A Radiographic Appraisal of the Effects of X-Radiation on the Mandibular Cartilage and Femoral Epiphyseal Cartilage of the Rat

Julio Aldo Battistoni
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A RADIOGRAPHIC APPRAISAL OF THE EFFECTS OF X-RADIATION ON THE
MANDIBULAR CARTILAGE AND FEMORAL EPiphyseal
CARTILAGE OF THE RAT

by

Dr. Julio A. Battistoni

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

1962

LOYOLA UNIVERSITY MEDICAL CENTER
LIFE

Julio Aldo Battistoni, was born in Chicago, Illinois on December 11, 1925. He graduated from Harrison High School in Chicago, Illinois, and from Bradley University, Peoria, Illinois, with a Bachelor of Science degree in June, 1949.

After a period in private industry, he began his dental education at Loyola University in September, 1956. He received the Doctor of Dental Surgery degree in June, 1960.

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ACKNOWLEDGEMENT

My sincere appreciation is extended to all those who in anyway aided in this investigation, particularly to the following:

I wish to extend my gratitude, first and foremost to Joseph R. Jarabak, D.D.S., Ph.D., Professor of Orthodontics, Loyola University, who as my advisor, offered his invaluable guidance and supervision during the course of this investigation.

To Gustav Rapp, Ph.D., Professor of Biochemistry and Physiology and Patrick Toto, D.D.S., M.S., Professor of Oral Diagnosis and Oral Pathology, and Chairman of Research, for their understanding, patience and constructive criticisms.

To Dr. Harry Wang, Professor of Anatomy, Loyola University, and Dr. Toto my many thanks for serving as members of my advisory board.

To Dr. Harry Sicher, M.D., D.Sc., Professor of Anatomy and Histology, for his continued inspiration to me and to all students.

To Joseph Gowgiel, D.D.S., Ph.D., Department of Anatomy, University of Chicago, and his laboratory staff, who gave abundantly of their time and facilities.

To my wife, Gracia, for her proficient assistance in the construction of this thesis, and for her encouragement, interest, and personal sacrifices without which this graduate work never could have been undertaken.
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INTRODUCTION

Since the discovery of x-rays by Roentgen, scientific investigators have been curious to learn how living organisms are affected by radiation. Experimentation has greatly resolved the why of the question, but we are now concerned with a more comprehensive query. What are the precise effects of radiation on living organisms?

In this study, an attempt has been made to determine the effects of x-radiation on the mandibular cartilage and the femoral, epiphyseal cartilage of a group of young albino rats. Roentgenographic and morphological methods were used in collecting this data.

The literature abounds with material related to the effects of radiation on living animals or organisms, but studies of effects on the mandibular cartilage of the rat have been few. A careful perusal of this literature has provided certain information which adds some insight into the particular problem being studied.

While the conclusions drawn here may not necessarily resolve the question of effect, it is hoped that these results may contribute to an eventual scientific understanding of the many aspects of this problem.
The discovery of x-rays was largely accidental. Wilhelm Konrad Roentgen, in 1895, while conducting experiments with electricity, discovered rays which were unknown, and termed these x-rays. Other pioneers in radiation studies who dealt with these unknown rays - rays which were destined to become extremely important in diagnosis and therapy - were Edison, Dally, Gillmore and Curie.

Dunlap, in 1957, categorized radiation into several forms of energy. One such form is known as electromagnetic radiation and thought of as wave motion, and the other is termed particulate radiation. This latter form functions by the movement of alpha and beta particles. While the properties of these forms of radiation are similar in many respects, their biological effects are qualitatively identical.

Robbins, in 1957, stated that the biologically effective electromagnetic radiations are all of relatively short wave length, and that the range of biologically useful wave lengths is narrow. Four divisions of electromagnetic waves are generally recognized. These are:

- Soft (long) roentgen rays
- Hard (short) roentgen rays
- Soft gamma rays
- Hard gamma rays

The two divisions of gamma rays are the most penetrating and also most effective...
in the treatment of disease. Generally, alpha and beta particles have little ability to penetrate and are therefore absorbed by lead shields or superficial tissues of the body. They are much less penetrating than the gamma rays given off by high voltage vacuum tubes.

English, in 1952, stated that at one time the tolerance to radiation was the amount the skin could absorb without visible signs of reddening or other noticeable effects after a certain period of time. The amount that caused a reddening of the skin was called the "skin erythema dose." We now use the roentgen to measure a unit of dosage. This unit (r), is the amount of radiation which will produce one electrostatic unit of charge in one centimeter of air under standard conditions.

Zirkle, in his study of the effects of x-rays on tissue metabolism, calls attention to the fact that his experiments refute the existence of a latent period where x-ray sickness is supposed to develop some time after irradiation. It was shown that immediately after irradiation symptoms became manifest.

Clark, in 1936, and Thoma, in 1948, wrote on "The Biological Effects of X-Radiation," and parts of their papers were devoted to the effects of x-rays on normal cells and on radiosensitiveness of cells. Thoma lists the tissue cells according to irradiation sensitivity from the highest to the lowest, as follows: lymphoid cells, leukocytes, epithelial cells, endothelial cells, connective tissue, muscle, bone and nerve cells. Clark also showed that cells may be classified according to their radiosensitiveness, and indicated that the epiphyseal plate is a very radiosensitive area.

It was Perthes, in 1903, who first recorded the effect of radiation upon the growth of long bones and produced retardation in the development of wings
in one-day-old chickens by exposure to roentgen rays.

In 1905, Tribondeau and Recamier showed a similar effect upon the cranial bones of the cat. These men irradiated one side of the face of a young cat and observed that there was retardation in the development of the dentition on the irradiated side. They also noted a generalized stunting of the entire skeleton, but since they lacked standardization of x-ray dosage, an interpretation of the work they did is very difficult. There were others who described qualitative stunting in a number of animals, but none attempted to express the quality or quantity of radiation in terms of measurable units.

Regaud, in 1922, and Flaskamp, in 1930, held that adult bone is largely resistant to irradiation, but Nageotte (1922) felt that x-rays alter adult bone, although not distinctively. Regaud and others found that adult cartilage is quite refractive to x-rays.

Bloom (1943) found that when rats were given 600r of x-rays, there was a disruption of continuity of epiphyseal cartilage of femur and tibia with the spongiosa, at nine days after treatment in some animals, resulting in temporary cessation of bone growth in length. Recovery or resumption of growth was irregular but was complete in all specimens by the end of seventy days.

O'Shaughnessy, in 1958, showed that animals subjected to 888r and 444r indicate that there is some effect of x-rays on body growth, but all other organs of the body showed normal activity.

Brooks and Hillstrom, in 1933, performed more standardized experiments by showing that bony shortening in rabbits three to four weeks old could be produced with varying doses of radiation.

Regan and Wilkins, in 1936, showed complete cessation of growth in long
bones of young rabbits treated with 2,600 roentgens in one exposure.

Bisgard and Hunt, in 1936, using rabbits three to five weeks old, found that 1,540 roentgens to the forelegs produced no shortening if the epiphysis were protected with lead, but in four week old rabbits, 400 roentgens produced gross retardation when the epiphysis were included in the field. These men stressed the great lessening of effect produced by fractionation of the dose.

Barr, Lingley and Gall (1943) reported on the effect of roentgen radiation on epiphyseal growth. These were experimental studies on the albino rat.

Barr and associates, in performing this study, wanted to enlarge on previous studies and determine the following points:

1. What dosage produces maximal effect on the epiphyseal plate without permanently damaging juxta-epiphyseal tissues?

2. What is the effect of varying dosages on longitudinal bone growth?

3. Does bone deformity, maldevelopment, or fragility occur after such treatments?

To this end, graded dosages of roentgen rays were applied to the growing epiphysis of albino rats and a series of roentgenographic and histologic studies pursued.

Their animals were divided into three age groups, and the purpose of this division was to determine the variability of response, if any, which might result from differences in growth rate at varying ages. All of the animals in a given treatment group received equal amounts of radiation, the individual group dosages ranging from 665r to 1800r. Each dose was administered at a single sitting to the right hind extremity in a field around the knee joint. This investigation was initially motivated by the possibility of a practical
use for the known sensitivity of the epiphyseal plate.

Hinkel, in 1942 and 1943, showed that moderate amounts of X-irradiation (750 to 1500r) at 200 KV given in a single dose through a portal 5 mm. in diameter over the distal femur to young rats produce slowing of the longitudinal and transverse growth. The effect depends chiefly on the age of the animal. No changes in bone salts were brought about.

Burstone, in 1950, did several excellent pieces of work concerning the effect of x-ray irradiation on the development of the mandibular joint, and on teeth and supporting structures of the mouse. He found that x-ray irradiation of the mandibular joint produces a marked inhibition in the process of ossification. X-Radiation of the condyle of the mouse with 1,500, 3,000 and 5,000r results in damage to the intermediate and hypertrophic zones with a subsequent marrow aplasia and fibrosis.

The cranial portion of the joint and the inter-radicular disc are relatively radio-resistant.

The growth potential may be restored to some extent approximately six weeks following irradiation with 5,000r.

Sixty-two days following X-Radiation (5,000r) there is a marked increase in the cellular activity of the resting zone and the intermediate zone.

The degree of radiation damage to the teeth and jaws of mice is dependent upon the age at which the animal is irradiated and the stage of histogenesis of the individual tooth.

Following exposures of 1,500 to 5,000r, the development of the basal and alveolar portions of the jaw is retarded or stopped completely.

The late post-irradiation changes include atrophy and fibrosis of the pulp.
and ankylosis of the root to the alveolar bone. Burstone also observed a twisting of the mandible to the side of the exposure, and found some anterional notching.

Skewing of the snout of the rat was also shown by Jarabak and Vehe in 1949. Following the sectioning of the facial nerve at the stylomastoid foramen, morphological changes occurred, and among other things, the snout skewed to the side of the resection.

Levy and Burstone (1949) showed the results of experiments involving young mice who were exposed to 5,000r. Those given 1500r were three-day-old mice and this caused a hemiatrophy of the mandible noticeable after two weeks. In all animals the irradiated side showed the more marked changes.

English and Associates, in 1954, studied the effects of a single dose of localized head x-ray radiation to twenty-one day old animals which were three littermate groups of white rats. The maximus dose was 1500r, administered at the rate of 4hr per minute. Striking changes were observed in the developing incisor teeth of irradiated animals, sacrificed 100 days following treatment. By means of roentgenograms, taken 43 days after irradiation treatment, it was revealed that all exposed animals already had a visible break in incisor tooth formation, located at the region which was forming at the time of exposure. The incisor teeth of sacrificed animals were separated into two segments: in the maxilla, the first segment was frequently lost at 100 days, leaving a stump-like tooth; in the mandible, the first segment persisted, while the second segment frequently erupted lateral to the first, producing the effect of a supernumerary incisor tooth. In histologic section, it was seen that extensive damage had been done to the tooth forming elements which were physiologically active at the time.
of exposure, as evidenced by stoppage of tooth formation. Also damaged was the gingiva. Despite the fact that these random odontogenic elements were completely obliterated, there appeared to be a general recovery of tooth forming tissues.

English, in 1956, used two series of rats in an investigation to determine whether radiation changes previously observed in developing teeth, following 1500r of 200 KVP local x-ray exposure, were due to direct effects upon the tooth forming cells or to indirect effects resulting from such factors as the production of toxic substances, or humoral changes in regions beyond the dental area. The bodies of the rats were protected from radiation by means of lead shields, except for the dental area in one series, and the posterior portion of the head in the other series. The pituitary gland is located in the latter field. Through the use of radiographic films, it was determined that gross developmental changes were observed in animals in which only the posterior part of the head was irradiated. That the dental changes were similar to those previously observed following 1500r of ionizing radiation was verified through histologic examination.

Louie, in 1956, stated that the degree of damage to the irradiated parts is related to the degree of sensitivity of the various cells to the rays, and also to the amount of radiation taken by the cells. The stage of development of the tooth and the metabolic activity of the cells are factors which are important to the resistance of the cells to radiation.

The effects may be manifested soon after the irradiation or may be delayed until very much later.

English and Hansen (1957) showed that there was a severe interruption in
tooth formation following irradiation of the mandible, and during the early recovery period abnormal tooth substance was formed.

Gates, in 1943, said that radiation produces minor alterations on bone itself because it is largely an inter-cellular substance of high mineral content. The difference between viable and non-viable bone may be only a slight variation in staining reaction and in the histologic appearance of osteocytes unless disruptive forces, such as trauma or infection, intervene. For this reason, effects of radiation on bone are less easily estimated.

Moreover, the actual intensity of a given dose of radiation may be greater in bone than in other tissues because of the secondary radiations from the calcium.

The final effect of radiation on developing bone depends on the number of cells damaged beyond recovery. The contour of irradiated growing bone is usually close to normal (Brooks and Hillstrom). Where there is deformity, it is due to two factors: muscle strain and greater injury to one part of the bone than to the rest.

The two histologic changes most frequently described in dwarfed bones of animals as an effect of radiation are early alteration of cartilage cells and disorientation of endochondral ossification. The earliest changes in epiphyseal cartilage cells, such as swelling, pyknosis and loss of columnar pattern, were observed one or two weeks after 600r (200 kilovolts) were administered to rats (Gall and Associates).

It has been stated that total body radiation has a lethal effect in certain doses. An abbreviation of LD\textsubscript{50} means the single dose that will be fatal to 50 per cent of exposed individuals. For man it is about 300r for 250
kilovolt x-rays; monkeys about 500r and mice about 600r (Loutit, 1959).

As might be expected, much higher dosages are tolerated when only a part of the body is exposed (Gorvy, 1953). Here the effect will depend on the amount and kind of tissue that is irradiated, its vulnerability to radiation and its role in the economy of the organism. For example, an individual can absorb a few hundred roentgens to an extremity with virtually no effect on his body as a whole. However, an equal dose to the abdomen would have serious consequences. Moreover, the rate at which radiation is received has a great deal to do with its effect. A much larger quantity of radiation can be tolerated if it is divided into small fractions.

B. GROWTH AND DEVELOPMENT

Strong (1925) stated that ossification in the maxilla and mandible is first observed 17 days and 55 minutes in utero. The mandible ossifies rapidly. Meckel's cartilage is apparently calcified at 18 days, but the mandible is more complete at 19 days 9 1/2 hours. Developing teeth are apparent 2 days after birth and they are adequately outlined at 8 days. A considerable separation exists at the symphysis even in 3 week old rats. The alveolar process is extensively developed at 18 days.

Strong explains that it is difficult to get exact measurements of ossification in fetal femur, but it has been shown that 2 or 3 epiphysis appear at the distal end of the femur in 8 day old rats.

At birth, the normal cartilage plates of the rat show no generally visible differentiation of the cartilage plates, but by the first week the cartilage cells between the diaphysis and epiphysis have become oriented into the typical
row formation. By the second week, the diaphysis and epiphysis, with their respective marrow cavities, are well formed. From this time on, for the next 2 months, growth in length of the bones is most rapid (Nunnemacher, 1939).

From the time of formation, the cartilage plate is composed of five zones. The relation of these zones to one another gives a good index of the state of activity of the cartilage plate. Beginning at the layer of epiphyseal bone lying on the distal side of the plate and proceeding toward the diaphysis, these are:

1. Zone of reserve cells, which is comparatively thin.
2. Zone of cell multiplication, which is generally the thickest. The divisions in this zone are responsible for the first increment in length of the bone.
3. Zone of individual cell growth.
4. In this zone, cells become full grown, hypertrophy, and are destroyed by the invading capillaries from the diaphyseal marrow. The matrix between the cell rows becomes calcified.
5. In this zone, cartilage removal takes place while endochondral bone is being laid down on the projecting trabeculae.

The condition of bony union is characterized by a complete absence of trabeculae on the diaphyseal side of the plate, and a smooth layer of lamellate bone on both the diaphyseal and epiphyseal side of the cartilage plate. The plate itself becomes progressively thinner and cartilage cell rows become sparse and short.

The proximal tibial plate forms between 5 and 15 days of age, due to the appearance and expansion of the epiphyseal center of ossification (Becks, et al.). It is generally known that the epiphyseal plate does not disappear until old age in the rat (beyond 600 days); however, marked changes occur and these are
associated with progressively decreased activity in this region. It also becomes almost completely sealed off from marrow, and so, too, from encroachment of blood vessels. Growth at the epiphyses practically ceases at 170 days.

Levy (1948) states that the growth and development of the mandibular condyle exhibits both intramembranous and intracartilaginous bone formation. The process differs from the development of long bones in that there is only one primary center of ossification. Moreover, the condylar cartilage constitutes the essential growth center of the mandible.

That bone forming the articular fossa, is formed primarily from intramembranous ossification. A layer of cartilage like tissue lies subjacent to the articular surface of the fossa; complete calcification of this tissue does not occur. The continued presence of this cartilage-like tissue probably accounts for the capacity of the fossa to adapt throughout life to changing stresses (Collins).

The mandibular condyle in the very young rat (5 days old) is composed entirely of hyaline cartilage. This cartilage continues to grow, but it is also being eroded by encroachment from the center of ossification. By 25 days of age, four zones have been differentiated in the cartilage:

1. the zone of embryonic cells
2. the intermediate zone
3. the zone of vacuolated cells
4. the zone of erosion

In old animals, the latter three zones disappear or become calcified. The zone of embryonic cells remains uncalcified up to old age.

The trabeculae, which are thin and delicate in younger rats, become
progressively coarse and fuse with advancing age and only small islands remain in the dense bone of ramus. The cartilage in contact with the fused trabeculae is calcified.

Sicher explains that the growth center in the condyle is represented by a cartilaginous disc covering the bony head of the mandible. This cap of hyaline cartilage, however, cannot be compared to an articular cartilage because it is, itself, covered by a fairly thick layer of dense fibrous tissue. This fibrous tissue borders immediately onto the articular cavity, and is in contact with the articular disc. Proliferation of the hyaline cartilage, and its replacement by bone, contribute both to the increase of the mandibular ramus in height, and to the increase of the over-all length of the mandible. The double effect of condylar growth is due to the fact that the condyle is obliquely implanted upon the body of the mandible by the obliquely ascending ramus.

By condylar growth, the over-all length of the mandible increases, and not the length of the mandibular body. Width of the ramus in an antero posterior direction is due to appositional growth, and this is also true of the coronoid process which keeps pace with the increased height of ramus. Growth of the mandibular body, from the lower border to the free border of alveolar process increases mainly by apposition of bone at the free border of alveolar process, growing into the space which is opened by the growth of the mandibular ramus in height. Apposition of bone at the lower mandible border is negligible.

In condylar growth, it is the proliferation of the cartilage, with eventual replacement by bone, which makes the mandible grow in height and over-all length, just as a long bone grown in length by proliferation of the epiphyseal cartilage. Replacement of the proliferated cartilage by bone, indispensable
for the proper function of the growing bone, contributes to the enlargement of
the bone as a whole.

If cartilage growth outbalances bone growth, then the mandible will be long
and the ramus high and narrow, having a tendency to become prognathic. The
opposite is true if bone growth is faster. The mandible will be short and the
ramus wide, and will tend to a retrognathic type of face.

The hyaline cartilage that was supposedly present on the articular surfaces
of the glenoid fossa and the condyle was in reality fibrous tissue. Underlying
this fibrous tissue on the condyle was a definite area of cartilage cells, while
under that of the glenoid fossa, cartilage cells were seen to be present
occasionally, but not in the definite order of the condyle area (Charles).

That the cartilage differs from ordinary hyaline cartilage is obvious
(Maximow & Bloom), as is the fact that the cells are the direct result of the
differentiation of the fibroblasts of the fibrous layer, which while it is
continuous with the periosteum of the bone, differs from it in being
considerably thicker and in being more vascular.

There can be no doubt that the growth which takes place at the posterior
border of the mandible and angle does not affect the general forward and
downward growth of the bone. Mandibular growth in a forward and downward
direction is solely controlled by the growth which takes place at the top of the
condyle. In the first place, the bone which is formed from the cartilage is
absolutely distinct from the bone of the angle and posterior border, and,
moreover, persists as a definite band or wedge of bone in direct line with the
body. If growth takes place at the posterior border, and absorption takes place
in front of the condyle, the continuity of the wedge of "cartilaginous" bone
must be destroyed, and the bone which now is seen to form a definite wedge would eventually appear on the anterior face of the coronoid process, and the ascending ramus would be interspersed with bone obviously different from the bone of the angle.

A description of the formation of the temporo-mandibular joint is offered by Symons. His observations are:

(1) The mandibular joint is produced by the growth of bony tissue of the mandible toward the temporal region.

(2) These areas of bone and the fibro-cellular condensations which surround them are separated by intra-articular fibrous tissue.

(3) The final approximation of the mandible to the temporal surface in the dorsal and cranial direction is brought about by the development of the secondary cartilage in the condylar process.

The mandibular joint is unlike other synovial joints in that its component parts as outlined in the mesenchyme are separated from each other and can only approach each other by the growth of cartilage on the upper surface of the primitive condyle.

In the strict sense, the rat has no "temporo-mandibular joint" because the articulation of the mandibular condyle is with the squamosal (E. Greene). It is, therefore, actually a "squamoso-mandibular joint." The term mandibular joint is used in order not to introduce confusion into the literature, since the term temporo-mandibular joint is correctly used for other species.

Collins stated that in the rat, as in man, the mandibular articulation is a ginglymoarthrodial joint. This type of joint allows ample hinge action for use of the molars in grinding, also gliding action for gnawing with the incisors.

The gross anatomy and physiology of the temporo-mandibular joint of the
female white rat is as follows:

1. The cranial portion of the joint is formed by an elongated groove in the squamosal bone. The long axis of the groove lies in an anteroposterior plane, and is directed upward and backward. The fossa faces downward and backward. It is divided into an anterior and a posterior part by a rounded eminence which is continuous with the zygomatic process of the squamosal. The part of the fossa posterior to this eminence accommodates the condyle of the mandible when the molars are being used in chewing. That part of the fossa anterior to the eminence accommodates the condyle of the mandible when the incisors are being used in gnawing. The eminence not only divides the fossa into two parts, but also divides it into two levels, an inferior and a superior level.

2. The interarticular soft tissues are composed of:

(a) The synovial membrane which covers the articular surface of the fossa and is composed of fibrous tissue which is continuous with the periosteum at the periphery of the fossa.

(b) The interarticular disc, which is a comparatively thick plate of fibrous tissue, conforms to the configuration of the fossa and the condyle. Its inferior concave surface is in contact with the condyle; its superior convex surface is in contact with the glenoid fossa. It is thicker at the margin than at the center.

(c) The synovial membrane, which covers the condyle and is composed of a layer of fibrous tissue, adheres to the cartilage of the condyle and is continuous with the periosteum.

3. The mandibular portion of the joint, formed by the condyloid process, consists of two parts; the condyle and the constricted portion which supports it, the neck. The condyle presents a surface for articulation with the articular disc of the joint. The anteroposteriorly directed long axis of the condylar head in the rat is, in the adult, approximately twice the length of the short axis. (This is in contrast to the position of the long and short axes of the condyle in man.) The neck is flattened from side to side and is strengthened by ridges which descend from the anterior and posterior borders as well as the sides of the condyle. The thickest of these ridges extends from the lateral side of the condyle to the base of the lower incisor.

The mandibular joint in the rat, as in other rodents, is adapted for
gnawing or cutting with the large incisors. In this animal, the incisors erupt continuously throughout life, replacing the tooth structure of the incisal edge which is being ground away. In performing this action, the condyle moves downward and forward into the anterior (longer) half of the fossa. The lower jaw is protruded until the incisors meet. The lower incisor may be occluded either posteriorly or anteriorly to the upper incisor. This type of action allows for use of the enamel covering the labial surface of the lower incisor against the dentine which forms the lingual surface of the upper incisor or the reverse.

In chewing with the molars the condyle moves backward and upward into the posterior (shorter half) of the glenoid fossa. This part of the fossa is shallow and is, therefore, better adapted for the movement of the jaw necessary in the use of the molars (Charles).

C. ROENTGENOLOGICAL STUDIES

A roentgenographic appraisal of the rat cranium shows that posteriorly and inferiorly is seen the tympanic bulla, the three semicircular canals and, in between, the denser petrous portion of the temporal bone, in which can be distinguished the bony canal of the cochlea. Superimposed upon the shadow of the tympanic bulla anteriorly can be seen the synchondrosis between the basi-sphenoid bones. There is no sella turcica in the rat, the pituitary gland lying upon the basi-sphenoid about the level where the shadow of the tympanic bulla superimposes this bone. Anteriorly is seen the synchondrosis between the basi-sphenoid and pre-sphenoid, and anterior to this is seen the shadow of the optic foramen. Above the pre-sphenoid the posterior root of zygoma goes downward and forward.
In front of and above the optic foramen is seen the cribiform plate of the ethmoid, and the inverted U shaped shadow of the anterior root of the zygoma. Below the ethmoid is the alveolar process of the maxilla bearing the three molar teeth. The first molar erupts the 19th day, the second one about the 21st day, and the third one about the 35th day. The incisor teeth erupt 8-10 days after birth, are rootless, and grow throughout life at the average rate of 2.2mm per week in the upper and 2.8mm per week in the lower incisors. The anterior of the incisor tooth is composed of enamel and dentin, whereas the posterior is dentin and cementum. The tooth is largely hollow and the gradual tapering of its wall to a fine point at the proximal or root end is characteristic of the normal.

Posterior to the shadows of the semi-circular canals is that shadow of the occipital condyle, which is lateral to the foramen magnum. Superior to this is the supra-occipital bone which forms an angle with the interparietal bone.

Increase in size of the skull is readily apparent by the 6th week, when the skull straightens out and the snout lengthens. It is obvious that there has been at work the same basic mechanism of growth as is seen in the shaft of a long bone, as it progressively increases in diameter in growth; that is, subperiosteal deposition of bone and central resorption in the medullary cavity (Mortimer).

The mandible forms the lower half of the face and consists of a rather limited area in the cranio-facial complex. In the rat, the mandible is comprised of two halves held together by an intermediate tendon composed of fibrous elastic tissue. The anatomy of the mandible and cranium are explained in definitive detail by E. Greene and Jarabak, and only those structures of the
mandible visible on a lateral radiograph will be discussed. The anterior end of the mandible consists of the alveolar process and this surrounds the lower central incisor. The superior surface continues as alveolar bone containing three molar teeth on each side. These molars erupt at approximately the same time as the maxillary molars. The inferior border continues posteriorly into a notch, the antegonial notch, and then bends downward to form the angle of the mandible. At the distal portion can be found the ascending ramus with two processes, the anterior or coronoid and posterior or condylar process. Separating these processes is a semilunar depression, called the mandibular notch. The head of the condylar process in the rat articulates in the two articular fossa of the temporal bone.

A roentgenogram of the femur of a rat at birth shows a well calcified shaft about 7 mm. long, having a tendency to flare out at the ends, which themselves are rather indistinct. There are no secondary centers of ossification at the knee joint, and the animal exhibits an immature skeletal development.

At eight days the three distal femoral centers of ossification which form the epiphysis appear and grow rapidly in size. They fuse into a bony plate by the end of the third week, simultaneously with the appearance of the epiphysis for the femoral head. At one month the secondary centers of ossification have assumed the shapes and proportions to be seen in the adult animal.

After six weeks, the femur undergoes a slight modification in shape and proportions. The bone continues to grow in length and thickness throughout life. This growth is most rapid in the first four months, but continues at a slower rate thereafter, so that careful measurements show growth even into old
While the epiphyses of nearly all the other long bones have fused by four and a half months, those of the distal femur, femoral head, and both lesser and greater trochanters remain unfused into old age.

Cephalometrics, the term applied to serial roentgenography, is now being used extensively in the field of longitudinal growth studies. This field was aided by the introduction and development of head holders by Broadbent (1931) and Hofrath (1931). Spence, in 1940, reported a serial study of the growth of the cranium of a normal rat. Jarabak, in 1942, and later Jarabak and Thompson, in 1948, reported on the development of a small animal head holder which was independently mounted.

Spence (1940) states that the value of studying the skull development of the living rat by roentgenographic method is twofold. First, the same individual can be observed at different age periods; second, x-rays reveal structural details such as sutures, sinuses and diploë, which cannot be studied as readily by other methods. Sites of growth as indicated by epiphyseal plates are also readily discernible.

The rat was selected because it is an ideal laboratory animal for experimental studies concerned with the dental apparatus, as demonstrated by Schour in his many investigations. The life span of the animal is relatively brief, and its rate of development is rapid. One year of its life is physiologically comparable to thirty human years.
CHAPTER III

METHODS AND MATERIALS

A. ANIMALS

Twelve female albino rats of the Wistar stock were used in this investigation. They were divided into three groups according to age. Four animals were in each group; two of these were experimental and two were controls. Group one consisted of animals twenty-one days of age when irradiated; animals in group two were twenty-eight days of age when irradiated; animals in the third group were thirty-seven days of age at the time of irradiation. The control animals were maintained and handled in the same manner as the experimental animals except for initial radiation.

All animals were maintained on a diet of Purina rat pellets and water, with a supplement of soft white bread given to them every two days. Food and water were available to the animals at all times during the experiment.

The rats were housed in small stainless steel animal cages. The tops of the cages contained food hoppers and 200 cc rubber-stoppered water bottles and canulas. The cages were cleaned every two days.

B. ANESTHESIA

The depth of anesthesia necessary in this investigation was obtained by injecting Nembutal (Abbott) of 50 mg cc. concentration intra-peritoneally. Adequate depth of anesthesia was usually obtained in about thirty minutes. It
was necessary, in some instances, to increase the original dose due to the variable reaction of the rats to Nembutal.

C. RADIOGRAPHIC AND CEPHALOMETRIC PROCEDURE

The cephalometer used in this investigation was constructed with the collaboration of Drs. Battistoni and Kozie, and ideas for it were derived from the instruments used by Spence (1940) and Jarabak (1942). The instrument consisted of two ear posts, two film cassettes, an animal cradle, x-ray tube head positioners and the x-ray tube.

The vertical film cassette was attached to the plastic table and was used to hold the film when lateral radiographs were made. The stationary ear-post was attached to the vertical cassette and extended out three-quarters of an inch. The moveable ear-post was attached to a precision grooved slide which allowed this ear-post to be adjusted medially and laterally on an accurate path. A set screw on the slide kept it from moving. The horizontal cassette was attached to the underside of the plastic table and held the film while femoral radiographs were being taken. (Fig. 1) The position of the horizontal cassette was fixed just beneath the plastic table. A one-centimeter long rod was attached to the center of each cassette to aid in enlargement of the radiographs. Three-sixteenths inch high lead letters were placed on the cassette at the time of exposure to record the date of exposure and the number of the animal.

In order that lateral and femoral radiographs could be taken without moving the animal, a moveable x-ray tube head was used. When taking lateral radiographs, the tube head was positioned on two one-inch steel dowel rods and
projected up from the wooden base board. (Fig. 2) These rods fit into two holes in the base of the tube head and held the head in a horizontal position and perpendicular to the vertical cassette. When femoral radiographs were taken, the tube head was placed into a cradle above the cephalometer. (Fig. 3) Metal lugs in the cradle positioned the tube head in the sagittal plane and perpendicular to the horizontal film cassette.

When taking a lateral radiograph, the anesthetized animal was placed on its back in the animal cradle. (Fig. 4) The animal's head was then positioned by placing the fixed ear-post into the animal's right ear hole and moving the moveable ear-post into the left ear hole. The slide was then locked by means of the set screw and the animal's head was suspended between the ear posts. String was then placed over the upper incisor teeth and slight tension was exerted in a horizontal plane to parallel the head in a dorso-ventral plane. (Fig. 4) The central ray from the x-ray tube then passes through the ear posts and perpendicular to the sagittal plane of the skull and to the film in the vertical cassette.

To take the femoral radiograph, the tube head is placed in the overhead cradle and the film is placed in the horizontal film cassette. The central ray from the x-ray tube then passes through the distal of the right femur and to the film. The rat is placed with its abdomen in contact with the table so that the knee also makes contact. This is done so that the femur will have as little distortion as possible on the radiograph. (Fig. 5)

D. IRRADIATION

At the start of the investigation, all experimental and control animals
were weighed and placed under Nembutal anesthesia. While under deep anesthesia, the animals were orientated in the animal cephalometer and each was radiographed from a lateral head aspect giving a lateral view radiograph with an accurate as possible superimposition of right and left sides of the mandible. The legs of the animals were also radiographed, care being taken to include both right and left femurs and tibias. Dorso-neutral radiographs of the head were also taken, and these were used by Dr. Kozio in another phase of this investigation. Shortly after taking the lateral head and femoral radiographs, the experimental animals were placed on a head board to which a head-positioning device had been attached (Fig. 6) The head positioner consisted of two round plastic ear posts, one attached to the board and fixed, and the second post movable in a sagittal plane and in line with the fixed post. The animal was oriented in the positioner in the following manner. Under anesthesia, the animal was secured with its right side in contact with the board. The head of the rat was then manipulated until the fixed ear post entered the left ear hole, and thereby secured the animal in the sagittal plane. The body legs and head of the animal were then secured with twine to hold the head as stationary as possible and to make movement negligible during the period the animal was being irradiated. By this method, the right and left mandibular condyles were orientated in the same plane both antero-posteriorly and superio-inferiorly in relation to the board.

The animal was then entirely covered by a one-eighth inch thick lead casket in which a one-quarter inch portal had been opened. The portal was centered over the left mandibular condyle of the animal, the position of which had been marked with dye, and maintained at about a distance of four millimeters from the skin. The animal and apparatus were then placed in the center of the
field under the x-ray tube. (Fig. 7)

X-radiation in the amount of 1500 roentgens was then delivered through the one-quarter inch portal to the condyles at a target distance of twenty-five centimeters, using 220 kilovolts and 15 milliamperes. The radiation was delivered by a General Electric therapy-size x-ray machine (Fig. 8 and 9) equipped with one millimeter of aluminum and one-half millimeter of copper filtration. The animal was removed from the head positioner and reorientated on the board and tied so that a like amount of radiation could be delivered to the distal femoral epiphysis of the right leg and this was delivered to the animal through a 5 x 7 mm portal. All parts except the distal of the femur were protected by the lead casket during the second irradiation. The animals were returned to their cages after regaining consciousness.

E. FILM DISTANCE

Film distance for the lateral radiographs was fixed at 7/8 inch from the tip of the fixed ear posts to the film in the vertical cassette. Film distance for the femoral radiographs was fixed at 1/4 inch from the femur to the film in the horizontal film cassette. The tube head was maintained at a distance of 12 inches from the subject for all lateral and femoral exposures.

F. RADIOGRAPHIC EQUIPMENT

A General Electric portable x-ray machine with a moveable head was used for all radiographs. The machine was operated at 65 kilovolts and 10 milliamperes. The exposure time for the lateral radiographs was two and one quarter seconds and the femoral radiographs had an exposure time of two seconds.
Kodak super-speed dental occlusal film was used for all radiographs. Films were developed in the manner advised by the manufacturer.

G. SEQUENCE OF RADIOGRAPHS

Lateral head, dorso-ventral head, and femoral radiographs were taken of all the animals at the start of the investigation. The animals in groups one and two were radiographed every two weeks thereafter, for a period of three months. The animals in group three were radiographed at one month intervals for a period of three months. The weight of the animals was recorded to the nearest gram each time they were radiographed. (Fig. 24 and 25)

H. MEASUREMENTS

All measurements were made indirectly from the lateral and femoral radiographs. The individual radiographs were put between thin sheets of glass and placed on the stage of a micro enlarger. The lens used was checked and found to be free of distortion and aberration for the entire field measured. (Fig. 10) All measurements were made with the image enlarged five times. Enlargement was aided by the image of the one centimeter long rod which was attached to the cassette during exposure and which appears on the radiograph below the image of the cranium and below the femur. The measurements made from the enlarged image were recorded to one hundredth of a millimeter.

Measurements of the cranium and mandible were made in Part I of this study. Part II of this study, undertaken by this investigator, measured the femurs and tibias. A constant method of measurement was used to measure the length of the femur and the tibia. For the femur this measurement was as a straight line from
the proximal tip of the greater trachanter to the distal end of the medial condyle. Any curvature that may have resulted was only noted macroscopically and not measured. The tibia was measured from the proximal end between the lateral and medial condyle to the distal point on the medial malleolus.
CHAPTER IV

FINDINGS

A. GENERAL FINDINGS

The weight charts of the experimental and control animals show that the radiated animals did not gain in weight as rapidly, nor did they attain the weight of the control animals. Two weeks after being irradiated, the experimental animals showed a weight gain comparable to the controls, but after the fourth week the control animals surpassed the experimentals in weight gain. This lead continued and the control animals weighed more (12-50 grams) at the end of thirteen weeks.

Periodic gross examination of the animals showed that the irradiated experimental animals showed signs of radiation dermatitis which made its appearance four weeks after initial radiation. The animals had a loss of fur in a circular area approximately five millimeters wide, inferior and mesial to the ear and roughly over the mandibular condyle. There was another area where hair was missing and the skin showed signs of erythema. This was in the region of the knee joint of the right leg. This area consisted of a band about 10mm. with which encircled the knee joint of the right leg of each of the radiated animals.

As the rats grew older, the radiation burn area at the site of the left condyle diminished in size and at thirteen weeks was no longer present. The burn area on the right leg also diminished in size and, at about ten weeks,
some short hairs appeared in this area. The effects of radiation were still visible here after fifteen weeks.

The radiated and control animals appeared to be in good health throughout the period of this study. Macroscopically, the radiated animals of Group I and Group II revealed that the right leg was shorter than the left leg. The control animals exhibited no unusual changes at the completion of the experiment, except for an increase in weight. Further, the control animals in Groups I and II showed a greater weight increase than the experimental animals. (Fig. 24 and 25)

B. GRAPHICAL ANALYSIS

This material was obtained from a graphical analysis of the growth curves of experimental and control animals. Measurements were made of femoro-tibial growth in three groups of animals, each group having experimental and control specimens. The right leg of each experimental animal was subjected to 1,500 r x-radiation. The growth curve of this leg was then charted and compared to that of the left, or control, leg of each animal. In all graphs, the growth curves for experimental findings are represented by the lines labeled R, and control findings by the lines labeled L. A graphical analysis of femoral length is presented first.

C. FEMORAL LENGTH

Group I Animal Number 2

The growth increment of the control leg of animal number 2 attained most of its potential during the first four weeks, with a decreased incremental growth
for the following nine weeks. By the second week of this experiment, the radiated femur began to show growth retardation which became considerably more acute by the fourth week. From this time until the eighth week, the growth curve of the experimental femur closely paralleled that of the control femur. From the eighth through the tenth week, there was no measurable growth of the radiated leg. The control femur, however, continued its steady growth increment. From the tenth through the thirteenth week, the radiated leg grew slightly. Measurement revealed that at the conclusion of the experiment the radiated femur was .75 mm shorter than the control femur. (Fig. 11 and 26)

**Group I  Animal Number 3**

The growth increment of the left femur (control) of animal number 3 reached most of its potential at the sixth week. The radiated femur also showed the greatest effect of radiation at the sixth week. Both femurs showed a leveling of growth from the sixth week to the eighth week. At the eighth week, however, the control femur appeared to resume its steady growth increment, growing 1.60 mm. in length between the eighth and the thirteenth week, while the radiated femur added only .85 mm. to its length during this period. Final measurement of the animal revealed that the radiated femur was .98 mm. shorter than the control femur. (Fig. 12 and 27)

**Group II  Animal Number 7**

In this animal, the growth curve of the radiated femur closely paralleled that of the control femur from the beginning of the experiment through the seventh week. Each appeared to attain most of its growth by the second week, after which the retarding influence of radiation on the right femur was more pronounced. The greatest difference in length was noted at the tenth week, at
which time the radiated femur evidenced a 1 mm. retardation. The growth curve of the experimental femur rose even more sharply than that of the control between the tenth and thirteenth week, allowing a measurable difference of only .75 mm. at the end of the thirteenth week. (Fig. 13 and 28)

Group II Animal Number 9

The growth curves of the femurs, control and radiated, of animal number 9 ascended in close proximity to the second week, when the pattern of growth increment began to assume a less vertical path for each. The two curves remained relatively equidistant until the eighth week, when the control femur seemed to again achieve a rapid, even growth which persisted until the end of the experiment, while the radiated femur leveled in growth increment. The largest variance in length was noted at the thirteenth week and measured as 1.25 mm. (Fig. 14 and 29)

Group III Animal Number 13

The radiated femur of animal number 13 manifested an inhibition in incremental growth at one month. This inhibition was more obvious when compared to the control femur at the end of the second month. At this time, a 1.30 mm. difference in length was noted. From the eighth week through the thirteenth and final week of the experiment, the two bones paralleled one another in growth increment. By the end of the third month, there was a leveling off in the growth of both femurs. The marked difference in the length of the two bones was measured, at the conclusion of this study, as 1.45 mm., only slightly greater than that difference which existed at the end of the eighth week. (Fig. 15 and 30)

Measurements were made from radiographs of the femurs of the control
animals in Groups I, II, and III to show their patterns of growth. An analysis of the graph showing the femoral length of the control animals indicates that there is a similarity of growth increment among the animals within each group. The youngest animals (#5 and #6) reveal the greatest acceleration of growth increment, while the remaining animals show a less active vertical growth pattern. (Fig. 16)

D. TIBIAL LENGTH

Group I Animal Number 2

The growth inhibition of the radiated tibia of animal number 2 was evident at the end of the second week, though the growth curves for the radiated and control tibias assumed an unlike vertical direction from the onset of this experiment. The growth curve of the control tibia rose sharply until the fourth week, while that of the radiated bone leveled markedly and never resumed its pronounced vertical ascent. At the conclusion of this study, the left tibia was 7.80 mm. longer than the radiated tibia. (Fig. 17 and 26)

Group I Animal Number 3

The growth curves of animal number 3 were quite similar to those of animal number 2. The inhibition of the radiated tibia was evident at the end of the second week, and this marked radiation continued until the end of the thirteenth week. Incremental growth of the radiated tibia from the fourth week to the end of the study was negligible, as is evidenced by the near-horizontal direction of the curve. Growth of the control tibia, however, gained new momentum in the sixth week and the curve rises sharply from then until the completion of the study. At that time, the difference in length between the left and right tibias
was 7.15 mm. (Fig 18 and 27)

Group II Animal Number 7

Animal number 7 also showed marked evidence of growth inhibition of the radiated tibia as compared to the growth curve of the protected tibia. The growth curve of the experimental bone leveled sharply at two weeks and maintained an almost horizontal direction until this experiment was concluded. The curve denoting growth increment of the protected tibia continued its sharp ascent through the thirteenth week. A difference of 7.80 mm. between the two tibias was measured at the end of the study. (Fig. 19 and 28)

Group II Animal Number 9

The growth curves of both tibias of animal number 9 rose sharply until the fourth week. At this time, the growth of the radiated bone progressed spasmodically, evincing almost no growth between the fourth and sixth week, renewed growth between the sixth and eighth week, negligible growth between the eighth and tenth week, and a marked growth increment between the tenth and thirteenth week. Although the growth curve of the protected tibia assumed a more horizontal slope between the fourth and thirteenth week, it maintained a steep vertical path. The total difference between the two bones at the end of this study was 8.0 mm. (Fig. 20 and 29)

Group III Animal Number 13

The difference in incremental growth between the radiated and the control tibia of animal number 13 was so slight that no measurements were recorded.

Macroscopic examination of the radiographs of the heads of experimental and control animals did not reveal any significant morphologic changes. A perusal of leg radiographs revealed that femoral and tibial morphology of the
control animals was apparently normal, though they were smaller in size.

If any morphologic changes could be ascribed to the experiment, they were a slight bowing of the shaft of the right femurs six weeks after being radiated. There was no evidence of bowing of the protected femurs.

Upon examination of the tibias of the control and radiated animals, it was noted that the radiated tibia of one of the experimental animals had a pronounced bowing. This bowing was not evidenced upon examination of the radiographs of the other specimens. (Fig. 21)

The radiated tibias of the animals in Group I and Group II showed some narrowing of the shaft in addition to a macroscopic shortening of the bones. (Fig. 22 and 23) This shortening of the tibia and narrowing of the shaft was not evidenced in the radiated leg of animal number 13 in Group III.
CHAPTER V

DISCUSSION

The purpose of this experiment was to determine if cartilage derived from fibrocartilage as seen in the mandible, and cartilage derived from division of cartilage cells as seen in the femoral epiphyses, responds similarly to x-radiation.

The influence of x-radiation on the growth of the condyle, at the fibrocartilagenous growth center, was studied in Part I of this study by Dr. Kesiekewicz. Part II, which is the material contained in this thesis, deals with the influence of x-radiation on the femora of the same experimental animals used in Part I.

Every effort was made to control the variables which might be introduced when radiating the animals, during the roentgenographic procedures, and when measuring the radiographs.

This investigation was motivated by the possibility of determining a practical means of utilizing x-radiation in retarding growth in the mandibular condyle in prognathic mandible.

The animals used in this study were divided into three age groups. The purpose of this division was to learn the variability of response to x-radiation which might result from differences in growth rate at varying ages.

A study of the weight charts revealed that the control animals surpassed the experimental animals in weight gain at the conclusion of this study. Since
the weight discrepancy became noticeable two weeks after initial radiation, and it has been established that massive doses of roentgen radiation will cause radiation sickness. It must be concluded and, this is based on a study by Barr and Associates in 1943, that the weight loss was due to a general malaise following exposure, and that the animals lacked a desire to eat.

Radiographs were taken of the legs of the experimental and control animals every two weeks, and at the same time as were those on the crania, so that a roentgenographic study could be made of the femoral development. The right leg, of which the distal femoral epiphysis was radiated, was used for the experimental study to determine the effects of radiation on epiphyseal cartilage. The left leg, which was protected from radiation, was used as a control so that a comparative graphical analysis could be made.

That the mandibular condyle is an important growth center in the development of the mandible and plays an important part in its morphology has been well discussed and corroborated in the literature by Sicher and others. Proliferation from fibrocartilage of condylar cartilage postero-superiorly causes a downward and forward growth of the mandible, and it is this movement which has been described as the direction of growth of the mandible. This proliferation of the hyaline cartilage and its replacement by bone contributes both to the increase of the mandibular ramus in height and to the increase of the over-all length of the mandible. Any interference with the proliferation of the cartilage can be followed by changes in direction of growth and morphology of the mandible.

There are few studies to be found in the literature dealing with a serial roentgenographic study of the rat mandible following condylar treatment of some
Jarebalsk in 1953, performed a study dealing with condylar resection. He stated that, after condylar resection, there was an increase in anterior mandibular height due to an increase in dimension from the occlusal surface of the lower first molar to the lower border of the mandible, and this was due to lower molar alveolar growth. Graphical analysis, as shown in Part I of this study, reveals that the experimental animals had a period of reduced condylar growth, and this was particularly true of the animals in Group I, which were the youngest animals at the beginning of this study. The animals of Group II did not reveal any significant decrease or increase in anterior mandibular height, but the experimental animal of Group III showed an increase in this dimension and this is discussed in Part I. It may be concluded that radiating the condyle of an animal during a period when growth is most prolific will cause a more marked inhibition in anterior and downward direction of growth than will be exhibited in an older animal where growth in the mandibular condyle has reached most of its potential.

The effect of x-radiation on normal cartilage cells, and radiosensitivity of cells has been investigated by numerous men. Clark, in 1936, showed that cells may be classified according to their radiosensitivity, and indicated that the epiphyseal plate is a very sensitive area. Burstone, in 1950, found that radiating the mandibular joint resulted in damage to the intermediate and hypertrophic zones. He showed that this produced a marked inhibition in ossification and growth. It was also stated that the degree of radiation damage to the condyle depended upon the age at which the animal was irradiated. Flakamp, in 1930, studied the effects of radiation on bone and stated that "retardation or cessation of growth of bone in young animals is quite a constant
effect of radiation, provided the dose is above the threshold of tolerance." Burstone further stated that growth potential of the radiated condyle was restored to some extent following radiation. Hinkel, in 1943, conducted experiments to quantitatively study the sensitivity of the epiphyseal cartilage of the rat femur and to show the influence of age on the effect produced. Besides confirming the findings of others that retardation of growth was in direct proportion of the dose, Hinkel clearly established that the age of the animal at the time of radiation did influence the effect of radiation on bone growth. Bloom, in 1940, showed that there was disruption in growth of tibia and femur following radiation of the epiphyseal cartilage. Recovery or resumption of growth was irregular, but was complete in all specimens at the end of seventy days.

A growing long bone, such as the femur or tibia, consists of a bony shaft, the diaphysis, and bony extremities, the epiphyses, which aid in the articulation with the adjoining bones. The diaphysis is separated from the epiphysis by plates of hyaline cartilage, the epiphyseal plates. The epiphysis are covered on their free surfaces by the articular cartilage.

Young cartilage can grow in two different ways; by interstitial growth and by appositional growth. Cartilage growing by the mechanism of interstitial growth increases in size in much the same way that a piece of bread dough rises. Appositional growth, as the name implies, means a mechanism whereby new layers of cartilage are apposed to one of its surfaces.

Epiphyseal and articular cartilage of the long bones are both derived from mesenchyme. During the development of an endochondral bone, the first sign of its maturation is a condensation of the mesenchyme to precartilage, and this
gives rise to the perichondral splint. It encircles and supports the shaft where the cartilage degenerates and is resorbed. Shortly after the cartilage is resorbed, bone formation commences in the connective tissue which fills the marrow cavity, and bone is deposited on the surface of the remaining calcified cartilage. Development of the bony shaft proceeds proximally and distally until it reaches the level where, later, the epiphyseal cartilage is found. Further growth in the development of a long bone is seen in the replacement of the cartilaginous extremities of the bones by spongy bone, and these extremities are known as metaphyses, which remain covered by articular cartilage. Discs of cartilage remain, separating both ends of the shaft from the articular ends, and these are the epiphyseal cartilages.

Longitudinal growth of a long bone occurs by interstitial growth of the epiphyseal cartilage, but it does not contribute to the growth of the articular cartilage or epiphyses. The articular cartilage grows in thickness by interstitial growth and widens its surface area by appositional growth at its border. Longitudinal growth of such a bone is primarily achieved by interstitial growth of the articular and epiphyseal cartilages, and a thickening of these plates of cartilage brings about a true lengthening of the bone.

Sicher (1947) says that partial replacement of the growing cartilaginous plates by bone does not lead to a lengthening of the bone as a whole, but to lengthening of the bony shaft and bony epiphysis. Even without replacement by bone, the proliferation of the cartilage increases the length of a long bone.

A study of the graphs showing growth of animals in this investigation reveals that the twenty-one day old animals in Group I had the most inhibition of longitudinal growth. The older animals in Group II and Group III did not
show as much inhibition in longitudinal growth. These are comparable observations to those made in Part I of this study, and also by Barr and associates in 1943, who reported on the effect of roentgen radiation on epiphyseal growth. They found that a known single dose produced slowing of the longitudinal and transverse growth, and that the effect depended chiefly on the age of the animal.

The duration for which the increments of growth were inhibited appeared to vary more among the animals of the three age groups than between animals of the same group.

An effect on the transverse growth of the femurs was not as manifest as was the effect on longitudinal growth. This, however, was not true of the tibias. Here the effect of radiation was evidenced not only in longitudinal growth, but also in a thinning of the diaphysis of the experimental animals of Group I and Group II, whereas the tibias of animals of Group III did not show a reduction in transverse growth. There is no doubt that the right proximal tibial epiphyses of experimental animals in all three groups were included in the field of radiation, and it is felt that a more remarkable change was evinced in the first two groups due to the younger age of the animals.

Although it is difficult to ascribe a specific reason for the bowing of a tibia on one of the experimental animals, we can speculate as to its cause. The most plausible explanation seems to lie in the fact that it was difficult, in every instance, to pin-point the target area. In lieu of this, it seems plausible to believe that the bowing of the tibia was due to radiation of this area rather than that of the cartilage site. It is also possible that the radiation caused a weakening of the bone and that the bowing was the result of
unequal muscle pull.

The results of this investigation tend to substantiate those of previous studies, which found that inhibition of growth due to x-radiation is due to interference with cartilage proliferation and ossification. This study, using the same dosage for all areas radiated, indicates that the effects of radiation do not differ in cartilage from fibro-cartilage growth centers and epiphyseal cartilage.
CHAPTER VI

SUMMARY AND CONCLUSIONS

This investigation is a two part study. Part I is a study of the influence of x-radiation on the growth of the mandibular condyle. Part II, which is the material contained in this thesis, deals with the influence of x-radiation on the femora of the same experimental animals used in Part I.

Twelve white rats of Wister stock were used as subject material. Six animals were subjected to 1,500 roentgens to the condyle and 1,500 roentgens to the femoral epiphysis. The remaining animals served as control specimens.

Accurate measurements were made from enlarged radiographs of the femurs, tibias, and crania, and these measurements were then transferred to charts in an attempt to show the effects of radiation.

The specific results which were obtained from this study are as follows:

1. Macroscopic changes were observed two weeks after the animals were subjected to 1,500 r. of x-radiation. The areas over the left condyle and in the vicinity of the right knee joint showed a loss of fur and erythema.

2. Reduction in weight gain occurs in animals subjected to massive doses of x-radiation.

3. The condylar cartilage of the mandible is an important growth center in the development and morphology of the mandible.

4. Any interference with the proliferation of epiphyseal cartilage
can be followed by changes in growth and morphology of the femur and tibia.

5. Young animals had a marked inhibition of longitudinal and transverse growth of long bones following exposure to a large dose of radiation.

6. Morphologically, the radiated lower extremities of experimental animals revealed a decided shortening, and in some cases an excessive bowing of the long bones was also manifested.

7. The effects of radiation do not differ in cartilage from fibrocartilage growth centers and epiphyseal cartilage.
BIBLIOGRAPHY


Mortimer, H. Pituitary and Associated Hormone Factors in Cranial Growth and Differentiation in the White Rat: A Roentgenological Study. Radiology, 28:15, 1937.


APPENDIX
FIGURE I

Photograph of animal cephalometer used to hold rat in position to be radiographed.

A. Vertical film holder
B. Horizontal film holder
C. Ear posts
D. Animal platform
E. Set screw
S. Precision-grooved slide
FIGURE 2

Photograph showing the x-ray tube head on steel dowel rods in position for taking lateral head radiographs.
FIGURE 3

Photograph showing x-ray tube head on supporting cradle in position for taking tibial and femoral radiographs and dorso-ventral head radiographs.
Figure 3
Photograph of animal in position to have lateral head radiographs taken.

F. Film in vertical film holder.

P. Moveable ear post

3. String under tension tied to upper incisors to hold head in a horizontal plane

X. X-ray tube head
FIGURE 5

Photograph of animal in position for femoral and tibial radiographs.

H. Film in horizontal film holder
FIGURE 6

Photograph of an anesthetized animal tied in position on a holding board and ready to be x-radiated in the region of the left condyle.

R. Moveable ear post used to keep head stationary and area of the condyle in the field of radiation.
FIGURE 7

Photograph of anesthetized animal covered by a lead casket in which there was a 5 x 7 mm. portal through which the right knee joint was radiated.
FIGURE 8

Photograph of an anesthetized animal that was radiated in the area of the left mandibular condyle.

L. Lead casket in which a 5 mm. portal was opened
FIGURE 9

Photograph of a General Electric therapy-size x-ray machine control panel which was used to regulate the amount of radiation given to the animals.

V. Voltmeter

A. Ammeter
Figure 9
FIGURE 10

Photograph of the radiograph projector and the measuring apparatus.

P. Micro-enlarger
S. Radiograph holder and stage
T. Measuring table
ANIMAL 2  FEMORAL LENGTH

Figure 11
Figure 13

ANIMAL 7 FEMORAL LENGTH

GROWTH INCREMENTS IN mm

TIME INTERVAL IN WEEKS
ANIMAL 9 FEMORAL LENGTH

GROWTH INCREMENTS IN mm

TIME INTERVAL IN WEEKS

Figure 14
ANIMAL 13 FEMORAL LENGTH

GROWTH INCREMENTS IN mm

TIME INTERVAL IN WEEKS

Figure 15
FEMORAL LENGTH
CONTROL ANIMALS

GROWTH INCREMENT IN mm

TIME INTERVAL IN WEEKS

Figure 16
ANIMAL 2 TIBIAL LENGTH

GROWTH INCREMENTS IN mm

TIME INTERVAL IN WEEKS

Figure 17
Figure 18

ANIMAL 3 TIBIAL LENGTH

GROWTH INCREMENTS IN mm

TIME INTERVAL IN WEEKS

ANIMAL 3 TIBIAL LENGTH

GROWTH INCREMENTS IN mm

TIME INTERVAL IN WEEKS
Figure 19

ANIMAL 7 TIBIAL LENGTH

GROWTH INCREMENTS IN mm

TIME INTERVAL IN WEEKS

L

R
Figure 20

ANIMAL 9 TIBIAL LENGTH

GROWTH INCREMENTS IN mm

TIME INTERVAL IN WEEKS

0 1 2 4 6 8 10 13

L

R
FIGURE 21.

Photograph of the enlarged radiograph showing evidence of tibial bowing as the result of x-radiating the knee joint.
FIGURE 22

Photograph of an enlarged radiograph showing a shortened tibia eight weeks after the knee joint was x-radiated.
FIGURE 23

Photograph of an enlarged radiograph showing tibial shortening thirteen weeks after the knee joint was x-radiated.
## WEIGHT CHART - CONTROL ANIMALS

<table>
<thead>
<tr>
<th>Time of Experiment</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III At One Month Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Number</td>
<td>Number</td>
</tr>
<tr>
<td>Begin</td>
<td>38</td>
<td>39</td>
<td>53</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>85</td>
<td>87</td>
<td>143</td>
</tr>
<tr>
<td>4 Weeks</td>
<td>147</td>
<td>153</td>
<td>177</td>
</tr>
<tr>
<td>6 Weeks</td>
<td>178</td>
<td>192</td>
<td>208</td>
</tr>
<tr>
<td>8 Weeks</td>
<td>194</td>
<td>211</td>
<td>213</td>
</tr>
<tr>
<td>10 Weeks</td>
<td>214</td>
<td>233</td>
<td>249</td>
</tr>
<tr>
<td>13 Weeks</td>
<td>227</td>
<td>229</td>
<td>250</td>
</tr>
</tbody>
</table>

Weight Change 189 190 186 179 184 146

Average Weight Change - 179 Grams

*Weight in Grams*
WEIGHT* CHART - EXPERIMENTAL ANIMALS

<table>
<thead>
<tr>
<th>Time of Experiment</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III At One Month Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
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<tr>
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<td>129</td>
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<tr>
<td>4 Weeks</td>
<td>121</td>
<td>124</td>
<td>158</td>
</tr>
<tr>
<td>6 Weeks</td>
<td>141</td>
<td>154</td>
<td>189</td>
</tr>
<tr>
<td>8 Weeks</td>
<td>156</td>
<td>166</td>
<td>193</td>
</tr>
<tr>
<td>10 Weeks</td>
<td>177</td>
<td>182</td>
<td>212</td>
</tr>
<tr>
<td>13 Weeks</td>
<td>178</td>
<td>207</td>
<td>237</td>
</tr>
</tbody>
</table>

Weight Change 135 164 160 155 178

Average Weight Change = 158.4 Grams

* Weight in Grams
ANIMAL NUMBER 2

MEASUREMENTS MADE FROM FEMORAL RADIOGRAPHS

<table>
<thead>
<tr>
<th>Area Measured</th>
<th>TIME INTERVAL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Begin</td>
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</tbody>
</table>

MEASUREMENT MADE FROM TIBIAL RADIOGRAPHS

<table>
<thead>
<tr>
<th>Radiated Tibia</th>
<th>TIME INTERVAL</th>
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<tbody>
<tr>
<td></td>
<td>Begin</td>
</tr>
<tr>
<td>11.20</td>
<td>12.38</td>
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</table>

<table>
<thead>
<tr>
<th>Projected Tibia</th>
<th>TIME INTERVAL</th>
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<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>11.20</td>
<td>13.42</td>
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</tbody>
</table>

Figure 26
ANIMAL NUMBER 3

MEASUREMENTS MADE FROM FEMORAL RADIOGRAPHS

<table>
<thead>
<tr>
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<th>TIME INTERVAL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Begin 2 Weeks 4 Weeks 6 Weeks 8 Weeks 10 Weeks 13 Weeks</td>
</tr>
<tr>
<td>Radiated Femur</td>
<td>7.82 9.81 11.75 12.87 13.45 13.87 14.57</td>
</tr>
<tr>
<td>Protected Femur</td>
<td>7.82 10.43 12.14 13.55 13.75 14.23 15.61</td>
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MEASUREMENTS MADE FROM TIBIAL RADIOGRAPHS

<table>
<thead>
<tr>
<th>Area Measured</th>
<th>TIME INTERVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Begin 2 Weeks 4 Weeks 6 Weeks 8 Weeks 10 Weeks 13 Weeks</td>
</tr>
<tr>
<td>Radiated Tibia</td>
<td>11.67 13.05 14.22 14.61 15.18 15.43 15.88</td>
</tr>
<tr>
<td>Protected Tibia</td>
<td>11.65 13.93 16.08 16.67 17.96 18.90 20.04</td>
</tr>
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</table>

Figure 27
ANIMAL NUMBER 7

MEASUREMENTS MADE FROM FEMORAL RADIOGRAPHS

<table>
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<th>TIME INTERVAL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Begin</td>
</tr>
<tr>
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<td>8.65</td>
</tr>
<tr>
<td>Protected Femur</td>
<td>8.65</td>
</tr>
</tbody>
</table>

MEASUREMENTS MADE FROM TIBIAL RADIOGRAPHS

<table>
<thead>
<tr>
<th>Area Measured</th>
<th>TIME INTERVAL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Begin</td>
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<tr>
<td>Radiated Tibia</td>
<td>12.77</td>
</tr>
<tr>
<td>Protected Tibia</td>
<td>12.77</td>
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</table>

Figure 28
# ANIMAL NUMBER 9

**MEASUREMENTS MADE FROM FEMORAL RADIOGRAPHS**

<table>
<thead>
<tr>
<th>Area Measured</th>
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<td>Begin</td>
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<td>Radiated Femur</td>
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<tr>
<td>Protected Femur</td>
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</table>

**MEASUREMENTS MADE FROM TIBIAL RADIOGRAPHS**

<table>
<thead>
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<th>Area Measured</th>
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<td>Radiated Tibia</td>
<td>12.43</td>
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<tr>
<td>Protected Tibia</td>
<td>12.48</td>
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</table>

*Figure 29*
# ANIMAL NUMBER 13

**MEASUREMENTS MADE FROM FEMORAL RADIOGRAPHS**

<table>
<thead>
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<th>Area</th>
<th>Measured</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Begin</td>
<td>1 Month</td>
<td>2 Months</td>
<td>3 Months</td>
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<tr>
<td>Radiated Femur</td>
<td></td>
<td>9.45</td>
<td>12.10</td>
<td>13.71</td>
<td>14.63</td>
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<tr>
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<td>12.75</td>
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</table>

**MEASUREMENTS MADE FROM TIBIAL RADIOGRAPHS**

<table>
<thead>
<tr>
<th>Area</th>
<th>Measured</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Begin</td>
<td>1 Month</td>
<td>2 Month</td>
<td>3 Month</td>
</tr>
<tr>
<td>Radiated Tibia</td>
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<td>14.05</td>
<td>16.43</td>
<td>18.38</td>
<td>19.66</td>
</tr>
<tr>
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<td>14.05</td>
<td>16.43</td>
<td>18.31</td>
<td>19.62</td>
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</tbody>
</table>

*Figure 30*
Figure 31

FEMORAL LENGTH
PROTECTED LEGS OF EXPERIMENTAL ANIMALS

TIME INTERVAL IN WEEKS

GROWTH INCREMENT IN mm

TIME INTERVAL IN WEEKS
HISTOLOGIC STUDY

The irradiated and unirradiated legs of a sacrificed experimental animal were removed in order to do a histologic study of the distal femoral epiphyseal cartilage and proximal tibial epiphyseal cartilage. This study was conducted in an attempt to show the effects of radiation on epiphyseal cartilage cells. The protected leg was studied so that some comparison could be made of normal and radiated epiphyseal cartilage. A cut was made through the decalcified femurs and tibias in order that both the distal femoral epiphysis and proximal tibial epiphysis could be included in the section.
APPROVAL SHEET

The thesis submitted by Dr. Julio Aldo Battistoni has been read and approved by 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.

5-16-62

Date

Signature of Adviser