parathyroid hormone.

Chang (1950) contributes to the effects of parathyroid grafts. He conducted the experiment of placing parathyroid grafts against the parietal bone of rats and mice to show definite local bone resorption. This effect was not produced by the transfer of tissues other than parathyroid tissue. Barnicot (1948) transplanted parathyroid tissue and parietal bone into the cranial cavity of mice to show definite bone resorption occurring contiguous to the parathyroid transplant whereas bone



deposition had been noted to be occurring on the other side of the implanted bone fragment.

Both Raiss and Hammack (1959) and Turner (1960) concur that the effect of parathormone is local mainly on the bone itself but may also have some effect on the kidney, intestines and other tissues.

Other investigators state the effect of parathormone is principally on the kidney by affecting the rate of phosphate excretion, namely Neufeld and Collip (1942). The work of Albright and Elsworth (1929) is substantiated by Goadby and Stacey (1934) all who claim that the principal effect of the hormone is phosphate excretion. Cohn, Cohn and Aub (1942) also state that the action of the parathyroid hormone is to control phosphorus metabolism through the control of the phosphate excretion through the kidney. They explain the hypercalcemia as being due to the slower rate of calcium excretion through kidney rather than claiming any effect of the parathyroid hormone with regard to calcium retention. Tweedy and Campbell (1944) using radiophosphorus to label phosphate in experimental rats showed that almost immediately after parathyroid hormone injection an increase in urinary phosphate is seen followed by somewhat delayed excretion of fecal phosphate. Phosphate take up by bone or promotion of phosphate retention in bone was not noted.

Selye (1942) assigns a secondary importance to the kidney. He states that the kidney is more important as a regulatory factor in the parathyroid hormone effect as nephrectomy greatly increased the effectiveness of parathyroid hormone. By itself the nephrectomy produces a slight osteitis



fibrosa cystica which can be ablated by a concomittant parathyroidectomy.

A most emphatic presentation is made by McLean, F. C., and Urist, M. R. (1961) who state with regard to the mode of parathyroid activity:

" These differences may now be regarded as resolved. The parathyroid hormone acts directly both on bone and on the kidneys; current effort in this area is directed toward elucidating the effects of the hormone on cells, and toward integration of the major effects into a single system responsible for what is recognized as the primary function of the parathyroids, i.e., homeostatic control of the blood calcium level. In view of the intimate association between the physiologic activities and functions of vitamin D and the parathyroid hormone, such an integrated system must incorporate both the vitamin and the hormone."

Selye in his textbook of Endocrinology (1949) lists a Calcium X (Greenwald and Gross) theory of parathyroid hormone activity. This theory is based on the assumption that parathyroid hormone enters into an actual chemical combination with calcium. There is formed a water soluble but non-ionized calcium compound, somewhat similar in its physico-chemical properties to calcium citrate. It is this hypothetical compound that was suggested as the interpretation for the dissolution of calcium from the skeleton to produce the hypercalcemia observed from the injection of parathyroid hormone.



The osteoblast theory of parathyroid hormone activity (Selye 1949) states that when the parathyroid hormone is given in large quantities, osteoblasts are transformed into osteoclasts which cause the destruction of bone with consequent discharge of skeletal minerals into the blood stream. Conversely, it is stated, if small quantities are given or if treatment is very prolonged, the osteoblasts are merely stimulated and bone is produced producing bone characteristically seen in marble bone disease or Albers Schonberg disease.

There is periodic mention in the literature of a possible hypophyseal hormone influencing the activity of the parathyroid glands. To this effect McLean and Urist (1961) state:

" the existence of a parathyrotropic hormone, elaborated by the anterior lobe of the hypophysis, is no longer supported by any evidence, and the idea of such a direct link between the pituitary and the parathyroids has disappeared from modern endocrinology."

Irradiated ergosterol has been noted to produce effects similar to parathyroid hormone. Schmidtman (1931) showed large doses to very young animals leads to bone resorption with spontaneous fractures. Small doses over long periods of time showed increased calcification in bones with the cortical bone being denser and thicker with a general increase in size. Selye (1932) comments to the effect that the histological picture of the denser and larger bones differs from that seen when parathyroid hormone is the inducing factor for this phenomenon.



Cameron (1949) stated that dihydrotachysterol, one of the products of ultraviolet irradiation of ergosterol, produces effects similar to parathyroid hormone. This drug has been shown clinically useful in replacement therapy to decrease parathyroid sensitivity.

Selye (1932) showed viosterol as a drug which also simulates the effects of parathormone. In its similarities to the parathyroid hormone, viosterol passes through the placental barrier, and the mammary glands, and also shortens the blood coagulation time.

The effect of nephrectomy on calcium and phosphorus has been investigated considerably. Grollman (1954) stated that nephrectomy stimulates the release of calcium from the bone to the extracellular fluid. Tweedy et. al, (1937 and 1939) and Neufeld and Collip (1942) show that parathyroid hormone produces no effect on the serum calcium level following nephrectomy or ligation of the ureters, however, cutting of the ligated ureters produced the normal parathyroid hypercalcemia. However, McLean and Bloom (1941) state as does Selye (1942) that in the rat complete nephrectomy does not prevent the action of the parathyroid hormone on bone, in producing osteoclastic absorption and thus mobilization of bone mineral.



## II. Thymidine, Feulgen Reaction and Tritiated Thymidine

Nucleic acids as supplied for the body are derived principally through the ingestion of nucleoproteins. Through the activity of proteases the nucleoproteins are divided into a protein and a nucleic acid. The protein component varies with the originating species as well as the tissue source. Upon hydrolysis these proteins may yield a considerable amount of the required basic amino acids.

The nucleic acids may be either ribose nucleic acid or the desoxyribose nucleic acids which were originally called yeast nucleic and thymus nucleic acids respectively. These two nucleic acids vary with respect to their pentose and pyrimidine components as may be seen on a table below as taken from Kleiner (1954):

### Hydrolytic Products of

Ribose Nucleic Acid		Desoxyribose Nucleic Acid
Phosphoric acid		Phosphoric acid
D-Ribose	Pentoses	D-2-Desoxyribose
Adenine	Purines	Adenine
Guanine		Guanine
Cytosine	Pyrimidines	Cytosine
Uracil		Thymine

On a cellular basis desoxyribose nucleic acid is located in the cell nucleus, more specifically in the chromatin of the cell nucleus. Ribose nucleic acid is present in the cell cytoplasm and in the plasmasome (nucleolus).







According to Kleiner (1954) the purine nucleotides are formed with the pentose joined to the nitrogen at position 9 of the purine and the phosphate is usually on carbon 3 of ribose, or carbon 5 of deoxyribose. In the pyrimidine nucleotides the sugar is joined to nitrogen number 1, and, again, the phosphate is probably on the number 3 carbon, on the pentose.

The names of the nucleotides or nucleosides are derived from the particular purine or pyrimidine present; for example:

Adenine nucleoside: Adenosine

Adenine nucleotide: Adenylic acid

Guanine nucleoside: Guanosine

Guanine nucleotide: Guanylic acid

Thymine nucleoside: Thymidine

Thymine nucleotide: Thymidylic acid

As early as 1924 Feulgen and Rosenbeck devised a technique with a high specificity for the detection of deoxyribose nucleic acid in tissues. Confirmation of the validity and specificity of this test was made by Stowell, (1946). To further affirm this specificity for deoxyribose nucleic acid, Wyckoff and Ebeling (1932) using ultraviolet studies were able to demonstrate the co-incidental location of thymonucleic (deoxyribose nucleic) acid with that of the Feulgen technique.



Tissue reacting positively to the Feulgen reaction is made Feulgen negative after such tissues are treated with purified pancreatic desoxyribonuclease.

Basically, the Feulgen reaction is the application of the Schiff reaction for aldehydes on a histological scale. Of the solutions used for this reaction, leuco-fuchsin is the most apt to create difficulties. Consequently a volume once proved satisfactory for the reaction should be reserved for future use. When properly prepared the solution should be of a light yellow color. The reagent is made as follows;

" Dissolve one gram basic fuchsin in 200 cc. boiling water;  
Cool to 50 degrees centigrade; filter and add 10 cc. M  
hydrochloric acid and two grams potassium metabisulfite  
 $K_2S_2O_5$ ; allow to bleach for 24 hours; then add 0.5 grams  
neutral activated charcoal; shake for one minute and filter rapidly  
through coarse filter paper."

The staining technique for the Feulgen reaction is as follows;

1. Bring paraffin sections through xylene and alcohol to water
2. Rinse one minute in N. hydrochloric acid at 60 degrees centigrade for twenty minutes
3. Rinse one minute in cold N hydrochloric acid
4. Immerse 2 hours in leuco-fuchsin solution



5. Drain and give 3 successive 10 minute treatments in closed Coplin jars in acid sulfite solution. This compound is composed of 6 cc. 10% potassium metabisulfite, 6 cc. N hydrochloric acid, and 120 cc. distilled water. Place 44 cc. of this in each jar.
6. Rinse in distilled water. Counterstain in 0.5% alcoholic fast green
7. Dehydrate in alcohol
8. Clear in xylene and mount

Thymidine was tritiated at Brookhaven by Hughes in 1955 and independently in Belgium. With respect to its use as a tracer for desocytidine nucleic acid Cronkite et. al. (1959) state;

" Although thymidine is apparently not a normal precursor of DNA, it can enter the synthetic chain and label DNA at the time of doubling prior to mitosis. Thus, if the label is sufficiently intense and permanent one can by autoradiography follow cells from the time of DNA synthesis to ultimate death."

Tritiated thymidine as supplied by Schwartz Laboratories contains 0.36 C/mM. The labeling was created on the pyrimidine ring presumably by the exchange with a hydrogen bound to carbon. Recrystallization with carrier thymidine indicates the compound to be free of labeled impurities. The compound is diluted with isotonic sodium chloride to a concentration convenient for injection.

Cronkite et. al., (1959), realizing the possibility of criticism with their conclusions related to the use of tritiated thymidine, established a set of premises which also may be open to question. These are as follows;

1. The tritium label on thymidine does not exchange
2. Thymidine base does not exchange after incorporation into DNA



3. DNA turnover is solely the result of mitosis and cell death.
4. Re-utilization of tritium-labeled materials in DNA synthesis is significant.
5. Re-utilization of large chunks of DNA is unlikely in most cell renewal systems.
6. DNA synthesis in normal cells destines a cell to divide once again.
7. Tritium labeled thymidine initially is uniformly distributed throughout the body and is either promptly incorporated into DNA or degraded.
8. The effective availability time of tritium-labeled thymidine for DNA synthesis is short and a small fraction of the time for synthesis of DNA.
9. There is no significant radiation injury of the cells; thus one can by autoradiography follow cells from time of DNA synthesis to ultimate death without perturbation of the normal sequence.
10. In vitro labeling determined the proliferative potential of the normal cells.
11. In vivo labeling after a single intravenous injection makes possible the study of the kinetics of proliferation.

Leblond et. al., (1959) recommend the use of tritiated thymidine as an investigatory tool and rejects the use of  $S^{32}$  and  $C^{14}$  on the basis of poor resolution. Also the doses of  $S^{32}$  and  $C^{14}$  required to produce the autoradiographic effect may be high enough to produce radiation damage. They state that with tritiated thymidine the doses used and the radiation thereby produced causes no visible radiation damage even after months in the body. Because of the high specificity for desoxyribose nucleic acid the sections need not be handled for chemical extraction thereby avoiding the possibility of damage to the tissue section. Unlike  $p^{32}$  which has a



high energy beta particle (1.7 MeV maximum) the photographic resolution obtainable from the low energy beta particle of tritiated thymidine (0.018 MeV maximum) is excellent.

Lajtha and Oliver (1959) state a problem of material stickiness peculiar to  $p^{32}$  which aggravates contamination from bench tops and glassware. They also agree with Leblond et. al. who highly recommend the use of tritiated thymidine as an investigatory tool.

Lajtha and Oliver further state that  $p^{32}$  has a half life of only fourteen days consequently a problem of potency curing storage arises. To the opposite end,  $C^{14}$  has a half life of 5,600 years. More ideally placed between the above two is thymidine with a half life of 12.26 years with a disintegration rate of 0.016% per day.

The suggested doses for injection as given by Lajtha and Oliver are of the order of 0.1-0.5 mc to a 5 Kg. dog and 19 micro curies to a 20 gram mouse.

Cronkite et. al., (1959), suggest the possibility of an active transport of tritiated thymidine directly into the cell synthesizing desoxyribose nucleic acid. They claim no evidence in support of or against this statement. The availability time, that is, the time tritiated thymidine is available and capable of being accepted into desoxyribose nucleic acid, is not believed to be in excess of a sixty minute period. During that time tritiated thymidine must be incorporated into the cell or be degraded.



Experimental doses of tritiated thymidine up to 2.0 micro curies per gram have not shown Cronkite et. al. any histological evidence of radiation damage. Even so they recommend that such an agent should be utilized mainly for experimental use on an animal level and if used on humans then only for those individuals who stand no chance of procreation.

### III. The Osteoclast

Previous to the early nineteenth century not a great deal of information had been available regarding bone catabolism. Although some mention of bone resorption is found recorded in the experiments and observations of Howship (1817), the literature was sparse. To acknowledge his efforts, the concavities seen on bony surfaces are to this day referred to as "Howship Lacunae".

Arey (1916-17) in acknowledgement of early researches states that Bidder (1843) quite probably shares distinction with Robin (1849) as some of the first to observe the bone resorbing cells. Arey states as follows:

" Since the discovery of the polykaryocyte by Robin in 1849 the polykaryocytes of developing bone have been regarded as the agents of bone resorption. For this reason these multinucleate cells have been termed osteoclasts."

Tomes and deMorgan (1852) reported some unusual findings during a surgical procedure involving hard and soft tissues where they state it seemed that the hard tissues were giving in to the soft. They seemed



confused by this as is evident from their paper, "It is difficult to resist the belief that the cells which lie in contact with wasting bone and dentine take up these tissues." In their paper they clearly indicate a cellular theory of bone absorption although they do not attempt to identify or classify the responsible cells.

The term "myeloplaques" owes its presence in the literature to Robin who in 1864 created this term to describe the morphological peculiarities of certain multinucleated giant cells. He noted the pleomorphism and variety of nuclear number but did not assign any specific function to the cells.

In his paper on the significance of the giant cell in bone Bredichin (1867) makes a very definite point that these cells are transitional stages in the transfer of bone matrix into bone marrow and granulation tissue, with a coincident multiplication of the nuclei of the component bone cells. Thus then it is to infer that the origin of the osteoclast would be attributable to the fusion of bone cells.

Further reference is made to Robin's myeloplaques by Rollett (1870). In a manual describing the histology of endochondral ossification Rollett remarks on the occurrence of "large masses of protoplasm with many nuclei".

Bassini (1872) and Kolliker (1873) claim that the earliest osteoclasts and for those found during the resorption of milk teeth owe their existence to connective tissue cells. Later, however, the osteoclasts were derived from repeated nuclear division of single osteoblasts.



Rindfleisch (1872) in a textbook of histological pathology states that in his observations of giant cell sarcomata, the polykaryocytes are bone cells which have gone free and gone into a peculiar hypertrophic state.

Wegner in 1872 stated an osteoclastic origin from marrow connective tissue. Then in 1883 Wegner with Pommer also state that the capillary endothelium also is to be considered as a site for osteoclast formation.

The suggestion that the myeloplakes described by Robin in 1864 may be the cells responsible for the absorption of bone was put forth first by Kolliker in 1873. He proposed the term "Ostoklast" and predicted that this cell would also be associated with pathological bone absorption. Kolliker states the nuclear count increases by nuclear division

Morrison (1873) working under the inspiration of Kolliker reported intermediates in the number of nuclei between the osteoblast and osteoclast thereby indicating a transition of osteoblast into osteoclast, as also did Kolliker. Morrison also indicated the possibility of osteoclasts arising from capillary endothelium as did Wegner and Pommer above.

Writing again in 1873 Rindfleisch in discussing pathological bone resorption notes the presence of Howship lacunae which may contain multinucleated giant cells. He does not however attribute bone absorption to such cells but rather assumes a dissolution of the bone matrix by the tissue fluid itself.

Maas (1877), Brodowski (1875), Wegner and Pommer (1883), Kolliker (1873) and Morrison (1873) all agree that in some part the origin of the



osteoclast is attributable to the capillary endothelium.

Muriser (1875) and Zeigler (1878) are in agreement with Bredichin (1867), all who imply the osteoclast originating directly from a fusion of liberated bone cells.

Cellular fusion was stated by Klein and Smith (1880) as the method of osteoclast formation. They claimed that sessile connective tissue cells, such as osteoblasts, fibroblasts and other mesenchymal cells including reticular cells were responsible for the creation of the osteoclast.

Pommer (1881) once again is in agreement with statements made by Kolliker, that is, osteoclasts represent single osteoblasts that had undergone repeated nuclear division. For those osteoclasts present during the resorption of milk teeth an origin from connective tissue cells was assumed.

Quite different from the previous researchers are the views of Kaczander (1882) who claims the osteoclast arises out of the coalescence of liberated cartilage cells. To help explain the greatly nucleated cell Kaczander further states that the chondrocyte may be seen as multinucleated even while in its capsule.

Adding his name to Kolliker (1873), Morrison (1873), Wegner and Pommer (1873), Brodowski (1875) and Maas (1877), Schaffer (1888) likewise agrees that the capillary endothelium is a contributor to the formation of the osteoclast.

Throughout the years of reporting, the capillary endothelium is



mentioned as a source of the cell contributing to the osteoclast. Again in 1906 Bidder states the capillary endothelium as the source of the precursor cell for the osteoclast. Likewise the lymphoid marrow cell is named from time to time as the cell responsible for the production of the osteoclast. Ranvier (1889), Renault (1893) and Duval (1897) all make mention of the lymphoid marrow cells in their papers.

Howell (1890) concurs with Morrison (1873) and Kolliker (1873) in his study concerning the occurrence and function of giant cells of the marrow. He therefore also states that the osteoclasts arise by a fusion or coalescence of the bone forming osteoblasts.

Jackson (1904) attributes the origin of the osteoclast to reticular cells of the bone marrow. He claims enlarged reticular cells initially containing two to three nuclei within a basophilic cytoplasm convert to multinucleated cells with an oxyphilic cytoplasm. In the osteoclast itself the increase in number of nuclei is attributed to nuclear mitosis. In essence then Jackson suggests a unicellular fusion theory of osteoclast formation.

A like viewpoint was upheld by Danchakoff (1909) who was also an advocate of the unicellular fusion theory for osteoclast formation.

Von Recklinghausen (1910) proposed a "thrypsis" concept to explain the presence of these giant cells. Here the osteoclast is regarded as a syncytium formed by a condensation and fusion of bone cells withdrawn from the bone substance. The bone owes its presence to the cell bodies



The osteoclast is present as a "thrypsis giant cell" and is there in spite of the bone resorbing phenomena. Haggquist (1934) revived the thrypsis concept as was proposed by Van Recklinghausen.

Maximow (1910) was a supporter of the unicellular fusion theory of osteoclast formation. Like Danchakoff (1909) and Jackson (1904), Maximow traced osteoclast development of early bone to enlarged reticular cells of the marrow. Maximow further believed large osteoclasts arose at the expense of smaller ones, and that he has never seen mitotic or amitotic nuclear division to explain the multi-nuclearity of the osteoclast.

Mallory (1911), Lacoste (1923) and Haythorn (1928) were of the opinion that the osteoclast held its origin not to the sessile connective tissue cells as fibroblast, reticular cells of other mesenchyme cells but rather to a wandering type cell. Mallory claimed the endothelial leukocyte, that is, the histiocyte as the precursor cell of the osteoclast. Lacoste showed transitions between small uni-nucleated wandering cells and osteoclasts. Mallory also suggests that the osteoclasts may have formed some distance from bone and the newly formed osteoclast wandered into the direction of the bone. Haythorn regarded the fusion of the monocytes as the mode of origin for the osteoclast.

Todd (1913), Geddes (1913), von Recklinghausen (1910) and Haggquist (1934) basically all advocate the osteoclast formation by the fusion of liberated bone cells or cartilage cells. Both von Recklinghausen and Haggquist suggest the thrypsis concept as was given above. Todd considered the osteoclast as merely masses of pre-osseous tissue which



became separated from the fully ossified bone during histological preparation. Geddes thought that two or more cartilage cells which became freed from matrix during endochondral ossification fused together, and, after incorporation with any number of osteoblasts became a hybrid, syncytial mass known as the osteoclast. Retterer (1917), considered osteoclasts to be islets of degenerated bone tissue formed from the nuclei of the osteocytes and of bone substance during the process of dissolution.

Lewis (1913) regards the osteoclasts as degenerating cells resulting from conditions which lead to the dissolution of bone and rejects the osteoclastic origin by cellular fusion.

Arey (1916-17) and (1919) claims osteoclasts arise from a cellular syncytium which owes its origin to depleted osteoblasts. As resorption continues the osteoblasts become absorbed into the osteoclast. Arey demonstrates the transition from basophylic osteoblasts into oxyphylic osteoclasts. Nowhere he states has he ever observed any amitotic or mitotic division.

Using *Rana pipiens* as the experimental subjects, Jordan (1925) proposed nuclear mitosis in the osteoblast to be responsible for the production of the osteoclast.

Weidenreich (1930) concurs with Kolliker attributing osteoclast formation to osteoblasts. This Weidenreich states, is accomplished by amitotic division.



A study of the mitochondria and Golgi apparatus was undertaken by Chang Hui Ch'uan (1931) and Smith (1933). Both authors indicate extremely like mitochondria and Golgi apparatuses in the osteoblast and osteoclast thus tending to indicate that the osteoclasts arise from the osteoblasts.

Ham (1932) considers that osteoclasts arise not of necessity from recognizable osteoblasts, but rather that each osteoclast and osteoblast represents the differentiation of osteogenic cells along different pathways.

In reporting of hyperparathyroidism, Jaffe (1933) claimed active bone resorption even in the absence of osteoclasts. The cell responsible for the active phagocytosis was the polymorphonuclear leukocyte. He does state that osteoclasts may be seen forming from the marrow connective tissue adjacent to the decalcified bone margins and that these osteoclasts were responsible for the phagocytosis of the organic matrix.

McLean and Bloom (1937) using parathyroid hormone to induce osteoclasia in rats show osteoblasts and reticular cells transforming into osteoclasts. They further indicated that osteoblasts were seen to change to fibroblasts and phagocytes. Fibroblasts were also seen to change to osteoblasts.

Subcutaneous transplantation of bone grafts led Barnicott (1941) to state that reticular cells have the ability to form osteoclasts. In that same year Bloom, Bloom and McLean suggest the marrow reticular cells as an indifferent source of the osteoclast. They also assert that the



osteoblast may give rise to the osteoclast.

Hancox (1946) was able to produce in vitro large multinucleated cells which wandered out of the embryonic bone explant. Hancox considers these cells as osteoclasts produced by the fusion of monocytes that originate in the outer zone of the graft. This outer zone consists of wandering cells which were microphage and macrophage. The former resemble blood monocytes by staining property and the latter may have their origin from the more centrally placed spindle cells of the graft.

The fine filamentous attachment that appeared to be joining osteoclasts led Barnicott (1947) to believe that separation was responsible for osteoclast production. That is to say osteoclasts form by fission or splitting to form smaller osteoclasts.

Maximow and Bloom (1948) in a textbook of histology state that the stromal cells of the marrow contribute to the origin of the osteoclast. At times a number of osteoblasts fuse to form the larger osteoclast whose size may also be added to by the incorporation of liberated osteocytes.

Hancox (1949) in a review of the literature on the origin of osteoclasts indicates that the balance of research tend to favor a unicellular origin for the osteoclast and more specifically by the fusion of unicucleated cells.

Full cellular conversion from one cell type to another is suggested by Heller, McLean and Bloom (1950). They state that osteoblasts, osteoclasts and osteocytes are only different and mutually convertible stages in the life of one cell type. The free conversion among reticular cells,



spindle cells, osteoblast, osteoclasts and osteocytes is also suggested.

Weinman (1955) states it is quite probable that osteoclasts, like osteoblasts are derived from undifferentiated elements of loose connective tissue. Weinmann makes no attempt to explain the high nuclear count. He denounces the capillary endothelium as a source of the osteoclast on the basis that the proliferating capillary buds, as are seen in an area undergoing resorption may easily be mistaken for osteoclasts.

Weinmann and Sicher (1955) state that during too rapid orthodontic movement osteoclasts arise from the differentiation of loose connective tissue of the adjacent marrow spaces or even from the periosteal surface of the necrotic bone. They condemn the theory that osteocytes may differentiate into osteoblasts, fibroblasts or revert to fibroblasts.

In that same year, (1955), McLean and Urist claim that fused osteoblasts or osteocytes contribute to the total osteoclast. A portion of the osteoclasts are believed to arise from the stromal cells of the marrow.

Cells relatively undifferentiated that have the potential to differentiate either into macrophages or fibroblasts are believed by Ham and Lieson (1957) to be the cells capable of producing the osteoclast. Thus the reticular cell of the marrow is also considered as a precursor cell for the osteoclast.

In pigeons where bone transformations are very rapid, McLean and Urist (1961) show a free conversion system from reticular cell to



osteoblast and then osteocyte in the preovulatory period. After ovulation of the first egg, osteoblasts and liberated osteocytes become osteoclasts. The osteoclasts may then during the second period of bone formation turn into osteoblasts. In the post-ovulation period osteoblasts, and osteoclasts again become reticular cells. All this is in line with the statements of Heller, McLean and Bloom (1950) where a full cellular conversion system is suggested.



## Chapter III

### Materials and Methods

Seven adult male Sprague Dawley rats weighing approximately 100 grams were utilized. The diet consisted of Wayne Lab Blox with an adequate amount of drinking water. The room temperature was maintained at approximately 75 degrees Fahrenheit.

All except one animal utilized as control were injected intra peritoneally at zero hour with 100 units of parathyroid hormone (1 cc. as supplied by Lilly). At the third hour again the same dose of parathormone was injected and followed immediately by .2 cc. (200 micro curies) of tritiated thymidine (0.36 Curies per m Mole). At the sixth hour and ninth hour again 100 units of parathormone were injected intra peritoneally into the remaining animals.

The time chosen for the injection of the tritiated thymidine was the third hour. In as no definite time is established for the injection of tritiated thymidine it was intended to first induce a proliferation of the young loose connective tissue cells to make them available to accept the tritiated thymidine.

An ether chamber was used for the sacrifices at 6, 12, 18, 24, 48 and 72 hours. From each rat the mandible and proximal end of one tibia



was removed using a scalpel and scissor for the sectioning of the tibia.

The specimens were fixed in cold 10% neutral formalin for twenty four hours. Decalcification of the specimens was accomplished in formic acid-sodium citrate (Custer's) solution. Embedment was in paraffin and sections were made at three microns. Slides were stained with hematoxylin and eosin and for the Feulgen reaction using light green as a counterstain.

Autoradiography was accomplished by the strip film technique which is slightly modified from that given by Fitzgerald (1953):

1. Under dark room conditions, a Wratten #1, Red Safelite 10 watt bulb is used. A humidity of 70% and below is recommended.
2. Kodak, AR-10 fine grain emulsion is placed on a cutting jig, and cut into twelve squares using a scalpel.
3. Using a scalpetl, a corner of the auware is picked up, stripped from the glass plate, and floated, emulsion side down, in a glass dish containing distilled water four inches deep.
4. The histologic slide (section side up) is inserted into the water. The slide is brought up under the floating emulsion and engaged.



5. The slide is then air dried for 10 minutes.

6. Ten slides are placed in a black light proof exposure box, section sides up. Lithium chloride is placed in the box for maintaining a low humidity, and black masking tape is used to seal the box.

7. The slides are exposed in this box for 30 days at low humidity and temperature. During the exposure time the box must be maintained in a position which keeps the sections upright.

#### Developing:

1. The slides are placed in a staining rack and developed for 5 minutes at 60 degrees F. (18 degrees C.) in Kodak D198 developer.

2. The slide is rinsed in distilled water for 30 seconds.

3. Place in acid fixer for 10 minutes.

4. Wash in running tap water for 30 minutes.

5. Cover the slides and staining dish with Kleenex (to prevent dust from settling on slides) and allow to dry in a stream of air.

6. When the slides are dry, they are dipped in water for



30 seconds and the excess emulsion is trimmed from the slide.

7. The slides are then dipped in 95% alcohol, xylene I, and xylene II.

8. Cover slips are finally mounted with Canada Balsam.



## Chapter IV

### Findings

Definite indications of cellular radioactivity were observed in the six hour specimens. In the molar areas radioactively labeled cells are found adjacent to the cementum, in the intermediate periodontal plexus, within the periodontal space adjacent to the supportive alveolar bone and in the endosteum in the bone marrow itself. Radioactively labeled cells are quite frequently seen in the near proximity of blood capillaries. Few osteoclasts are found in Howship lacunae along the alveolar bone in the jaw sections. Those osteoclasts that are seen are located mainly at the distal root surfaces of the molar teeth. Osteoclasts found in Howship lacunae in the calcified cartilage of the tibia do not show any radioactive labeling of their nuclei.

At twelve hours there was an increase in the number of osteoclasts in the Howship lacunae on the alveolar bone surface. An increase in the number of young connective tissue cells identified as indifferent cells is noted. Radioactively labeled cells are again seen adjacent to the cementum, in the intermediate periodontal plexus, within the periodontal space adjacent to the supportive alveolar bone and in the



endosteum in the bone marrow. Also such cells show an increase in number of those containing radioactively labeled nuclei.

At eighteen hours many osteoclasts are found in Howship lacunae some of which now show radioactively labeled nuclei. There are young loose connective tissue cells, some showing labeled nuclei occupying the eroded bone surfaces previously occupied by osteoclasts. The labeling of the young loose connective tissue cells remains the same as that seen adjacent to the cementum, in the intermediate periodontal plexus, on the surface of the supportive alveolar bone and in the endosteum of the bone marrow.

Considerable bone resorption has occurred on the alveolar bone surface at twenty-four hours. Only few of the nuclei in the osteoclasts are radioactively labeled. The young loose connective tissue cells including those with labeled nuclei are seen also to differentiate into fibroblasts in the periodontal ligament. Delicate but distinct collagenous fibers are found parallel to the long axis of such cells. Similarly, young loose connective tissue cells occupy the eroded surfaces of the alveolar bone previously occupied by osteoclasts. Young loose connective tissue cells appear to present a diminishing amount of radioactive labeling.

At forty-eight hours the number of osteoclasts have diminished. However, the scalloped surfaces on the alveolar bone resulting from previous



osteoclastic activity now are filled with young loose connective tissue cells. The number of young loose connective tissue cells has increased in number while the radioactive labeling is decreased.

Evidence of recovery is observable with the seventy-two hour specimen. Osteoclasts previously observable are now difficult to find. The alveolar bone is covered with young connective tissue cells some of which may have differentiated into osteoblasts. Bone apposition is also evidenced by the presence of reversal lines and the formation of osteoid tissue.





## Chapter V

### Discussion

It would be anticipated that the injection of 400 units of parathormone over a nine hour interval into rats weighing approximately 100 grams would produce a considerable disturbance of the hard tissues on a histologic level. Surprisingly enough such was not seen to occur. The few osteoclasts noted were seen mainly at the distal root surfaces of the rat mandibular molars.

During physiological tooth movement, the compression side of the alveolus demonstrates a loss of the bone tissue and an accumulation of osteoclasts. Normal movement of the posterior teeth in a rat is in a distal direction. This then explains the appearance of the osteoclasts at the distal root surfaces of the rat molars.

In view of the small number of osteoclasts produced one might suspect the parathormone of bovine origin may lack potency due to a sensitizing effect upon the recipient animal. Weinman and Schour (1945), Selye (1949) and others make note of this lack of response and suggest the presence of the sensitizing effect of the hormone.

Oral epithelial tissue when observed after radioactive labeling with tritiated thymidine shows a much different cellular response. Schoenheider



(1960), indicates an rapid epithelial cell turnover. In this study the observation of the loose connective tissue revealed few mitotic figures and radioactively labeled cells as compared to that seen by Schoenheider in the epithelium.

Examination of the six-hour specimen reveals evidence of radioactive labeling of the connective tissue. It is probable that the labeled cells near the cementum are destined to become cementoblasts, however no specificity for any definite cell type can be predicted. The same would apply to the indifferent cells observed in the intermediate periodontal plexus, those adjacent to the alveolar bone and tissue in close proximity to the endosteum.

Apparently these non-specific, indifferent cells of the loose connective tissue possess the ability to differentiate into the particular cell needs of the connective tissue. Their increase in number is indicated by an increase in number of radioactively labeled cells, as seen in autoradiograms, thereby making themselves available in quantity for differentiation into more specific cell types.

At twelve hours it was important to observe that there was an increase in the number of young connective tissue cells identified as indifferent cells. Moreover, increased numbers of these radioactively labeled cells are seen in the same areas as reported in the six hour specimen. In as much of the tritiated thymidine is available for a period of 60 minutes



for incorporation into the DNA of the cell, (Hughes, Bond, Beecher, Cronkite, Painter, Quastier and Sherman 1958), the increase in the number of labeled cells is due to mitotic division. As the number of daughter cells is increased, the radioactive thymidine contained in each mother cell is now equally divided among the two daughter cells.

Finally, radioactively labeled osteoclasts are observed at eighteen hours. Up to this point all observable osteoclasts showed no labeling. This includes those osteoclasts found in the tibia in the six-hour section. As the labeling now present in the osteoclast could not have been obtained from free tritiated thymidine, the only other source would be that contained within the chromatin of previously labeled cells.

The histogenesis of the osteoclast has been attributed to many sources. Wegner (1872), Pommer (1883), Morrison (1887), Brodowski (1875), Kelliker (1873) and Schaffer (1888) felt that the capillary endothelium was responsible if not in whole at least then in part. As was stated by Maximow and Bloom and as it was seen in this study, labeled indifferent cells were also seen adjacent to capillary walls. It is likely then that the contributor to the osteoclast in this case is not the endothelial wall itself but rather the indifferent (undifferentiated cell of Maximow) cell found in close proximity to the capillary wall.

Cellular fusion as the source of the osteoclast was a popular opinion as was evidenced by the following list of contributors: Bredichin (1867),



Morrison (1873), Kolliker (1873), Bassini (1872), Muriess (1875), Zeigler (1878), Pommer (1881), Howell (1890), Jordan (1925), Weidenreich (1930), Chang Hui Ch'uan (1931), Smith (1933), Bloom, Bloom and McLean (1941), Maximow and Bloom (1948), and McLean and Urist (1955). If a cell is liberated and then fused to form the osteoclast it becomes interesting to try to consider what mechanism or what cell was responsible for the initial liberation of these cells to make them available for fusion.

Fewer in numbers were those who attributed cellular degeneration for the creation of the osteoclast. These were Retterer (1917), Rindfleisch (1872 and Lewis (1913). Von Recklinghausen (1910) and Haggquist (1934) felt that artifacts of liberated bone cells were the causes for the production of osteoclasts. Mallory (1911), Lacoste (1923), and Haythorn (1928) believed a wandering cell or monocyte formed the basis for the osteoclast.

The most common and popular belief for the source of the osteoclast is the loose connective tissue cell. Even the more contemporary writers share the opinion as is indicated by the more recent investigations as conducted by Bloom, Bloom and McLean (1941), Maximow and Bloom (1948), Weinman (1955), Weinman and Sicher (1955), McLean and Urist (1955) and Ham and Leison (1957).

The indifferent cells, (undifferentiated cells of Maximow), were observed in the six hour specimen as being adjacent to the capillary



endothelium. Maximow and Bloom (1953) refer to undifferentiated cells that are believed to possess the potencies of mesenchymal cells, even in the adult animal. They state these cells are often smaller than the fibroblast, but have the same general appearance: in the loose connective tissue they are found along the blood vessel walls particularly along the capillaries. It is evident from this that the cells seen in the six hour specimen were indifferent cells as described by Maximow. Further and most interesting is the fact that these same cells show positive evidence of radioactive labeling.

The osteoclasts which demonstrate radioactively labeled nuclei do not have all the nuclei labeled. Neither do all of the loose connective tissue cells demonstrate a positive label. It is apparent from this that the contributors to the osteoclasts arise from a pool of cells some which have accepted the radioactive label and from others which had not. This supports the reports of Bloom, McLean, Maximow, Wienman, Sicher, Ham and Leison, which consider the fusion of cells as the method of osteoclast formation.



## Chapter VI

### Summary and Conclusions

This investigation was conducted in order to determine the origin of the cell or tissue responsible for the elaboration of the osteoclast. It was also desired to learn whether autoradiograms using tritiated thymidine, could be employed in such a study.

Six adult Sprague Dawley rats weighing approximately 100 grams and one other such rat as a control were utilized. Of the six animals that received parathormone the subject sacrificed at six hours received a total of 400 units of parathormone intraperitoneally. The intraperitoneal injections of parathormone were given at 0, 3, 6, and 9 hours. Immediately following the second injection of parathormone all six subjects received 200 micro curies (0.2 cc) of tritiated thymidine (0.36 curies per  $\mu$  Mole) also given intraperitoneally.

Sacrifices were conducted in an ether chamber at 6, 12, 18, 24, 48 and 72 hours. From each animal the mandible and proximal end of one tibia were removed for histologic study. The specimens were fixed in 10% neutral formalin for 24 hours, decalcified in Custer's solution, embedded in paraffin and cut at thicknesses of three microns. Slides were stained with hematoxylin and eosin and for the Feulgen reaction light green was used as the



counter stain. Autoradiograms were then made over the tissue sections.

Examination of the slides revealed that radioactive labeling of cells occurred basically in four areas, 1. adjacent to the cementum of the rat molars, 2. within the intermediate periodontal plexus, 3. within the periodontal space adjacent to the supportive alveolar bone and 4. in the endosteum of the bone marrow.

Radioactive labeling of indifferent cells is seen as early as the six hour specimen. The twelve hour specimen reveals an increase in the number of labeled indifferent cell. Osteoclasts are observed in the Howship lacunae in greater numbers than in the six hour specimen however no radioactively labeled osteoclasts were noted. At eighteen hours the first radioactively labeled osteoclasts are seen. The indifferent cells (undifferentiated cells of Maximow) maintain their labeling and location as described in the six and twelve hour sections.

The 24 hour specimen revealed considerable bone resorption of the alveolar bone surfaces with young loose connective tissue cells occupying the areas previously occupied by osteoclasts. The osteoclasts again show a labeling of some of their nuclei as was also seen in the 18 hour section. In the intermediate periodontal plexus, young loose connective tissue cells are seen to differentiate into fibroblasts.

Forty-eight hour sections revealed fewer osteoclasts with young connective tissue cells filling in the scalloped surfaces of the alveolar



bone. Greater numbers of the loose connective tissue cells are observed but the radioactive labeling is decreased.

Seventy-two hour slides indicated recovery from the effect of the parathormone. New bone formation is indicated by the reversal lines in the bone and the presence of increasing numbers of osteoblasts.

It was concluded from this study that the origin of the osteoclast is due to the indifferent cell or the undifferentiated cell of Maximow. Further to explain the statement that cellular fusion is the method by which the osteoclast is formed, it has been seen that not all nuclei of the radioactively labeled osteoclast were labeled.



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Figure 1

Twelve hour microautophotograph, 100X, showing labeled cells adjacent to alveolar bone. Note also the presence of a labeled mitotic figure at the middle of the field.



**Figure 2**

**Oil emersion microautophotograph taken from midfield of Figure 1 showing labeling of cell adjacent to bone and a labeled mitotic figure.**



Figure 4

Oil emersion microautophotograph taken from midfield of Figure 3 showing prominent labeling of osteoclast.



**Figure 5**

**Twelve hour, 400X, microautophotograph showing a labeled cell adjacent to the cementum and another labeled cell adjacent to the capillary endothelium.**



Figure 6

Twelve hour, 400X, micrautophotograph showing dentin, cementum and peridental space with labeled cells adjacent to cementum.



**Figure 7**

Twelve hour, 400X, microautophotograph of an alveolar inter-radicular area of bone showing a labeled cell adjacent to the cementum, and labeled cells adjacent to capillary endothelium.



Figure 8

Eighteen hour, 400X, microautophotograph of alveolar bone marrow spaces.

Note labeled cells and labeled mitotic figure at right.



# APPROVAL SHEET

The thesis submitted by Dr. John J. Magon has been read and approved by three members of the Departments of Anatomy and Oral Biology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

May 22, 1963  
Date

Esther D. Teto  
Signature of Adviser