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## A Study of Gamma Irradiated Mouse Submandibular Gland

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A STUDY OF GAMMA IRRADIATED MOUSE  
SUBMANDIBULAR GLAND

by

Mario Vincent Santangelo

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

1960

DEDICATION

To The Memory of

My Father

Anthony

## LIFE

Mario Vincent Santangelo, the second of three children, was born October 5, 1931 in Youngstown, Ohio.

His education began at Madison School. He attended Hayes Junior High School and was graduated from Rayen School in Youngstown, Ohio in June, 1949. He enrolled at the University of Pittsburgh, Pittsburgh, Pennsylvania in September, 1949 for his undergraduate studies in a pre-dental curriculum.

In September, 1951 he began his professional studies at Loyola University, School of Dentistry, Chicago College of Dental Surgery, Chicago, Illinois and was graduated in June, 1955 with a Doctor of Dental Surgery degree.

Upon completion of his professional education, he entered the United States Air Force as a dental officer in August, 1955. Awarded a commission of 1st Lieutenant, he served with the 7425th U.S.A.F. Hospital, Hahn Air Base, Germany. In March, 1956 he was appointed Base Dental Surgeon and was promoted to the rank of captain. He remained in that capacity until completion of his tour of duty in August, 1957.

In September, 1957 he received an appointment to the faculty of Loyola University, School of Dentistry, in the departments of Oral Diagnosis, Oral Pathology and Roentgenology. He then started graduate studies for a Master of Science degree in the Department of Oral Anatomy. In April, 1960 he became a member of the American Academy of Oral Pathology.

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## CHAPTER I

### INTRODUCTION

The effects of both single massive doses and smaller fractionated doses of x-ray radiation on both experimental animals and humans have been extensively reported. Relatively few of these studies have included the effects of radiation on dental and associated oral structures such as the salivary glands.

The salivary glands are comparatively sensitive to ionizing radiation. The clinical effect of x-ray radiation manifests itself as a physiological dysfunction of the gland. For this reason x-ray radiation has been used therapeutically as a means of reducing the function of the gland and thereby decreasing salivary flow in humans.

It is known that the sensitivity of a particular type of cell to radiation damage is proportional to its proliferative activity. Since the proliferative activity of the salivary gland is not known, the cellular turnover of the submandibular gland of the mouse was studied by the use of a radioactive tracer, tritiated thymidine, and the autoradiographic technic. It was then possible to compare the effects of radiation on the submandibular gland with those of other tissues.

Although much is known on the effects of x-ray radiation, few reports are available on the damaging effects of gamma radiation. This study was,

therefore, intended to investigate the effects of low chronic gamma radiation on the submandibular gland of the mouse and to decide whether these effects would manifest themselves as an alteration in the morphology of the salivary gland or a disturbance in the secretory function of the gland.

A thorough study of the cytology and histochemistry of the submandibular gland served as a basis for the comparison of any morphologic, histochemic, or histopathologic changes which might follow total body gamma radiation. The gamma radiation was administered in daily doses of twelve roentgens for periods of one hundred and two hundred days with accumulative doses of 1,200 and 2,400 roentgens respectively.



## CHAPTER II

### REVIEW OF THE LITERATURE

A study of the literature revealed an inadequate description of the morphology and histogenesis of the submandibular glands of mice. Authors made reference to the submandibular glands of mice as being similar to that of other rodents (Fekete, 1941; Sreebney, 1954). However, these authors recognized sex differences in the structure of the gland which were mainly in the type of epithelium lining the intralobular ducts. In the female the glandular epithelium was found to be striated and lined the terminal tubules as well as the intralobular ducts. In the male the cells of the terminal tubules and intralobular ducts resembled columnar cells and were described as looking like mucous cells. This sexual dimorphism was not observed in young mice from five to seven weeks of age.

Kohn et. al. (1957) reported that fifty-seven per cent of the glandular mass of male mice consisted of secretory tubules with the remaining forty-three per cent made up of the other structural elements of the gland. On the other hand, in the female only twenty-seven per cent of the glandular mass was composed of tubules with the greatest component of the gland being the acini. These investigators also observed that in the male the tubules were longer and columnar in shape containing numerous secretory granules. These observations were not as evident in the female.

Oppel in 1900 claimed that the submandibular glands of the mouse and rat were serous producing and did not contain mucous secreting cells. Stormont (1928) considered all the cells of the submandibular glands of the muskrat, rabbit, rat, and mouse as "special serous cells". This was the extent of the literature available on the mouse submandibular glands.

Radioactive isotopes are used to label compounds that play a role in the metabolism of an animal. These labeled compounds can be followed into organs and structural elements by autoradiography. The principle of autoradiography is based on the ability of these labeled organic compounds to emit rays and to produce an ionization of the silver bromide crystals impregnated in the photographic emulsion when placed over a tissue specimen. The autoradiographs when processed will then microscopically show an accumulation of black silver granules over those areas in the tissue section which metabolized the radioactive isotope.

The autoradiographic technic has been used widely as a means of study of cellular metabolism. Since first introduced its function has been as a means of tracing extremely small amounts of radioactive materials to the cytological level. London in 1904 first described a technic whereby anatomical specimens were placed in contact with photographic plates, exposed, removed from the plate and developed. Similarly in 1924 Lacassagne and Lattes, following this same principle and using pressure to insure good contact with the emulsion and the tissue section, studied histologic sections. Belanger and Leblond (1946) described a method whereby liquid photographic emulsion was painted in a thin layer over histological sections for locating radioactive elements in

tissue. This technic was modified slightly by Joffes and Warren (1955), Messier and Leblond (1957), and Joffes (1959) in that the mounted microsections were dipped into containers of liquified emulsion.

In 1947 Evans reported the mounting technic of autoradiography. In this technic the tissue specimens were floated on a photographic emulsion to insure that when developed they remained in a permanent relationship.

Pelc (1947), Boyd and Williams (1948), Doniach and Pelc (1950) and Fitzgerald et. al. (1953) described the stripping method, a procedure in which stripping film emulsion was used. The emulsion with its gelatin base was cut into small squares and stripped dry from the glass plate to which it was attached. These emulsion squares were floated on a distilled water bath with the emulsion surface against the water to allow for swelling and stretching out flat. The emulsion was placed directly over the microsection by bringing the histologic section underneath the emulsion. This allowed for a permanent relationship between the specimen and emulsion. The resolution of the resulting autoradiograph was increased considerably by this technic.

Harris et. al. (1950) introduced the freeze-dry method of preparing histological specimens to prevent the loss of soluble radioactive tracers which have a short half-life. Holt and Warren (1953) also prepared histological specimens by the freeze-dry technic following administration of  $\text{Fe}^{59}$ ,  $\text{P}^{32}$ , and  $\text{S}^{35}$ . This method theoretically prevented any displacement or leaching of the radioisotopes and thereby better autoradiographs were obtained.

Since autoradiography was first introduced, numerous studies have been conducted using various radioactive tracers. Some of those most frequently

used to label injected compounds include the following:

(1) Radiophosphorous ( $P^{32}$ ) which has a half-life of fourteen days.

Since one cellular location of phosphorous is in the desoxyribonucleic acid (D.N.A.) molecule, this tracer becomes a part of the chemical structure of D.N.A. making it a suitable tracer in D.N.A. synthesis studies. However, this tracer also enters the ribonucleic acid (R.N.A.) molecule and for this reason it is necessary to first remove the R.N.A. by ribonuclease to limit its localization to D.N.A. One of the greatest disadvantages to its use is that the beta rays which are emitted leave its origin in the nucleus at a wide angle producing electron tracks relatively far from its cell origin. This gives poor resolution to the autoradiograph. In 1948, Leblond et. al. injected  $P^{32}$  into animals and were able to localize by the autoradiograph newly synthesized D.N.A. in those tissues where mitoses were most numerous. They noted the greatest activity in the cortex of the thymus gland and in lymphatic tissue. Pelc and Spear (1950) demonstrated the uptake of  $P^{32}$  by avian fibroblasts using tissue cultures. Howard and Pelc in 1951, using the radioisotope  $P^{32}$ , localized nuclei and chromosomes of the bean root cell, *Vicia faba*, by the autoradiograph. Adams and Miller in 1952 grew spores and fungi on media containing  $P^{32}$ , washed the cells on emulsions and localized the radioactivity in mycellum and condida from the same culture when the autoradiographs were developed.

(2) Radioactive carbon ( $C^{14}$ ), a soft beta ray emitter, gives excellent resolution in the autoradiograph; however, its greatest disadvantage is in its extremely long half-life of 5,500 years. Investigations utilizing  $C^{14}$  includ-

ed those of Boyd et. al. (1948) who studied blood cells and those of Boyd and Levi (1950) in which the incorporation of  $C^{14}$  in liver was studied. In 1953, Fitzgerald traced paramecia using glycine labeled with  $C^{14}$ ,  $S^{35}$ , and tritium. Levi in 1954 traced  $C^{14}$  and  $S^{35}$  quantitatively to single cells of yeast and algae.

(3) The isotope  $I^{131}$  with a half-life of eight days was used by Fitzgerald in 1948 to study the morphology of the thyroid gland. Bennett and Gorbman (1951) investigated the survival of grafts of thyroid gland tissue in mice by demonstrating the concentration of  $I^{131}$  in the transplanted glands. Damerton (1953) demonstrated a concentration of  $I^{131}$  in live homographs of thyroid tissue transplanted to the anterior chamber of the eye.

(4) Campbell in 1951 used  $S^{35}$ , half-life of eighty-seven days, to study its uptake by cartilage. Swarm et. al. (1959) investigated the retention and redistribution of the isotope  $S^{35}$  in transplants of cartilage.

(5) Arnold and Jee (1959) reported the localization of  $Ra^{226}$  in bones of dogs. They found that  $Ra^{226}$  was deposited in highest concentration in the rapidly calcifying bone matrix found beneath the osteoblastic surface. However, it was also observed to be deposited in low, relatively uniform concentrations throughout the pre-existing old bone.

(6) Tritium ( $H^3$ ), the isotope of hydrogen with a mass of three, is an emitter of very weak beta rays with a maximum beta particle energy of 17.9 KEV. It has a maximum range of six microns, an average range of one micron, and a half-life of 12.26 years. Pyrimidine bases such as cytosine and thymine or its desoxyribonucleoside, thymidine, have been tritiated. "The method con-

sists of sealing a gram of an organic compound with a few cubic centimeters of tritium gas and allowing the sample to stand at room temperature and at sub-atmospheric pressure for from three to ten days. Under these conditions exchange occurs between the hydrogen atoms of the organic compound and the tritium gas. The compounds are labeled non-specifically and possibly non-uniformly, but this does not interfere with tracer studies concerning their in toto utilization and distribution in organisms and cells", Kisielewski and Smetana (1958). When thymidine is tritiated it is presumably labeled in the pyrimidine portion of the molecule where hydrogen is bound to the carbon in the pyrimidine ring. The specific activity of tritiated thymidine is approximately 300 curies per mole. Before injection it is diluted with isotonic saline solution to the desired concentration.

Tritiated thymidine is a particularly suitable tracer for biochemical localization studies especially for D.N.A. metabolism, chromosomal replication and generation times of cells. The reason is that it is a stable radioactive label with a high specific activity. A great advantage in using tritiated thymidine is the damage of radiation to cells which utilize the label is reduced making it possible to use doses allowing cells to be traced over periods of months. Tritiated thymidine is incorporated into cells without any apparent signs of radiation induced chemical degradation or damage to cells utilizing the label, Kisielewski and Smetana (1958). Following the introduction of tritiated thymidine the sections need not be subjected to any chemical extractions as with other labeling compounds since D.N.A. is the only molecule which will utilize the precursor thymidine. This is verified by the fact that by

treating tissue sections with D.N.Ase, all traces of radioactivity with the autoradiograph are eliminated.

Hughes et. al. (1958) stated "In order to distinguish the renewal of cells from the renewal of cellular constituents, the label must be incorporated into a fixed component of the cell which is not lost during the cells' lifetime and present knowledge suggests that a label incorporated into D.N.A. should be most useful for this purpose." Cronkite et. al. (1959) concluded from their studies that "although thymidine is apparently not a normal precursor of D.N.A., it can enter the synthetic chain and label D.N.A. at the time of D.N.A. doubling prior to mitosis. Thus if a label is sufficiently intense and permanent one can by autoradiography follow cells from the time of D.N.A. synthesis to ultimate death."

When tritiated thymidine is injected intraperitoneally it is apparently rapidly absorbed by the cells which are undergoing D.N.A. synthesis since it is a specific part of the D.N.A. molecule. Those cells which pick up the label become permanently marked and are subject to dilution only by cell division. This was corroborated by Leblond et. al. (1959) who stated that it is known that mitosis is preceded by a synthesis of D.N.A. If a precursor of D.N.A. which has been made radioactive is administered at the time of D.N.A. synthesis, the nuclei become radioactive and may be recognized by autoradiography even before mitosis takes place. Such nuclei retain the label during actual mitosis and pass it on to the daughter cells. The interval between completion of D.N.A. synthesis and the beginning of mitosis measures the time required for the appearance of labeled mitotic figures.

Personal communication with Samuel Lesher, Ph.D., of the Argonne National Laboratory explained from his studies using tritiated thymidine that the estimated time that tritiated thymidine was available for use was one hour following injection. It was shown that ninety-five per cent of the label was either used by the synthesizing cells or excreted by the body after one hour.

Thymidine has been shown to be rapidly incorporated intact into the nucleus D.N.A. of a wide variety of proliferating cells, making it an ideal carrier for introducing radioactivity into cells (Reichard and Estborn, 1951; Friedkin et. al, 1956; and Friedkin and Wood, 1956). Lajtha et. al. (1959) found from their studies that thymidine is a specific component of D.N.A. and is important in that it labels D.N.A. only. The findings of Painter and Drew (1959) confirmed the specificity by demonstrating the incorporation of tritiated thymidine into D.N.A. by bacteria and HeLa cell cultures.

Taylor et. al. in 1957 used tritiated thymidine to label the distribution of newly formed D.N.A. in mitotic chromosomes of bean root tip cells, *Vicia faba*, and demonstrated that the thymidine was incorporated "exclusively into D.N.A." They concluded that the labeled thymidine became part of a physical entity that remained intact. They followed their distribution during replication and subsequent nuclear divisions using photographic emulsions. Autoradiographs showed an apparent equal and uniform distribution of newly synthesized D.N.A. in both daughter chromosomes resulting from the first mitosis in the presence of labeled thymidine. Subsequent duplication in the absence of the labeled precursor of D.N.A. limited the tritium label to only one of each two daughter chromosomes. These observations indicated that the D.N.A. was synthe-



sized as a unit extending throughout the length of the chromosome and that each unit remained intact through succeeding replication and nuclear divisions. Each chromosome is composed of two such units, complementary to each other and each replication resulted in a chromosome with four units. This chromosome then divided in such a way that each chromatid regularly received an "original" and a "new" unit. This pattern of replication was analogous to the replicating scheme proposed for D.N.A. by Watson and Crick in 1953. As mitoses through subsequent generations recurred, the concentration of the label in the nuclei became more and more dilute due to this pattern of distribution to the new daughter cells.

Painter et. al. in 1958 investigated the distribution of tritiated thymidine in *Escherichia coli* during cell multiplication and reported that over several generations no loss of label occurred in the total population.

In 1958, Hughes et. al. studied cell renewal in the gastrointestinal tract of mice using tritiated thymidine. Brecher et. al. in 1958 determined the mitotic cycle and cell renewal of epithelium of the gastrointestinal tract. In 1959, Leblond et. al. investigated the cell renewal in various tissues and organs of mice and rats with tritiated thymidine.

Quastler et. al. (1958) described the influence of radiation upon cell renewal, maturation, and decay in the gastrointestinal tract of mice. Bond et. al. (1958), Fliedner et. al. (1958) applied tritiated thymidine to study radiation induced aberrations of the proliferative potentials and mitosis in dogs and mice.

Fitzgerald and Vinijchaikul in 1959 reported that in rats approximately

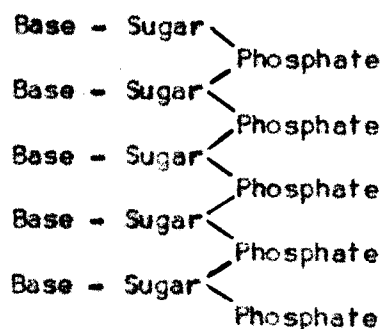
one per cent of pancreatic acinar cells concentrate radioactivity in their nuclei after injection with tritiated thymidine. They concluded that "the low percentage of labeled cells after tritiated thymidine is consistent with the low mitotic index of normal pancreas and is indicative of a low turnover of D.N.A. and cells."

The application of radioactive isotopes in the study of biosynthesis of D.N.A. has made possible studies determining its role in chromosomal replications. The behavior of D.N.A. during mitosis has been of interest because little is known about the process by which D.N.A. exerts its influence on cells. D.N.A. is a constituent of the chromatin material which has been found solely in the nucleus of cells. Since it is associated with the chromosomes of the nucleus, it is responsible for the genetic specificity of cells. In combination with proteins it forms the nucleoprotein of the genes which these cells pass on to daughter cells during mitosis, Stowell (1945) and Schultz (1941). Hughes et. al. (1958) stated that since D.N.A. is generally believed to contain the genetic characteristics which cells pass on to daughter cells it should remain unaltered. Evidence seems to show that D.N.A. is never replaced in a cell. The amount of D.N.A. remains constant in the chromatin material and is metabolically stable, Pelc (1959).

D.N.A. synthesis is believed to be intrinsically associated to cell division and it is generally accepted that D.N.A. is only synthesized by cells which are preparing for mitosis. It is only during interphase between mitoses that synthesis of D.N.A. occurs. Hughes (1957) using tritiated thymidine reported that chromosomes of bean root cells were "transmitted to daughter

cells as intact half-chromosomal units." These units act most likely as the templates to form new genetic material. Pelc in 1959 stated that D.N.A. directs and influences the activity of cells by serving as a template but it remains unaltered.

Watson and Crick (1953) established a basic chemical formula for D.N.A. D.N.A. is composed of alternate sugar and phosphate groups joined together in regular phosphate di-ester linkages. Each sugar is attached to a nitrogenous base. Four different types are found in D.N.A. Adenine and guanine are purines, while thymine and cytosine are pyrimidines. In certain organisms a fifth, 5-methyl-cytosine, may be present. Each purine or pyrimidine is present in nucleic acid as a phosphoriboside or nucleotide. The individual nucleotides are joined by phospho-ester linkages to form nucleic acids as follows:



Although the incorporation of radioisotopes into the D.N.A. molecule have led to a better understanding of its function by tracing them by the autoradiographic technic, studies of the histochemistry of this nuclear component were introduced by Feulgen and Rossenbeck in 1924. It is based on a modification of the periodic acid Schiff (P.A.S.) reaction for aldehydes. As origin-

ally reported the usefulness of this reaction was to demonstrate the concentration of D.N.A. in nuclei of cells. In general, investigators believe the Feulgen method is a specific reaction for D.N.A. Milovindov in 1938 cited some four hundred references of research confirming the specificity of this reaction. Stowell in 1945 stated that the amount of evidence available indicated that the Feulgen method for D.N.A. was one of the most specific histo-chemic reactions. Mazia and Jaeger (1939) demonstrated that D.N.A. was attached to chromosomes in *Drosophila* and showed that only D.N.A. was stained by the Feulgen technic. The studies of Wyckoff et. al. (1932) agreed that the Feulgen method was specific for D.N.A. localization in nuclei using ultraviolet light. The works of Caspersson in 1939, using ultraviolet photometric means, demonstrated that the amount of D.N.A. in the nuclei of cells could be estimated quantitatively by the amount of light absorption in Feulgen stained specimens. Other investigations supporting the specificity of this reaction were that in tissues treated with D.N.Ase prior to staining, rendered the tissue Feulgen negative.

Modifications have been introduced by deTomasì (1936) in the preparation of the stain. Coleman in 1938 used activated charcoal to remove extraneous coloring impurities from the stain to prevent staining of the cytoplasm.

Stowell (1945) described the Feulgen reaction in two parts. The first step was a mild hydrolysis which split the purine bases and the carbohydrate of the D.N.A. and liberated the aldehyde group of the aldo-pentose sugars. D.N.A. readily loses its purine bases in cold acid. The second step was a reaction between the liberated aldehyde groups and the leuco-basic fuchsin

reagent (fuchsin sulfurous acid). In the formation of this reaction, basic fuchsin is reduced and decolorized by the action of sulfur dioxide. After immersing the partially hydrolyzed tissue in this fuchsin sulfurous reagent, the portions of nuclei containing D.N.A. stained an intense reddish purple.

Since clinical evidence was first introduced supporting the hypothesis that ionizing radiation produces damaging effects on humans, an increased interest in research on this subject has resulted. A survey of the literature disclosed a number of studies on the effects of x-ray radiation on living cells. Those reviewed will be limited mainly to the salivary glands.

A study by Ivy et. al. in 1923 showed that x-ray radiation caused a reduction in saliva with a subsequent reduction in the organic component and the total solids in the saliva of dogs radiated about the head. The minimum dose required to depress the secretory activity was 110 Kv, 10 Ma, 25 cm. focal-skin distance, portal of entry 28 cm. square, one millimeter aluminum filter, and an exposure time of forty seconds. No histologic changes were immediately observed following administration of these relatively large doses of x-ray radiation. Fifteen days following radiation they observed a slight fibroblastic proliferation, an infiltration of round cells, and a reduction in the stored mucins in the alveoli. Two months after the x-ray radiation exposure, they noted a marked increase in connective tissue. However, there was a relative lack of round cell infiltration at this time.

The work of Case and Boldyreff (1925), studying the effects of x-ray radiation at a dose of 450 milliamperes-minute in dogs, showed a marked decrease in salivation which was reversible. The decreased function of the sali-

vary glands had reached its peak about three or four weeks after radiation. After this time the gland gradually returned to its normal function.

The study of Tsuzuki (1926), employing hard x-ray radiation, demonstrated an atrophy of the acinar cells of salivary glands of rabbits following a forty-two per cent erythema dose to the rabbit. Following a fifty-six per cent erythema dose, a fatty degeneration was observed in the excretory duct epithelium.

English and Tullis in 1951 described the effects of total body x-ray radiation on the salivary glands of twenty-four swine. At a mean age of 157 days these swine were exposed to 450 to 500 roentgens of radiation. Nine hogs were found dead and the necrotic changes observed in the acini and ductal cells of the salivary glands were suggestive of autolysis rather than radiation. Seven animals were found moribund after the eleventh to fifteenth day and were sacrificed. A decreased number of granules in the mucous cells were observed. Eight animals survived the exposure after thirty days and were sacrificed at this time. Histologically it appeared that the salivary glands recovered from any damage which might have previously existed.

Pindborg et. al. in 1954 reported the effects of x-ray radiation on the salivary glands of mice. Mice subjected to single doses of 625 roentgens of radiation at a rate of 14.5 roentgens per minute showed no observable changes in any of the three major salivary glands which could be definitely related to x-ray radiation. Their observations included "alteration in nuclear size of the submandibular gland acini, vacuolation of alveolar cells in each kind of gland, shrinkage in size of acini or tubules with reduction in number of cells.

None of these changes were sufficiently regular to be considered significant."

Shafer in 1953 investigated the effects of both single massive doses and multiple smaller doses of x-ray radiation applied selectively to the heads of rats. Rats were exposed to single doses of x-ray radiation ranging from 480 roentgens to 3,500 roentgens in the region of the salivary glands. They were sacrificed twenty-one days later. Alterations observed in the sublingual and parotid glands were inflammatory cell infiltration, congestion, edema, and a degeneration of the cells of the parenchyma which was suggestive of albuminous degeneration. The submandibular gland, however, was found to be resistant to radiation damage. Using repeated doses of x-ray radiation with an accumulative dose ranging from 4,500 to 8,000 roentgens, the changes in the sublingual and parotid glands were similar to those found in the lesser single doses. He noted that the changes were more severe in nature. The submandibular gland, however, remained relatively resistant to any observable histologic alteration with even the multiple doses of ionizing radiation. These findings appeared to be contrary to those of English (1954). English observed morphologic alterations in the submandibular glands of rats after sixteen hours to one hundred days following a dose of 1,000 to 1,200 roentgens. These changes were characterized primarily in the size, shape, and staining qualities of the nuclei of the acinar cells.

Burstone in 1953 found that the cell nuclei of radiated mouse salivary glands showed a chromatin splitting similar to that produced by the action of D.N.Ase. Similar changes were also observed in the cells of the intralobular ducts.

English et. al. (1954) cited the effects of 1,000 to 1,750 roentgens of x-ray radiation administered locally to the heads of dogs. They noted an increased fibrosis, atrophic and morphologic changes in the salivary glands after periods of up to eight months following radiation. After fourteen and three quarter months the effects of radiation became less apparent and after fifteen and one half months, the salivary glands appeared to return to normal.

The work of Cherry and Glucksmann in 1959 showed that in daily doses of 850 roentgens with accumulative doses of 3,000 roentgens to 7,650 roentgens of x-ray radiation, both ductal and acinar changes occurred. The excretory duct cells were seen to be degenerated and collapsed. The acini also showed degenerative changes characterized primarily as a reduction in mass accompanied by an increase in the interstitial connective tissue. There was also an inhibition of the secretion of saliva. The degenerative changes of the nuclei were karyorhexis, chromatolysis and pyknosis. At doses of less than 3,000 roentgens the excretory and intercalated ducts were not markedly affected and the acini showed only transient changes which were capable of repair.

Burstone (1950) injected colloidal gold with an activity of seventeen microcuries per cubic centimeter in mice which were only one to two days of age. He noted that after eight to sixty days the mucous secreting salivary glands appeared relatively normal. Some degree of acinous swelling and a decrease in number of nuclei were observed however. The serous glandular epithelium appeared condensed with some of the acini containing empty spaces. The nuclei appeared to be shrunken. The ductal elements remained relatively unchanged.



Kasboun in 1953 observed salivary glands of humans who had received radiotherapy for malignancies. Following x-ray therapy of from one week to two years, he studied the salivary glands removed during radical neck surgery. In his studies he noted a fatty change, atrophy of the gland and an accompanying sialadenitis. He also reported that there was a history of decreased salivation in all the patients from whom the glands were removed.

Clinical studies on humans irradiated in the region of the neck for malignancies showed a quantitative change in the salivary flow. It was felt that these quantitative changes also had accompanying qualitative changes in the saliva. del Regato in 1939 reported that the modification in the secretion of saliva as a result of irradiation of the salivary glands should be considered as one of the factors in the production of dental lesions. He suggested that a qualitative rather than quantitative change might result from radiation.

Schneyer (1953) studied the function of the salivary gland after administration of radioactive iodine in the treatment of thyroid carcinomas and hyperthyroidisms. He measured the salivary amylase levels following the administration of  $I^{131}$  with an activity of from 8 to 51 microcuries. After the fourth day of administration of the  $I^{131}$ , he noted that the salivary amylase activity was decreased considerably. This was reversible, however, and returned to its normal level between the fourth and tenth days. He concluded from his study that the salivary gland function was suppressed by radioactive iodine.

A study by Schlack and Ellinger in 1951, following a total body x-ray

radiation of two hundred roentgens, showed an increased incidence of dental caries in rats put on a cariogenic diet. However, rats on a non-cariogenic diet and exposed to the same amount of radiation did not show such increased incidence of caries.

Bloom (1948) studied the effects of x-ray radiation on the bone marrow of mice. With an exposure of 350 roentgens, histologic examination showed a depleted marrow characterized by greatly widened sinuses and small amounts of gelatinous material. Debris of dead erythroblasts and granulocytes was noted three hours following treatment. It reached a maximum within fourteen hours. In the metaphysis and epiphysis there was practically no hematopoiesis observable.

## CHAPTER III

### MATERIALS AND METHODS

The salivary glands used in this study were secured from CAF-1 mice. Such mice were hybrids developed by a cross between the C<sub>57</sub> Black and Albino mice. They were kept in wire cages with pine shavings as bedding. They subsisted on a diet of Wayne's Laboratory Blox and water ad libitum. Twenty-four mice were used in the tracer series and twelve in the radiation series. The tracer series of mice also served as the control for the radiation series.

#### Tracer Series

At two hundred days of age, the mice were injected, intraperitoneally, with 0.05 cc. of an isotonic saline solution of tritiated thymidine with an activity of fifty microcuries. Two mice were sacrificed, using ether, at the following intervals: fifteen, thirty, forty-five minutes, one, two, four, twelve, twenty-four, forty-two, forty-four, forty-six, and forty-eight hours. The parotid, submandibular and sublingual glands were grossly dissected; and a three cubic millimeter sample was removed from the submandibular gland and fixed in a ten per cent neutral formalin solution. The specimens were dehydrated in alcohol, embedded in paraffin and sectioned in series at three microns. Five sections, each tenth in series, were mounted on microscopic slides, deparaffinized with xylol, stained by the Feulgen method to localize nuclear desoxyribonucleic acid (D.N.A.), and placed in distilled water. Such slides

were brought to the dark room immediately.

The dark room was maintained at low humidity, seventy per cent, and at room temperature, twenty degrees centigrade. The only light was supplied by a #1 Wratten, red, ten watt safelite. The film used was Kodak AR-10 fine grain stripping film. The emulsion was attached to a five inch by seven inch plate of glass. With a sharp scalpel the emulsion was cut into approximately two and one-half inch squares. Each square was carefully removed and floated emulsion-surface down on a water bath, eighteen degrees centigrade, for two minutes, to allow for slight swelling and stretching out flat. The slides then were removed from the distilled water and immersed in the water bath beneath the floating emulsion squares. The slide was lifted carefully until the emulsion square draped intimately over the slide. Such slides were air dried for ten minutes. Ten such slides were placed section side up in every other slot of a twenty-five slot black light-tight plastic box. Five grams of lithium chloride were wrapped in tissue paper and placed in the last five slots of the box. This dessicant was used to maintain a low humidity in the exposure box in order to minimize background exposure of the emulsion. Finally, the box was sealed with black tape and stored on end, section side up, at room temperature and at low humidity (seventy per cent).

After a self exposure period of thirty days, the slides were brought to the dark room for processing. The stripped slides were transferred into glass staining racks. The developing solution was freshly prepared using Kodak developing formula, D 19b, as follows:

Elon 2.2 grams

Sodium sulfite (anhydrous) 72 grams

Hydroquinone 818 grams

Sodium carbonate (crystalline) 120 grams

Potassium bromide 4 grams

Water to make 1,000 cc.

The chemicals were dissolved in water in the order given and the solution was used without dilution. The stripped slides were developed for five minutes at eighteen degrees centigrade and rinsed thoroughly for thirty seconds in distilled water. Following washing, they were transferred into a stock preparation of Kodak acid fixer for ten minutes. The final processed stripped slides, autoradiographs, were washed in running tap water for twenty minutes and allowed to dry in a dust free atmosphere. To allow for easier trimming of the excess emulsion, the slides were dipped in distilled water for thirty seconds and the emulsion trimmed with a scalpel. The slides were mounted with cover slips using Canada balsam.

The autoradiographs were studied microscopically using oil immersion at 1,000 magnifications. All acinar cells of the submandibular gland were counted using a hand counter on a few of the autoradiographs. After counting various typical fields it became apparent that reproducibility was high and that an average of two hundred and fifty acinar cell nuclei were present in each of the typical oil immersion fields. Thereafter, a total of twenty separate oil immersion fields were selected at random for counting so that a minimum of five thousand acinar cells in each section were counted. This procedure of tabulating was followed for each of the autoradiographs. The counter was then

used to tally the number of nuclei with evidence of radioactivity (labeled cells), indicated by the presence of small black silver granules or grains above the nuclei, in each of the twenty fields. The total number of radioactive tagged nuclei in five thousand cells were then recorded as percentages. The percentages of tagged nuclei for each of the specimens were then plotted in a distribution curve with the percentage of labeled nuclei on the ordinate and time after injection on the abscissa.

In addition to the specimens used in the tracer series, five cubic millimeter samples of the submandibular glands were fixed in formalin, Carnoy solution, and one per cent trichloroacetic acid. The specimens were embedded in paraffin and sectioned at five microns. The formalin fixed tissues were stained with hematoxylin and eosin (H & E) for routine histologic study. The periodic acid Schiff (P.A.S.) method, as modified by Coleman, was used to stain mucopolysaccharides. Specimens fixed by formalin and by Carnoy solution were stained by the Brachet method for ribonucleic acid (R.N.A.). The trichloroacetic acid fixed specimens were stained for alcohol insoluble sulfhydryl groups using the reagent 2,2'-dihydroxy-6,6'-dinaphthyl disulfide, (D.D.D.), developed by Barnett and Seligman (1952). Silver impregnation and Mallory trichrome stains were also employed to specifically define collagen fibers.

#### Radiation Series

Twelve mice one hundred days old of the same litter and sex as the tracer series were placed in solid plastic cages containing pine shavings as bedding. They subsisted on the same diet as the controls. They were housed in the "gamma room". The "gamma room" was designed with heavy leaded walls and in

the floor at the center of the room was a deep recess housing several canisters containing cobalt 60. An automatic, mechanical, timing device simultaneously sealed off the entry into the room and raised the cobalt 60 gamma radiation source from its recess in the floor. At 10:40 P.M. each night the cobalt 60 source was raised and at 7:00 A.M. it was lowered into its recess. At the beginning of the study the cobalt 60 had an activity of 5.7 curies. To maintain a constant radiation dose level of twelve roentgens each day, the decay of cobalt 60 was compensated by increasing the time the source remained raised in the "gamma room" by five minutes every thirty days.

The mice were placed at a distance of ninety-two inches from the cobalt 60 source of gamma radiation. At this distance the mice were exposed to twelve roentgens over an eight hour period as measured by a dosimeter. Preliminary studies revealed these mice to live out their normal life span at doses of twelve roentgens each day. The normal life span is eight hundred and fifty days mean while the maximum life span is thirteen hundred days.

Six mice were sacrificed after one hundred days of radiation. At this time the mice were two hundred days old and had received a total of 1,200 roentgens of low chronic gamma radiation. All animals survived the radiation period. The other six mice were sacrificed after two hundred days of radiation. At this time they were three hundred days old and had received a total cumulative dose of 2,400 roentgens of low chronic gamma radiation. These mice also survived the radiation. The submandibular glands were removed and prepared for histologic study as in the control group.

## CHAPTER IV

### FINDINGS

#### Normal Histology of the Submandibular Gland

Morphologically the submandibular gland of the mouse may be classified as a tubulo-alveolar gland. The submandibular gland and sublingual gland are supported by a common capsule. Arising from the capsule, septa penetrate the substance of the submandibular gland and divide it into lobules. The collagenous fibers forming the septa envelope the interlobular ducts and accompanying arteries and veins, producing a characteristic triad. (Plate VII Figure 1) The collagenous fibers become more delicate as they continue around the intra-lobular ducts, intercalated ducts and the acini. (Plate VIII Figure 1)

In sections stained with hematoxylin and eosin the acinar or alveolar cells assume more or less a triangular shape. The dark staining, large round or oval nuclei are situated close to the broad base of the cell. The nuclei usually contain one or more visible nucleoli. A relatively thin basement membrane completely encircles the acinus. Between basement membrane and the acinar cells are stellate shaped myoepithelial cells which send out long processes forming a syncytium. The cytoplasm of the acinar cells stains basophilic and contains vacuoles and granules. The acinar cells continue into the cells lining the intercalated ducts. These small ducts are lined by flat to low cuboidal cells. The nuclei, which occupy a central position in the ductal



cell, are oval in shape. Their cytoplasm contains few granules. These low cuboidal cells change rather suddenly into the densely granular low columnar epithelium of the intralobular ducts. The cells of these ducts rest on a thin basement membrane and have a large round or oval nucleus in close proximity to their base. In the cytoplasm between the nucleus and the lumen of the ducts are located coarse, densely packed, heavily eosinophilic granules. (Plate III Figure 1) At the base of the cell the cytoplasm is characterized by a brush or striated border. The intralobular ducts may appear "S" shaped, "U" shaped, and curved in sections of the gland. In the interlobular ducts the cells change from low columnar into high columnar. Their nuclei have a basal position in the cell. The cytoplasm is less granular than that of the cells in the intralobular ducts. (Plate II Figure 1)

The secretory granules of the acinar cells and intralobular duct cells stain positive for mucopolysaccharides by the P.A.S. (Coleman) method. These granules are more numerous, densely packed and more intensely stained in the intralobular duct cells than in the acinar cells. (Plate IV Figure 1)

The R.N.A. positive substance appears, characteristically, as curved, parallel, perinuclear filaments. These filaments extend from the perinuclear area peripherally to the cytoplasmic membrane. This extension of filaments ranges from the base of the cell to the secretory pole of the cell. Focal accumulations are not only perinuclear but are also found around the intercellular central canaliculi of the individual acini. However, the cell center is free of such structures, and also the areas of the cell occupied by the vacuoles and secretory granules. (Plate V Figure 1) The striated borders of the

Intralobular duct cells are R.N.A. positive after Carnoy fixation. After formalin fixation, however, these borders appear R.N.A. negative.

With D.D.D. for alcohol insoluble sulfhydryl groups the acinar cells show a definite perinuclear positivity that extends from the nucleus through the cytoplasm as granules or as a continuous reticulum surrounding non-staining granular spaces to the cytoplasmic membrane. The striated borders of the intralobular duct cells stain moderately intense. (Plate VI Figure 1)

The Feulgen method reveals the greatest concentrations of D.N.A. in the nuclei of the acinar cells. The chromatin material is granular and stains intensely. (Plate IX Figure 2) The nuclei of the ductal elements are much larger in diameter, paler staining and the chromatin material is not as densely packed as in the acinar cells. The spindle-shaped stromal nuclei are almost as intensely stained as those of the acinar cells.

The stroma and the capsule of the gland were studied by the Mallory method and by silver impregnation. The greatest numbers of supporting fibers are seen in the capsule. The numbers of fibers decrease as they form the septa dividing the gland into lobules. Fibers, arranged in bundles, but of a more delicate nature, envelope the periductal elements. (Plate VII Figure 1) The fibers become very thin and thread-like, resembling a fibrillar reticulum, as they support the intralobular ducts and acini. (Plate VIII Figure 1)

#### Tracer Study

Following injection of the labeled compound, tritiated thymidine, those nuclei which metabolize the tracer become labeled and are recognized by the presence of small black grains over the nuclei in the autoradiograph.

(Plate IX Figure 1) Although such labeled nuclei are very few in number, they usually are found in clusters. (Plate X Figure 1) The distribution of the clusters of labeled nuclei are found at random, indicative of an asynchronous pattern of cell division.

The proliferative activity and D.N.A. biosynthesis of the submandibular gland of the normal mouse studied by the autoradiographic technic shows that between fifteen minutes and one hour following injection of tritiated thymidine the percentage of labeled nuclei of the acinar cells rises slowly to 0.30 per cent. From one hour through four hours following the introduction of the labeled thymidine, an insignificant rise in the percentage of labeled nuclei to 0.34 per cent is noted. The percentage of labeled cells doubles between four hours and twelve hours from 0.34 per cent to 0.74 per cent indicating that mitosis has occurred at this time. Despite an insignificant increase in labeled nuclei from twelve to forty-eight hours, 0.74 per cent to 0.76 per cent, there is evidently a plateau of cells in interphase after twelve hours.

(Plate I Figure 1)

#### Effects of Gamma Radiation

The mice in the one hundred day radiation period with an accumulative dose of 1,200 roentgens reveals neither any gross changes nor any significant histologic alteration in the submandibular gland. The cytology of the gland studied with H & E shows no detectible change following this radiation period. With the special histochemic stains the nuclear concentration of D.N.A., the R.N.A. activity, and the cytoplasmic mucopolysaccharide granules as demonstrated with P.A.S. appear normal microscopically when compared to the control animals.

The experimental animals which received gamma radiation for two hundred days and an accumulative dose of 2,400 roentgens did show moderate to severe degenerative signs. At autopsy the submandibular glands appear much smaller than those of the control animals. Most of the acini are atrophic. The staining ability of the nuclei and cytoplasm of the acinar cells is reduced with a loss of basophilia. In some of the acini the nuclei appear to be compressed against the base of the cell. A great number of the acinar cell nuclei exhibit karyolysis. In others there is complete nuclear destruction. The cell membranes in many of the groups of acini are indistinguishable. The cytoplasm shows severe hydropic degeneration in some cases, almost completely obliterating the cytoplasm. (Plate III Figure 2)

The epithelium of the intralobular ducts also exhibits severe histopathologic and histochemic changes as a result of radiation. These changes are manifested as signs of degeneration characterized by a marked variation in staining ability. With the special histochemic stains for alcohol insoluble sulfhydryl groups using D.D.D. (Plate VI Figure 2), R.N.A. activity (Plate V Figure 2), and the mucopolysaccharide granules with P.A.S. stain faint. The cells as a whole show signs of swelling of the cytoplasm. The characteristic striated or brush borders of these cells are almost completely lost. The cells of these ducts which normally exhibit an extremely granular, densely packed, P.A.S. positive cytoplasm reveal a marked diminution in the number of granules. (Plate IV Figure 2) The intralobular duct cells show karyolytic changes also. Some of the ducts are completely atrophied. The cells of the interlobular and intercalated ducts also show moderate atrophic changes and stain pale.

The supporting argyrophilic fibers of the submandibular gland manifest slight changes, namely a decrease in numbers of fibers supporting the gland. (Plate VII Figure 2) (Plate VIII Figure 2)

The gland as a whole does not show any significant evidence of edema or increased fibrosis. Although the larger blood vessels appear normal, some of the capillaries which accompany the intralobular ducts appear dilated and congested. There is a slight infiltration of lymphocytes with occasional scattered plasma cells most frequently found perivascularly, signs of a mild sialadenitis.

## CHAPTER V

### DISCUSSION

The submandibular gland of the mouse shows prominent sexual differences. The gland of the male, however, was found to be similar in many details to that of other rodents. For this reason only male animals were used in this study.

The study of the function of this gland by histochemic stains revealed that there was an abundance of P.A.S. positive, secretory granules in the cytoplasm of the acinar cells and of the cells of the intralobular ducts. The granules in the ductal cells stained more intensely than the fewer granules of the acinar cells. This may be due to the greater number of granules in the intralobular duct cells or to the presence of more free aldehydes that were available in the intralobular duct cells.

The R.N.A. activity was greater in the cytoplasm of the acinar cells than in the intralobular duct cells. The R.N.A. distribution within the cytoplasm was characterized by parallel, curved, filamentous structures that appear to terminate at the cell wall. The filaments had a definite perinuclear concentration. R.N.A. activity was absent in the striated borders of the cells in the intralobular ducts. Although the secretory granules were negative to R.N.A. stain, they were located in close proximity to the R.N.A. positive filaments. This observation is interesting in the light of recent reports of the relation of ribosomes located on plasma membranes and the function of plasma

membranes in synthesizing secretory granules. Gay, 1960

The alcohol insoluble sulfhydryl groups stained with D.D.D. showed a similar cytoplasmic distribution as the R.N.A. positive material. This suggested that some of the enzymes produced by R.N.A. might contain alcohol insoluble sulfhydryl groups or that R.N.A. activity and alcohol insoluble sulfhydryl groups were associated.

The nuclei of the acinar, ductal and stromal cells of the submandibular gland were the only parts of the cells which stained positive by the Feulgen method for D.N.A. This finding supported other reports that the nucleus is the only part of the cell which contains D.N.A., a specific component of chromatin. D.N.A. was found in greater concentration in the nuclei of the acinar cells than in those of the intralobular duct cells. The greater intensity of staining may be indicative of an increase in quantity of D.N.A.

Following intraperitoneal injection of tritiated thymidine those nuclei of the cells of the submandibular gland which were, during interphase, synthesizing D.N.A. at the time of injection and during the first hour were recognized by the presence of black silver granules in the autoradiograph. The labeled D.N.A. was transmitted to the daughter cells during mitosis which led to dilution of the labeled material through repeated divisions. The labeled cells were distributed in a random manner indicative of an asynchronous pattern of mitoses. However, the dividing cells seemed to be distributed in small clusters. Furthermore, more labeled cells were observed in the acini than in the ducts. This indicated that the acinar cells had a faster turnover than the intralobular ducts.

The acinar cells became labeled fifteen minutes following injection of the labeled thymidine. Although only 0.04 per cent of the acinar cells became labeled after fifteen minutes, it was evident that some of the acinar cells were synthesizing D.N.A. at the time of injection. At the end of one hour, at which time ninety-five percent of the tritiated thymidine is lost, 0.30 per cent of the acinar cells were labeled. This represented the percentage of acinar cells undergoing D.N.A. synthesis. Although a rise in the percentage of labeled cells was observed from one hour through four hours, 0.30 per cent to 0.34 per cent respectively, it was too slight to be considered of significance. The doubling which occurred between four hours and twelve hours, from 0.34 per cent to 0.74 per cent respectively, in the percentage of labeled acinar cells indicated that mitosis had occurred during this time. Although the percentage of labeled cells doubled within this seven hour period there can be no doubt that the rate of new cell formation was very slow.

This fact was further supported by the finding that there was no marked variation in the numbers of labeled acinar cells during the periods of twelve, twenty-four, and forty-eight hours. This was interpreted as a plateau of the labeled cell population and demonstrated a long interphase period.

It could be seen that the small percentage of acinar cells which synthesized D.N.A. and subsequently doubled yielded only 0.74 per cent new cells to the population of the submandibular gland. This finding, supported by the extremely long interphase beyond twenty-four hours, was sufficient evidence to conclude that the proliferative activity of the submandibular gland of this strain of mice was extremely low.



In the pancreas, a gland comparable to the salivary glands, Fitzgerald and Vinijchaikul in 1959 reported that the cell replacement rate of acinar and islet cells was extremely low in rats. They found that only one per cent of both acinar and islet cells were labeled in twenty-four hours.

Organs which are considered as being proliferative and undergo mitosis frequently are the most sensitive to the effects of ionizing radiation. Since the submandibular gland cannot be considered a highly proliferative organ it is felt that early chronic radiation damage may not manifest itself as alterations in its structure. In view of the active secretory function of the salivary glands, it seems likely that functional changes occur early and later are followed by structural alterations.

The concept of damage by ionizing radiation is based upon the electron loss from an atom leaving an orbit N, M, L, K, and traveling at speeds proportionate to the amount of absorbed radiant energy. This leaves the atom in an ionized state for short periods of time,  $1 \times 10^{-9}$  seconds.

There are molecules within the nucleus and cytoplasm of a cell which absorb radiant energy and become ionized. This may cause splitting of molecules and otherwise derange their structures. This direct absorption has been shown to be the cause for chromosomal splitting and rejoining, creating abnormal distributions which either prevent further mitosis or following division the daughter cells may be of a new cell race. It has been reported that absorption of radiation creates a regular splitting of chromosomes at specific sites regardless of the quantity of energy delivered. This is interpreted as striking a "target" and implying the transmission of energy to a sensitive site in a

molecule.

The ionization of water, which constitutes seventy-five per cent of a cell, produces unstable, highly reactive free radicals, principally hydroxyl and to some extent peroxides. These diffuse about thirty angstroms within a cell and react with nearby molecules to produce abnormal products which damage the cells' function. These products are considered to be the toxic agents causing not only local cell injury but also "radiation sickness". It seems reasonable to interpret radiation damage on a histologic structural level. However, the relative activities of cells may be proliferative as in the gonads and secretory in the submandibular gland. Therefore, the difference in the reactivity to ionizing radiation measured histologically must be interpreted in the light of the particular cell function.

The salivary gland of humans has long been known to be sensitive to ionizing radiation. Humans irradiated for the treatment of oral and paraoral malignancies develop a xerostomia, dry mouth, early. Also such irradiated individuals show an increased incidence of dental caries. Characteristically such caries are found in the cervical areas of the teeth, which normally are immune. Investigations have been conducted using x-ray radiation to the salivary glands of experimental animals in an attempt to secure evidence of gland injury or dysfunction that may be related to those observed clinical findings in humans.

While the effects of both massive doses and smaller repeated doses of x-ray radiation have been studied, the intent of this study was to determine the effects of low grade chronic gamma radiation on the structure and function

of the submandibular gland of mice.

The first group of mice following twelve roentgens daily total body irradiation for one hundred days were subjected to a total accumulative dose of 1,200 roentgens. This represented chronic gamma radiation on the order of one-tenth of the lifespan of the animal. The submandibular glands of these mice appeared completely unaffected by this large dose of accumulated ionizing radiation. English (1954), however, using one thousand roentgens of x-ray radiation in single doses showed pathologic changes in the submandibular gland of rats. Although the ionizing effect of radiation is not similar in these studies it may be significant that in equal doses of ionizing radiation the salivary glands may be more resistant to chronic radiation than to acute radiation. However, it is possible that the rat submandibular gland may be more sensitive to radiation than that of the mouse or that the submandibular glands of this strain of mice may be extremely resistant to radiation. The findings in the gamma radiation study did, however, appear to be in harmony with those of Shafer (1953). He observed no histologic changes in the submandibular glands of rats selectively irradiated only about the head with single doses of x-ray radiation ranging from 480 to 3,500 roentgens. It would appear from the similar studies on rats of Shafer and English that the effects of x-ray radiation are contradictory.

It was felt that these findings might be correlated to the very slow proliferative activity of the submandibular gland. In its cellular turnover the submandibular gland is different from organs such as the testes and tissues such as intestinal epithelium which have rapid turnover rates and show radia-

tion damage early. The observation of the lack of any structural change in the irradiated submandibular gland may be therefore related to its low cell turnover. However, the less obvious and possibly more critical effects of radiation may be detected in the secretory activity of the cells of the acini and ducts. It was believed that the effects of radiation might be manifested early in a functional disturbance of the gland. However, after the one hundred day radiation period no significant disturbance in the secretory activity was observed in either the number or chemistry of the secretory granules.

In the second part of this gamma radiation study, the mice were subjected to an accumulative total body gamma radiation of 2,400 roentgens over a two hundred day period which is approximately one-fifth of the lifespan of this strain of mice. Following this accumulated dose, changes in the submandibular gland compatible with radiation damage usually found and reported to occur in other organs as a result of radiation were observed. The changes observed were quite dramatic when compared to the one hundred day irradiated mice. From these findings it would seem that somewhere between 1,200 roentgens and 2,400 roentgens was a critical period in which structural changes first manifested themselves. The first observable change was an atrophy of the submandibular gland when examined grossly. The histologic alterations in the gland were characterized primarily as degenerative changes in the parenchyma. The most dramatic changes appeared to be those which occurred in the cells of the intralobular ducts. The intracellular secretory granules were greatly decreased in number and in their staining ability. These ductal cells showed a marked diminution in the normally abundant, P.A.S. positive, mucopolysaccharide

granules. This loss of granules may be interpreted as reduction in the function of the gland. Structural changes also were seen in the ductal cells. The nuclei showed karyolytic and pyknotic changes with a migration of the nuclei closer to the lumina. The cytoplasm also lacked the normal basal striations which normally characterize these ductal cells. Although these degenerative alterations were not uniform throughout the gland, it was evident that injury to the ductal cells varied from reduced secretory activity to the severer structural changes. Since the ductal cells showed the most marked damage in the gland, it would appear that they are the most susceptible to damage by radiation. Cherry and Glucksmann (1959), however, feel that the ductal cell changes appeared to be less pronounced than the acinar cell changes in rats irradiated with x-ray radiation in daily doses of 850 roentgens with accumulative doses of 7,650 roentgens administered in the region of the neck. This may be significant evidence of the difference in reaction of ionizing radiation between the submandibular glands of the rat and the mouse.

As in the ductal cells, the acinar cells of the submandibular glands of the two hundred day gamma irradiated mice also showed a variation in their reaction to ionizing radiation. The intensity of the damage did not appear to be as dramatic as those which occurred in the cells of the intralobular ducts. The acinar cell changes also ranged in severity from degeneration and complete collapse of the acini to the much less severe changes characterized by loss in the staining ability of the cells. The function of these cells also appeared to be reduced. The histochemic changes which were observed in the gland tend to lend definite proof of a decreased function of the gland as a result of the

Ionizing effect of gamma radiation.

The histologic changes in the submandibular gland of mice after an accumulative dose of 2,400 roentgens do not coincide with those of Shafer (1953). Shafer did not find any histologic changes in rat submandibular glands after repeated doses of x-ray radiation from 4,500 to 8,000 roentgens. Although it is known that the effects of ionizing radiation are accumulative, it may be significant that even though such large doses of radiation were given to rats, the radiation was administered twice weekly. The submandibular glands of such rats had time for recovery from the effects of ionizing radiation between such weekly treatments. Another significant difference in the methods used in these two studies was that the rats were sacrificed twenty-one days following the last treatment. It was possible that the submandibular glands of these rats could have recovered from the damaging effect of the radiation. In this study the mice submandibular glands were not given any chance for recovery since they were radiated daily and sacrificed the day they received their two hundredth day of radiation.

The supporting argyrophilic fibers of the gland were decreased in number as a result of two hundred days of radiation. However, this decrease in periductal fibrous tissue was slight and a rather insignificant finding when compared to the severe changes in the parenchyma.

The inflammatory reaction which was observed was slight in nature in contrast to the severe changes observed in the parenchyma. This does not appear to be consistent with what is believed to be the earliest reaction of an organ or tissue to ionizing radiation in which case inflammation is severe. Although

severe inflammatory response has been reported in acutely radiated glands, it must be pointed out that there were available large numbers of such cells to infiltrate the gland. It is known that the effects of radiation on the reticulo-endothelial system causes a suppression of production of hematopoietic cells. (Bloom, 1948) This will be reflected in time as a reduction or total lack of inflammatory cells in any site of injury such as the salivary glands. The lack of an inflammatory cellular reaction as observed in severely damaged submandibular glands in chronically radiated mice is a manifestation of the failure of the reticulo-endothelial system to react. This failure implies either suppression or injury of a more severe nature to those undifferentiated mesenchymal cells found perivascularly throughout the gland and lymphoid tissues of the body. Moreover, the absence of the granular series of cells is compatible with the bone marrow injury reported by Bloom in chronic gamma irradiated mice.

## CHAPTER VI

### SUMMARY AND CONCLUSIONS

This study attempted first, to ascertain the rate of cellular turnover in the submandibular gland of mice and, secondly, to investigate the effect of chronic low grade gamma radiation on this gland.

The conclusions derived from this study were:

A. The histology of the submandibular gland of the male mouse was similar in many respects to that described in other rodents.

B. The rate of new cell formation in the submandibular gland of the male mouse was very slow. At the end of the first hour following injection of the radioactive tracer, tritiated thymidine, only 0.30 per cent of the acinar cell nuclei became labeled. The rise to 0.34 per cent after four hours was insignificant. The percentage of labeled nuclei doubled after twelve hours to 0.74 per cent indicating cellular division had occurred. The percentage of labeled nuclei remained relatively constant from twelve hours through forty-eight hours indicating a long interphase period. Therefore, it is concluded that the acinar cells of the submandibular glands of this strain of mice had a slow proliferative capacity.

C. There was additional evidence that radiation of biologic systems was accumulative.

D. No histopathologic or histochemic changes in the submandibular gland



were observed following twelve roentgens daily gamma radiation for one hundred days.

E. Moderate to severe histopathologic and histochemic alterations were seen after two hundred days of gamma radiation at a rate of twelve roentgens per day. The mouse submandibular gland showed functional changes, namely a reduction in numbers and chemistry of the secretory granules. The severer structural alterations were characterized as degenerative and necrotic changes of both the intralobular duct cells and the acini.

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Figure 1      Distribution of labeled acinar cell nuclei with time.

Note the doubling of labeled nuclei between four and twelve hours and the plateau of resting nuclei after twelve hours.

APPENDIX

PLATE I

A. Graph

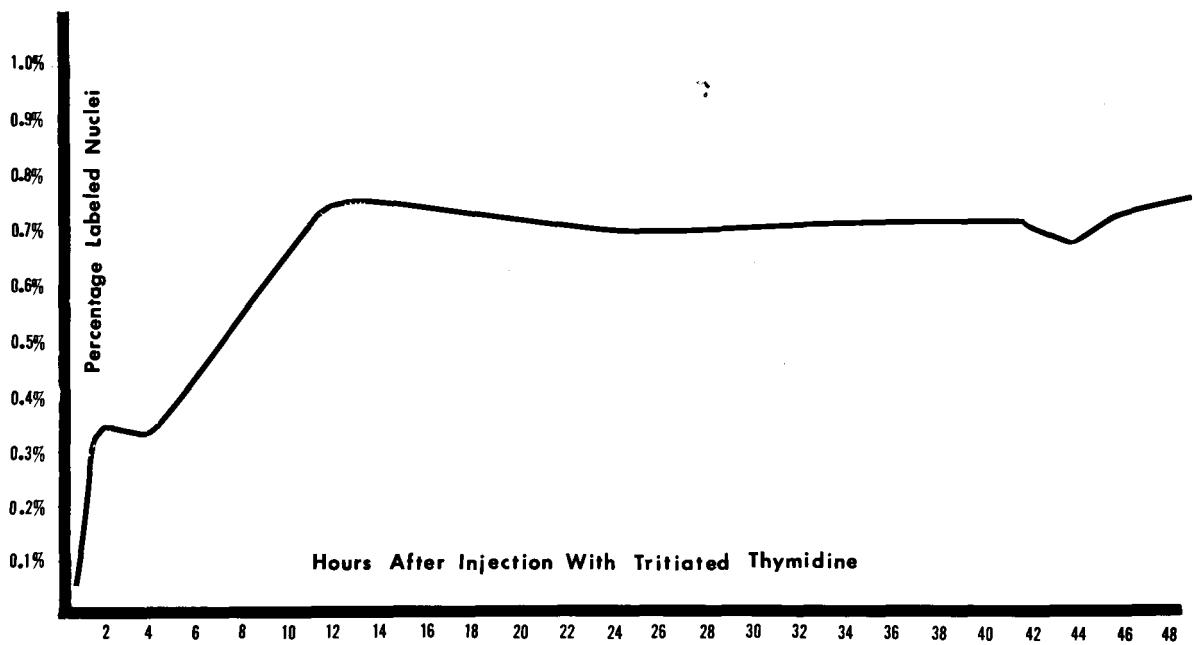


Figure 1

Figure 1      Photomicrograph x100: Control Specimen  
Hematoxylin and Eosin (H & E)

Observe the various shapes which the intralobular ducts assume and the intervening acini. Note the rich vascularity of the gland and the septa which divide the submandibular gland into lobules.

## PLATE II

## B. Photomicrographs.

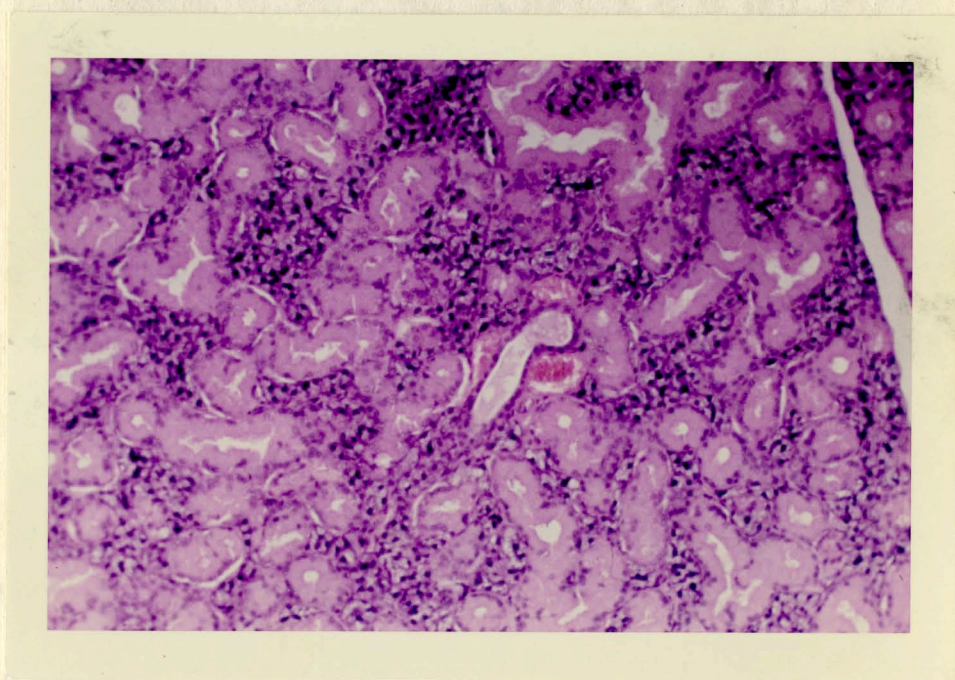


Figure 1

Figure 1 Photomicrograph x1000 (Oil Immersion): Control Specimen  
Hematoxylin and Eosin (H & E)

Observe the densely packed, coarse eosinophilic, secretory granules in the intralobular duct cells and the relatively few such granules in the acinar cells. Also note the small capillary.

Figure 2 Photomicrograph x1000 (Oil Immersion): Radiation Specimen  
Hematoxylin and Eosin (H & E)

Observe:

- A. Atrophy of some of the acinar cells.
- B. Compression of some acinar cell nuclei and karyolysis of others
- C. Hydropic degeneration of acinar cell cytoplasm.
- D. Reduction in number of eosinophilic granules in the cytoplasm of the intralobular duct cells.



## PLATE III

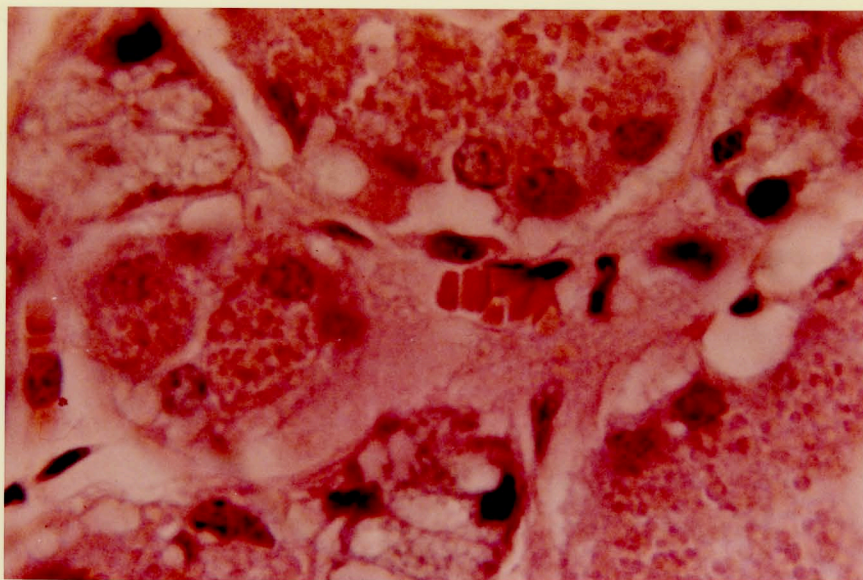


Figure 1

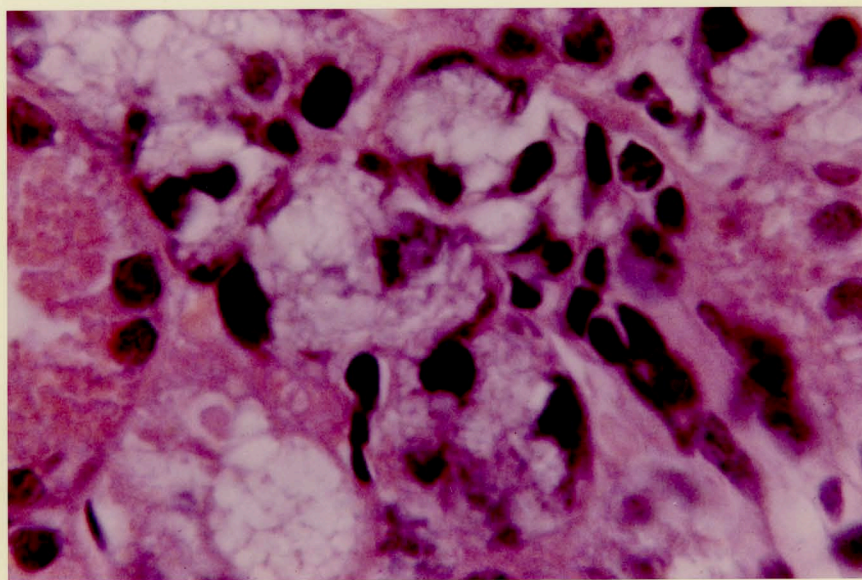


Figure 2

Figure 1      Photomicrograph x400: Control Specimen  
Periodic acid Schiff (P.A.S.)

Note the densely packed and intense staining mucopolysaccharide granules in the cells of the intralobular ducts. Such granules are fewer in number and lighter staining in the acinar cells.

Figure 2      Photomicrograph x400: Radiation Specimen  
Periodic acid Schiff (P.A.S.)

Note the marked diminution in the numbers of P.A.S. positive mucopolysaccharide secretory granules in the intralobular duct cells. There is also destruction of the cell wall on the lumen side. Note the acinar cell changes characterized by a decrease in number of mucopolysaccharide granules, hydropic degeneration and complete destruction of some of the acini.



## PLATE IV

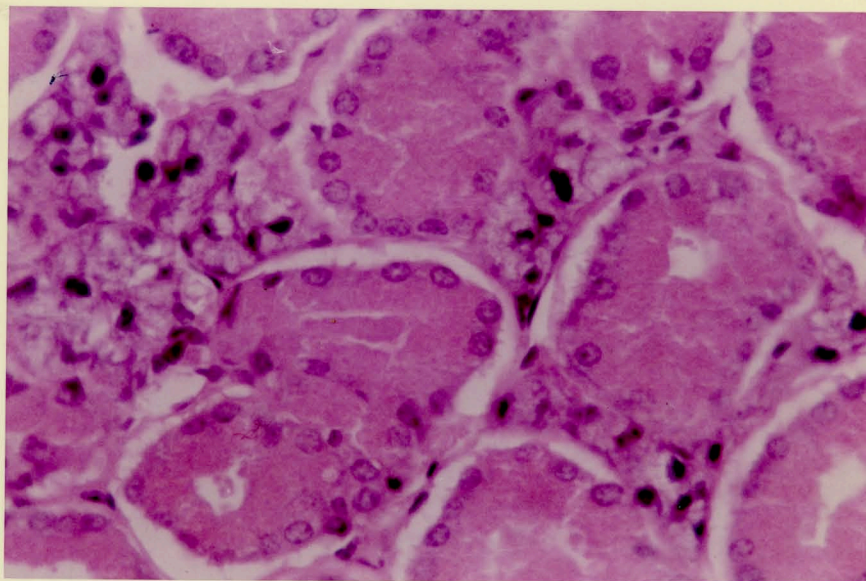


Figure 1

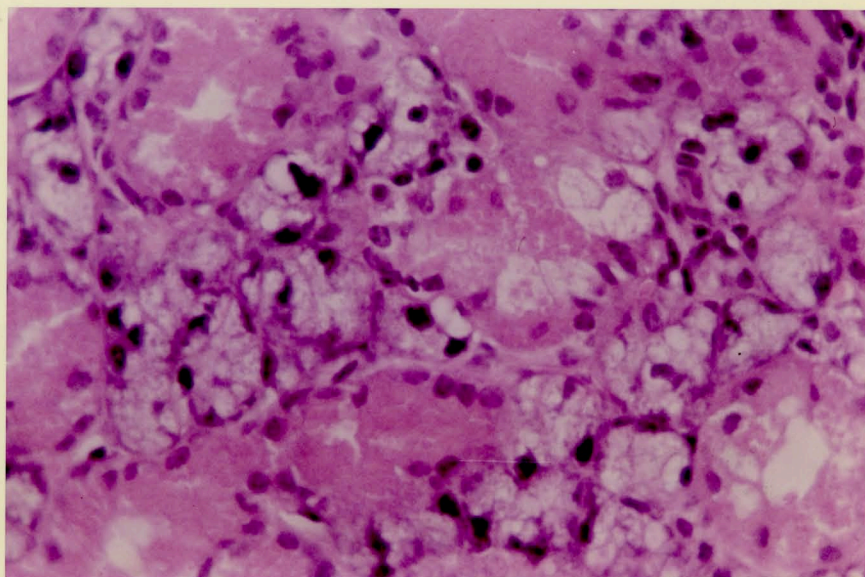


Figure 2



Figure 1 Photomicrograph x400: Control Specimen  
Ribonucleic acid (R.N.A.) (Carnoy Fixation)

Observe that the greatest concentrations of the R.N.A. positive material is located perinuclear. Note also the greater activity of R.N.A. in the acinar cell cytoplasm than in the ductal cells.

o

Figure 2 Photomicrograph x1000 (Oil Immersion): Radiation Specimen  
Ribonucleic acid (R.N.A.) (Carnoy Fixation)

Note the disruption of the R.N.A. positive material in the acinar and intralobular duct cell cytoplasm.

## PLATE V

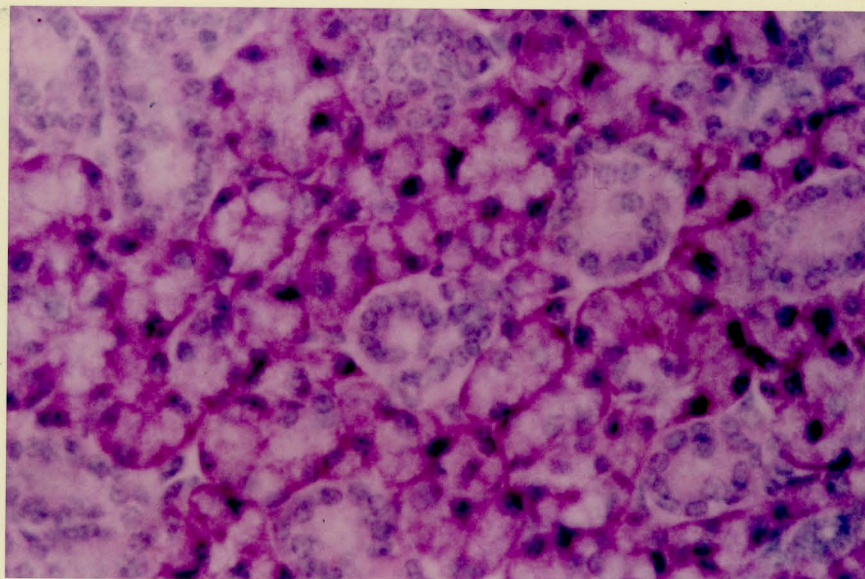


Figure 1

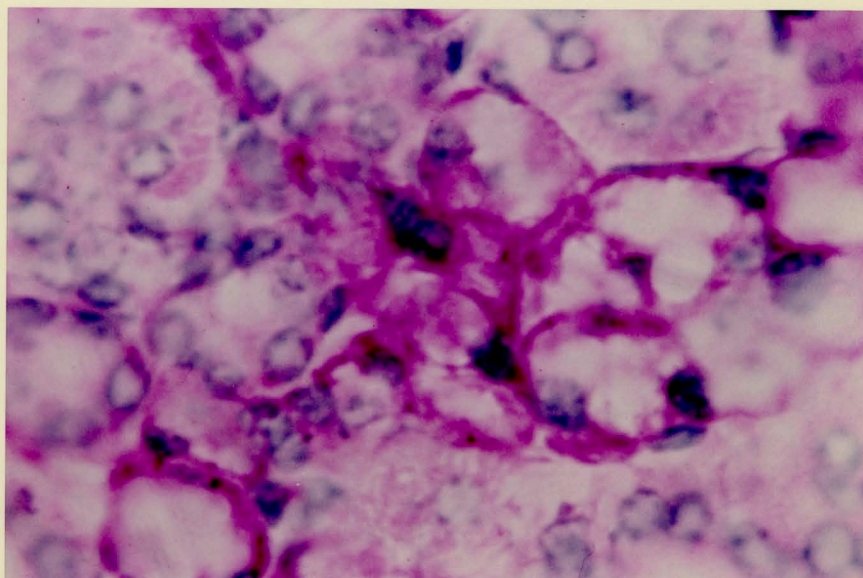


Figure 2

Figure 1 Photomicrograph x400: Control Specimen  
D.D.D. for alcohol insoluble sulfhydryl groups

Note the distribution of the alcohol insoluble sulfhydryl groups in the cell wall and the perinuclear (Nucleus unstained) cytoplasm.

Figure 2 Photomicrograph x400: Radiation Specimen  
D.D.D. for alcohol insoluble sulfhydryl groups

Observe the disruption of the alcohol insoluble sulfhydryl groups in both the acinar and intra-lobular duct cytoplasm.



## PLATE VI

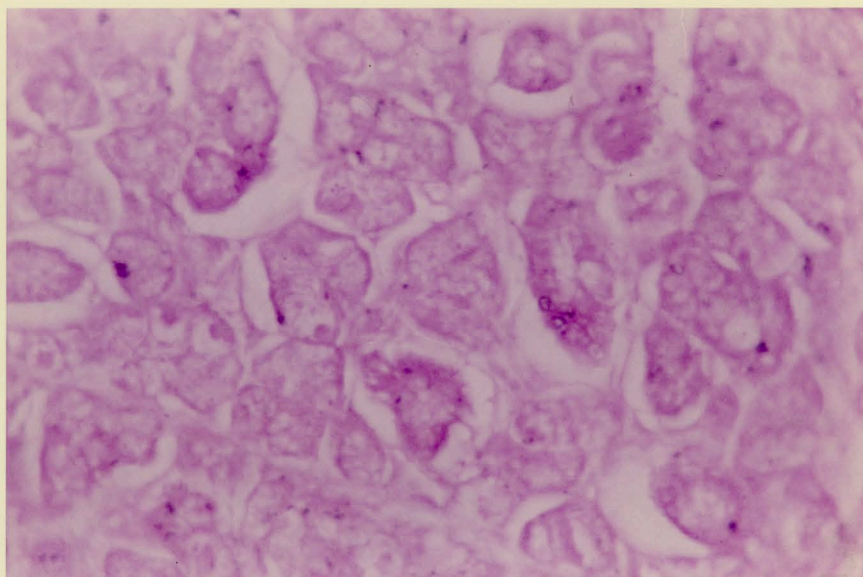


Figure 1

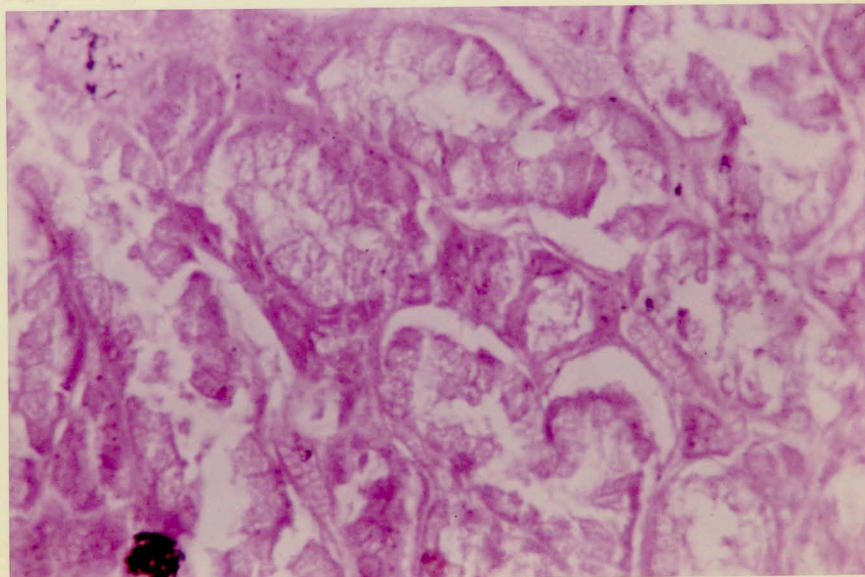


Figure 2

Figure 1 Photomicrograph x100: Control Specimen  
Mallory Trichrome Stain

Observe:

- A. The connective tissue septa penetrating the gland dividing it into lobules.
- B. The great numbers of fibers supporting the collecting interlobular duct, artery and vein.
- C. The delicate nature of the fibrillar reticulum supporting the acini and intralobular ducts.

Figure 2 Photomicrograph x1000 (Oil Immersion): Radiation Specimen  
Mallory Trichrome Stain

Observe the slight decrease in the numbers of collagenous fibers supporting the collecting duct, blood vessels, and acini.



## PLATE VII

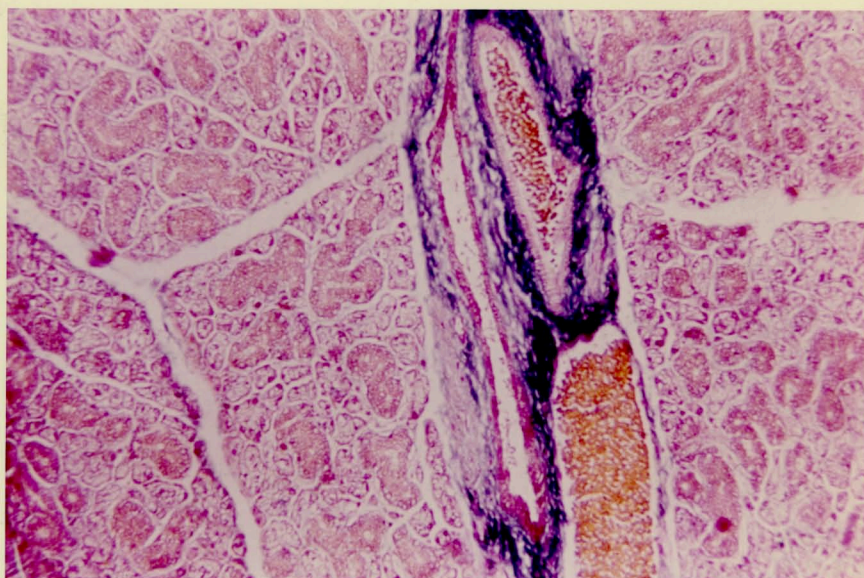


Figure 1

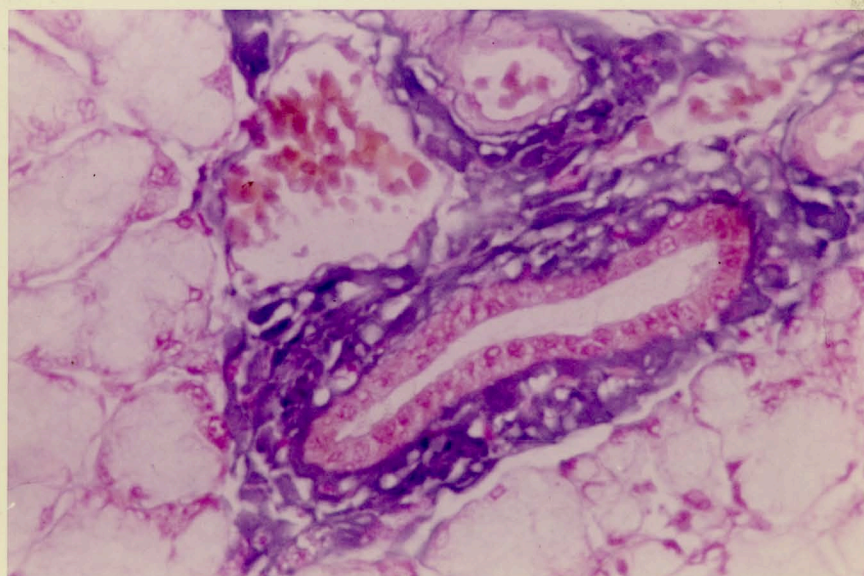


Figure 2

Figure 1      Photomicrograph x400: Control Specimen  
Silver Impregnation

The silver stain points out the extremely delicate argyrophilic fibers supporting the acini and intra-lobular ducts.

Figure 2      Photomicrograph x1000 (Oil Immersion): Radiation Specimen  
Silver Impregnation

Note the decreased numbers of argyrophilic fibers supporting the acinar and intralobular duct cells.



## PLATE VIII

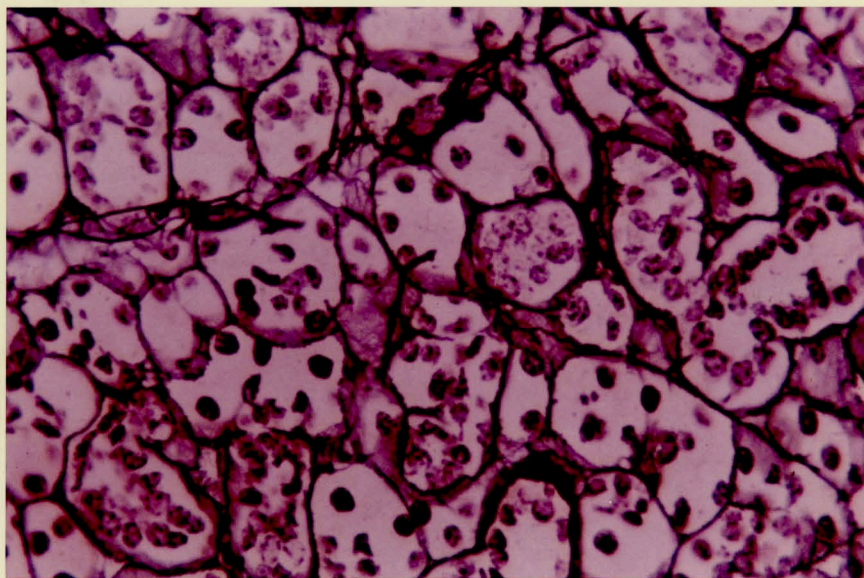


Figure 1

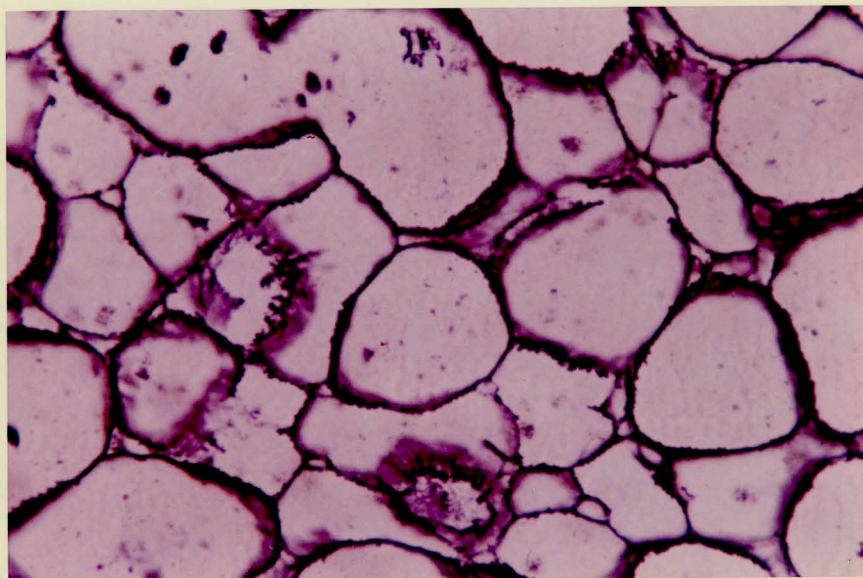


Figure 2



Figure 1      Photomicrograph x400: Tracer Series  
Feulgen Stain

Autoradiograph showing the labeled acinar nucleus in the center of the field. Note the extremely few numbers of such labeled nuclei in a typical field of the submandibular gland.

Figure 2      Photomicrograph x1000 (Oil Immersion): Tracer Series  
Feulgen Stain

Observe the granular appearance of the chromatin material in the acinar nuclei stained specifically for deoxyribonucleic acid (D.N.A.). Note also the black grains of the labeled nucleus in the center of the field.

## PLATE IX

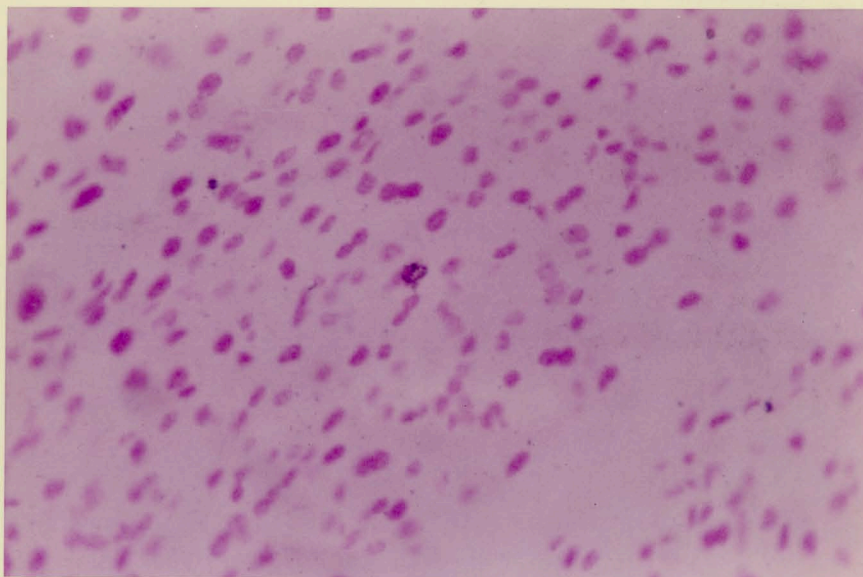


Figure 1

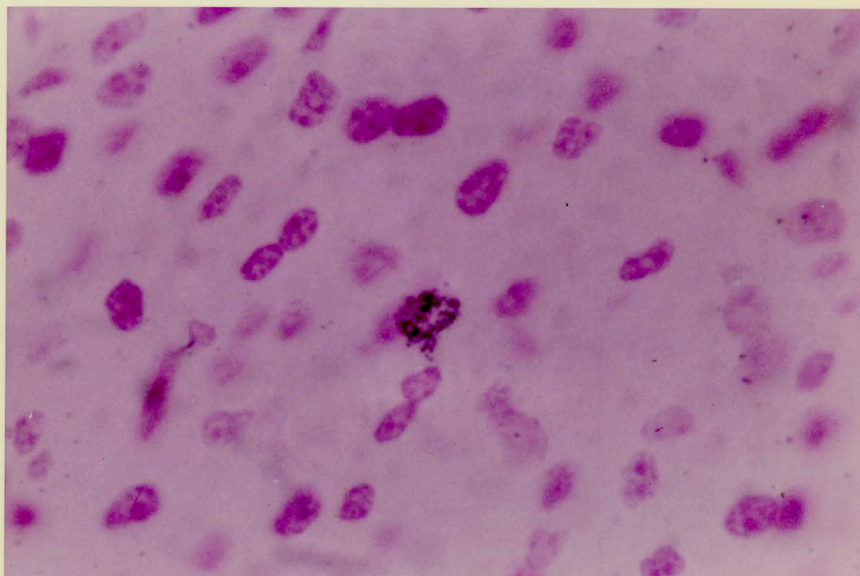


Figure 2

Figure 1      Photomicrograph x1000 (Oil Immersion): Tracer Series  
Feulgen Stain

Autoradiograph twelve hours after injection of  
tritiated thymidine. Note the pattern of distri-  
bution of the labeled nuclei. Note the small  
cluster of such labeled nuclei in the center of the  
field.



## PLATE X

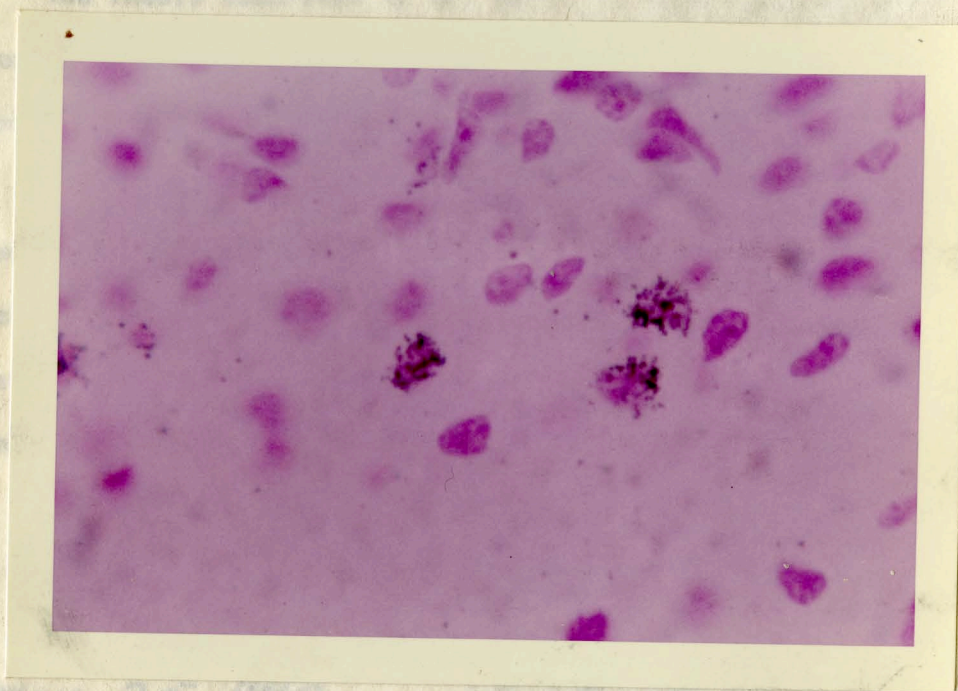


Figure 1

# APPROVAL SHEET

The thesis submitted by Dr. Mario V. Santangelo has been read and approved by three members of the Departments of Anatomy and Oral Anatomy.

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 25, 1960  
Date

Gustav D. Jato  
Signature of Adviser