THE EFFECTS OF 250-KVP X-IRRADIATION ON THE FUNCTIONING OF THE THYROID

by

Joan Cunningham

A THESIS
SUBMITTED TO THE FACULTY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF ARTS

Houston, Texas
June, 1961
Thesis
Biol.
1961
Cunningham
c. 2
ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. R. W. Talmage of the Biology Department of the Rice University for his cooperation and enthusiastic interest during these studies and for his patience and aid in the preparation of the manuscript.

I also owe a great debt of gratitude to Dr. W. C. Dewey of the M. D. Anderson Hospital for his helpful criticism, and especially for his patient counsel and encouragement both as a scientist and as a friend.

I would like to thank Dr. R. J. Shalek of the M. D. Anderson Hospital for allowing me the use of the equipment in the Physics Department, and Dr. W. K. Sinclair, now at Argonne National Laboratory, for stimulating interest in the initiation of this project.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. THYROID PHYSIOLOGY</td>
<td>2</td>
</tr>
<tr>
<td>III. THE REGULATION OF THYROIDAL ACTIVITY</td>
<td>9</td>
</tr>
<tr>
<td>IV. THE EFFECTS OF IODINE-131 ON THYROID FUNCTION</td>
<td>12</td>
</tr>
<tr>
<td>V. THE EFFECTS OF EXTERNAL IRRADIATION ON THYROID FUNCTION</td>
<td>15</td>
</tr>
<tr>
<td>VI. STATEMENT OF PROBLEM</td>
<td>17</td>
</tr>
<tr>
<td>VII. MATERIALS AND METHODS</td>
<td>18</td>
</tr>
<tr>
<td>A. Irradiation Procedure</td>
<td>18</td>
</tr>
<tr>
<td>B. Thyroid Uptake Measurements</td>
<td>19</td>
</tr>
<tr>
<td>C. Plasma I-131 Measurements</td>
<td>20</td>
</tr>
<tr>
<td>D. Whole-Body Counts</td>
<td>20</td>
</tr>
<tr>
<td>E. Biological Half Life of Triiodothyronine</td>
<td>20</td>
</tr>
<tr>
<td>F. Electrophoresis</td>
<td>21</td>
</tr>
<tr>
<td>G. Parabiotics</td>
<td>22</td>
</tr>
<tr>
<td>VIII. RESULTS</td>
<td>23</td>
</tr>
<tr>
<td>A. Comparative Study of Radiation Dose Versus Thyroid Uptake of I-131</td>
<td>23</td>
</tr>
<tr>
<td>B. Progressive Changes in I-131 Uptake after Irradiation with 10,000 Rads</td>
<td>23</td>
</tr>
<tr>
<td>C. Effect of Radiation (10,000 Rads) on Protein-Bound I-131 Distribution in Plasma</td>
<td>24</td>
</tr>
<tr>
<td>IX. DISCUSSION</td>
<td>29</td>
</tr>
<tr>
<td>X. SUMMARY</td>
<td>33</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>34</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>36</td>
</tr>
</tbody>
</table>
INTRODUCTION

In the past few years there have been more reports of thyroid physiology and actions than on any other endocrine gland. This renewed interest in thyroid activity is probably due to new methods of study and, particularly, to the availability of radioactive iodine for use in studies.

With the increasing use of radioactive iodine in the treatment of certain thyroid abnormalities, interest has arisen as to how the radiation affects processes in thyroid physiology. In addition to internal radiation, using radioactive iodine, external beam irradiation has been employed in the treatment of thyroid abnormalities.

The work to be reported in the following pages has been an attempt to show some of the effects of 250-kvp x-irradiation on various phases of thyroid physiology and to compare and contrast these findings with those of other investigators who have employed both internal and external irradiation techniques. In the studies presented here, emphasis has been placed on the changes in plasma levels of circulating protein-bound iodine and the uptake of radioactive iodine-131 by the thyroid.

To provide a background for a better understanding of the results to be presented, it was felt important to review several aspects of past work in other laboratories.

The reviews to follow deal, in order, with iodine metabolism and thyroid hormone synthesis, regulation of thyroid activity, effects of radioactive iodine, and effects of external beam irradiation.
Several excellent reviews of general thyroid physiology are available (1-4); therefore general information has been omitted in this presentation. However, since iodine metabolism bears a pertinent relationship to the experimental data presented, a brief review follows.

Within the organism there exists what is commonly known as the "iodide pool", from which the thyroid directly derives its iodide supply and which serves as a compartment for exchange of body iodide with exogenous iodide. The uptake of iodide from the blood is mainly dependent on two processes in the thyroid: (a) diffusion of iodide from the blood to the iodide space in the gland; and (b) organic binding of trapped iodide. The first process is a real diffusion which can go in both directions but is dependent on some mechanism in the gland that can maintain a high concentration gradient towards the blood (6). The mechanism for this is largely unknown but may be related to some "loose binding" of iodide to thyroid proteins, or may be an active transport of iodide. It has been shown by Wyngaarden (7) that binding of iodide occurs in vitro and that homogenized thyroid proteins show a higher affinity for iodide than do other tissue homogenates. However, an active transport of iodide is a more plausible explanation of the trapping mechanism (8). It is known that certain anions, such as thiocyanate and perchlorate, inhibit the thyroidal iodide pump and Wollman (8) concluded that this inhibition is competitive in nature. He also noted that the thiocyanate ion does not enter the thyroid tissue in a concentration above that of the blood. This competitive inhibition by a
substance which is not concentrated by the thyroid suggests that the accumulation of iodide is not due to its absorption onto thyroidal proteins.

Following the trapping of iodide by the thyroid, a second process is begun; that of the conversion of iodide to iodine. It is felt that this conversion of iodide to iodine is an enzymatic process, although very little is actually known about the mechanism. It has been postulated that the conversion is mediated by a thyroid peroxidase and Dempsey (9) in 1944 obtained histochemical evidence of peroxidase activity in the thyroid cells. Peroxidase activity in the rat thyroid cells and colloid has also been described by DeRobertis and Grasso (10). However, Glock (11) was unable to demonstrate peroxidase activity in the thyroid tissue. Astwood (12) has stated that "the difficulties of demonstrating a peroxidase in the thyroid tissue extracts are twofold: tests for peroxidase are not specific and the quantity of peroxidase presumed to be needed for the oxidation of iodide is very small."

The third step in the biosynthesis of the thyroid hormone(s) consists of the incorporation of the iodine into the tyrosine molecule. It is not yet known whether iodination in the thyroid gland occurs when tyrosine is free or when it is bound in the thyroglobulin molecule. But the iodination of the protein-bound tyrosine is favored and supported by the work of Taurog and co-workers (13-14). This iodination results in the formation of mono- and diiodotyrosine, probably as a part of a protein molecule.
The next step in the synthesis of thyroid hormone(s) is the coupling of two diiodotyrosines with the elimination of an alanine molecule to form thyroxine. Triiodothyronine can be synthesized by two pathways: (1) by the coupling of one molecule of monoiodotyrosine and one molecule of diiodotyrosine; or (2) by the elimination of one atom of iodine from thyroxine. There is evidence for and against each of the above pathways and the actual mechanism is not known. Roche, Michel and co-workers (15) demonstrated that thyroid slices were able to deiodinate free mono- and diiodotyrosine, but had no action on these amino acids when they were bound in the thyroglobulin molecule, and they were unable to demonstrate any action of the thyroid deiodinase on thyroxine or triiodothyronine. They therefore concluded that triiodothyronine could not arise by deiodination of thyroxine.

Following biosynthesis, the thyroid hormone(s) are held within the follicular colloid as a part of the thyroglobulin molecule. This phase of storage is terminated by the proteolysis of the thyroglobulin molecule into its component peptides and amino acids. The enzymatic processes involved in the hydrolysis of thyroglobulin are complex and involve at least two enzymes and possibly more (16). Free MIT and DIT are rapidly deiodinated by the thyroidal dehalogenase and do not leave the thyroid gland in the normal organism (16). The principal components secreted by the gland into circulation are triiodothyronine and thyroxine, both of which are resistant to dehalogenase activity. These leave the gland by diffusion and, in addition, thyroxine leaves as a result of competitive surface binding favoring the serum thyroxine-binding globulin (TBG) over thyroglobulin (17). Under abnormal circumstances
iodotyrosines may appear in the blood; thyroglobulin may be released after surgical or radiation injury and iodinated peptides or iodoproteins exhibiting the electrophoretic mobility of albumin and of gamma globulin may appear (18-19). The iodoprotein resembling serum albumin was first described in detail by Tata (18) in humans with thyroid carcinoma. A similar, but not identical, iodoprotein has been found in the blood of rats bearing a transplantable thyroid tumor (20).

Iodoproteins have also been found in the blood of humans treated with large doses of inorganic iodide but these, as yet, have not been characterized (21).

Under normal conditions, thyroxine is bound to certain circulating proteins. Relatively little work has been done on the thyroxine-binding protein (TBP) in the rat, and the reports in the literature at present are not in complete agreement. It has been reported that only a single peak of TBP is seen in the albumin zone, when exogenous thyroxine is added to rat serum, whether the concentrations are low or high. Other reports are of a thyroxine-protein component in the alpha globulin zone, and Myant (23) found good separation of the thyroxine zones in alpha globulin and albumin. Triiodothyronine is also bound in serum, primarily to TBP but also to albumin and globulins.

Another site of hormone binding has been designated as thyroxine-binding pre-albumin (TBPA), by Esser and Heinzler (26). TBPA has been found to bind thyroxine more strongly than TBP or any other protein fraction (27). TBPA will also displace thyroxine from TBP. Tata (27) has shown that pre-albumin had thirty times the thyroxine-binding affinity of albumin and six times that of TBP.
Since the interaction of thyroxine with TBG, TBPA, and albumin is reversible, an increase in concentration of this hormone is accompanied by its redistribution with a greater proportion bound to albumin, the component whose avidity for thyroxine is least and whose capacity is the greatest. Conversely, the addition of TBG to serum containing thyroxine results in the abstraction of the hormone from the protein of lesser affinity, albumin (28).

The physiological significance of the binding thyroid hormone(s) has been studied in great detail and the most acceptable function seems to be that the protein binding provides a vehicle of transport of the hormone(s) from their site of production to the site of action, while at the same time it provides a "buffer" to regulate their entry into tissue cells (27).

The process whereby thyroid hormone(s) are exchanged from extracellular compartments to intracellular components is unknown. There are a number of ways by which this transfer could take place: (a) through the intermediate of unbound or "free" thyroxine, present in small quantities and unassociated with proteins; (b) through a specific exchange wherein thyroxine is oriented by its transport protein in a manner which allows its receipt by the cell at selected binding sites which mark the entrance to intracellular pathways of utilization and degradation; or (c) by means of the entry of the entire thyroxine-binding protein complex into the cell (1). The first of these three possibilities is most generally accepted at the present time. Robbins and Rall (20) have devised a method whereby the "free" thyroxine can be calculated. By this method they have measured thyroxine disappearance
in subjects with alterations in serum concentrations of thyroxine and/or thyroxine-binding proteins. From this work they were able to relate the metabolic status directly to a calculated value for "free" thyroxine which is dependent upon the concentration of serum protein iodine and the quantity of T4G within the circulation. However, the discovery of the additional binding substance, TBPA, and the lack of defined thyroxine-binding constants of the transport proteins cast doubt on the validity of such calculations. "Free" thyroxine must exist in the organism due to the fact that traces of the hormone are found in the urine in the absence of proteinuria and by its dialyzability from serum to serum across a dialysis membrane.

Tata has produced evidence to support the theory that thyroid hormone-protein interactions in blood affect hormone action. It has been observed that in the chicken, in contrast to other animals, thyroxine and triiodothyronine have equal biological activity. Also in contrast to other animals, thyroxine and triiodothyronine are bound equally by chicken serum proteins, and disappear at similar rates from chicken blood. These observations give support to the previous belief that the quantitative difference in action between thyroxine and triiodothyronine in most animals is due to a difference in their interaction with serum proteins.

It is also of interest to consider the role which thyroid hormone-protein interactions might play in the homeostatic mechanisms involving the thyroid. From the work of Robbins and Rall (22) the controlling factor would be the concentration of "free" or diffusible thyroid hormone(s) in the blood and extracellular fluids. The "free" thyroxine
level would then determine the rate at which thyroid hormone(s) are distributed to the loci of action and the rates at which they are degraded and excreted. Through either direct or indirect influences on the pituitary-thyroid system, the "free" thyroxine level would also determine the rate of hormone production or secretion. The thyroxine-binding proteins would play a passive role in holding a quantity of secreted but nonutilized hormone in the extracellular fluids in ready equilibrium with "free" hormone(s). Because of the nature of the protein-hormone equilibrium, however, a third point of influence on the "free" thyroxine level is introduced. That is, the steady state could be disturbed not only by a change in production or a change in disposal of thyroid hormone(s), but also by any change which influences the binding reaction. The latter could come about through a change in the binding proteins or an extraneous agent which affects the interaction. Changes in thyroid-hormone(s) disposal and production would then occur in such a way as to restore the normal "free" thyroxine level. In the transition state, hormone secretion and disposal would be abnormal and total serum hormone concentration would, at first, be normal. In the new steady state, hormone secretion and disposal would be abnormal (22). This scheme is partially supported by the data from studies during pregnancy, estrogen treatment, androgen treatment, nephrosis, hepatitis, and idiopathic elevation of TBS.
THE REGULATION OF THYROIDAL ACTIVITY

Several factors contribute to the regulation of thyroidal activity. Among these are the central nervous system, the anterior pituitary, and autoregulation by the thyroid itself. The role of the hypothalamohypophyseal system has been reviewed recently (29-30) and will not be considered here.

Probably the greatest control over thyroidal activity is exerted by the anterior pituitary. This control is mediated through the thyrotrropic hormone (TSH). Several excellent reviews are available on this subject (31-33); therefore only a few general remarks will be made in this presentation.

The secretion of TSH by the pituitary stimulates the thyroid to secrete thyroid hormone(s). The actual site or sites of action of the TSH on the thyroid gland has yet to be elucidated. However, the earliest action of TSH appears to be an acceleration of proteolysis of colloid (1). TSH incubated in vitro with thyroid tissue (a) increases the tissue-to-medium iodide-131 ratio of propylthiouracil-blocked bovine thyroid slices, (b) increases the fraction of I-131 which is bound to protein by bovine thyroid slices, and (c) increases the total I-131 accumulation by minced bovine thyroid tissue.

In spite of the overpowering effects of TSH on various stages of thyroid activity, the thyroid gland retains some degree of autonomous behavior. Experiments by VanderLaan and Caplan (34) and Halmi (35) indicated the presence of an intrathyroidal factor capable of altering
the function of the thyroidal iodide pump. This conclusion is supported by the demonstration that a low iodine diet or prior propylthiouracil ingestion causes an increase in the thyroid-serum ratio of the hypophysectomized rat either in the complete absence of TSH or in response to a constant dose of TSH. It was found (34) in these various experimental situations that the T/S ratio is inversely proportionate to the concentration or organic iodine in the thyroid and it was proposed that the accumulation of iodide is stimulated by depletion of thyroidal hormonal stores. However, more recent findings suggest that, regardless of total organic iodine concentration in the thyroid, a small amount of recently bound iodine appears to inhibit the iodide pump. This tends to support the suggestion by Hamal (35) that one or more specific iodine-containing compounds within the thyroid (rather than total organic iodine concentration) directly depresses the "iodide pump" in opposition to the stimulating effect of TSH. Hamal suggested that the intrinsic depressor substance may be an iodothyronine, but Wollman and Reed (36) have made observations that implicate the iodothyronines.

In addition to the thyroidal autoregulation of the "iodide pump", there is also evidence that hormone synthesis and secretion are altered independently of or in directions opposite to TSH action. The ratio of MIT/DIT within the thyroglobulin molecule is greatly altered by dietary iodine supply; the ratio being extremely high in animals fed low iodine diets.
A third autoregulatory mechanism is suggested by the observation that the release of organic I-131 from the thyroid during propylthiouracil treatment falls by an amount constantly proportionate to the declining store of hormonal I-131 in the gland. This is clearly non-hypophyseal in origin, since any fall in the circulating level of thyroid hormone caused by propylthiouracil would lead to an increase in TSH output and a consequent increase in the release of hormone from the thyroid (1).
THE EFFECTS OF IODINE-131 IRRADIATION ON THYROID FUNCTION

A number of investigators have reported morphological and functional changes in the thyroid gland after the administration of various doses of radioactive iodine. In all of these investigations it was found that an increased degree of glandular damage occurred with an increasing dosage of iodine.

Among the reports concerning the damage to the thyroid produced by radioactive iodine varying degrees of attention have been paid to factors which have a bearing on the changes produced. These factors include the age of the animal, the amount of dietary and "carrier" iodine, the functional and morphological state of the thyroid at the time of irradiation, the dose of radioactive iodine administered and the amount retained per unit weight of thyroid tissue, and the total amount of radiation received.

Gorbman (60) has shown that 200 microcuries of radioactive iodine destroyed the thyroid of mice receiving a diet containing moderate amounts of iodide, whereas 28 microcuries destroyed the thyroid of such animals on a low iodide diet. The maximal labeled iodine uptake in the thyroid of animals on the former diet was 6 to 7.5% and on the latter diet was 58%. In each instance the thyroid was destroyed by 4 microcuries of I-131 per milligram of thyroid tissue.

Maloof (51), studying the effects of various doses of I-131 on the function and morphology of the thyroid of the rat, found no apparent change in function or morphology in the animals receiving 1 μc of I-131; impairment of function but no alteration in morphology in animals
receiving 5 μc (5800 rep); and both functional and morphological changes in groups receiving 20, 50, and 100 μc. The changes in morphology included increase in cell height, and bizarre nuclear changes. The thyroids of these animals did not enlarge in response to the administration of thiouracil in spite of the persistent hypertrophy of the cells, suggesting that cellular division was impaired by the radiation.

In a recent paper by Jovanovic, et al. (38) it was found that 20 μc of I-131 produced a decrease in oxygen consumption over the first two months, but this returned to normal by the end of the 80-day testing period. A permanent decrease in oxygen consumption and marked histological changes were noted in the animals receiving 300 μc (doses estimated to be: 20 μc = 20,000 rads and 300 μc = 170,000 rads).

An extensive study of changes in iodine metabolism was made by Feller, et al. (61). The criteria employed to assess thyroid function was: (a) histological appearance of the gland, (b) concentration of plasma PBI, (c) the I-127 content of the gland, (d) the retention of I-131, and (e) the ability to concentrate a second dose of I-131.

As judged by all of these criteria, no damage was observed in the thyroid glands of rats examined six days after an injection of 24 μc, nor in rats receiving 30 μc and being examined ten days after the injection (dosage estimates were 19,000 and 28,000 reps, respectively).

A decrease in the concentration of plasma-protein-bound iodine was observed twenty-four hours after the injection of 875 μc of I-131. The authors attribute this decrease to an interference in the formation and/or release of thyroxine by the gland.
Also at this dose, a subsequent rise in plasma-protein-bound iodine was frequently observed on the second or third day after the injection. This observation was attributed to the release of breakdown products of thyroid tissue into the circulation. This was occupied by a rapid loss of chemical I-127.

The third change in the protein-bound iodine was observed eight days after the injection of 875 μc of I-131. Here the levels of PBI were similar to those observed after thyroidectomy. (The radiation dose delivered to the center of the thyroid by 875 μc of I-131 was calculated to be 280,000 by the authors.)

In addition to the changes observed in the plasma levels of protein-bound iodine, the thyroid glands of animals which received 300 and 875 μc of I-131 were found to have been unable to concentrate a second dose of I-131 when injected three days after the first injection.
THE EFFECTS OF EXTERNAL IRRADIATION ON THYROID FUNCTION

Probably the first work to be published on the effects of irradiation on thyroid was by Bower in 1923 (40). His group implanted radium needles into the thyroids of dogs and found necrosis in the area of the needles. The radiation dosage was undetermined.

Relatively little work has been done on actual effects of external irradiation, probably because the thyroid gland is considered to be one of the most resistant tissues of an organism.

Walters (41) studied histological changes following "385 r units" of x-irradiation and found that normal thyroid tissue in dogs does not change morphologically in this dose range.

Hursb, et al. (53) published an extensive report in 1951 on the effects of local x-irradiation on thyroid physiology. These investigators found an increased uptake of I-131 following 3000 to 6000 r, and the amount of increase was greater the larger the dose of radiation and the longer the post-irradiation testing time. In addition, they could not detect any noticeable difference in the total-plasma I-131 in the control and irradiated animals. They studied the ability of the thyroid to bind inorganic iodide by dialyzing thyroid homogenate and found that irradiation had not affected this process.

In an effort to study dosage ranges above 6000 r they employed the protracted dose technique, since they were unable to have the animals survive in this region with a single dose. Even with this method, however, all animals which received more than the 6000-r dose died. The cause of death was not apparent and histological studies of the tissues of the neck failed to show any abnormalities.
Thyroid response to total-body irradiation has been studied by Botkin (144). It was found that total-body irradiation of 1000 r resulted in an increase of organic I-131 in the gland as early as two hours post-irradiation, and persisted until twenty-four hours post-irradiation. Following this there was a gradual decrease in activity through the sixth day. The same pattern existed in the serum I-131. The authors gave the following explanation of these results: "These alterations in functional activity of the thyroid may possibly be due to systemic changes caused by the radiation and are probably mediated through the hypophysis."

The release of I-131 from the thyroid following local irradiation has been studied by St. Aubin (43). Doses of 10,500 and 21,000 r were given to dogs and it was noted that the I-131 was secreted by the thyroid at a faster rate in the irradiated animals than in the controls, and that there was an increase in the amount of PBI-131 and total I-131 in the plasma of the irradiated dogs eight days after treatment.

Brayer (39), in 1959, published an abstract concerning a substance present in the serum of irradiated chick embryos which, when injected into unirradiated embryos, would produce the same result as the irradiation. The material was not characterized, and very little information was given about the substance.
STATEMENT OF PROBLEM

With the various results in the literature on the effects of x-irradiation on thyroid function, this project was undertaken in an effort to correlate the phases of thyroid metabolism changes due to carefully controlled external beam x-irradiation administered locally to the thyroid gland in rats. Emphasis has been placed on the changes which occur in the serum protein-bound iodine and the ability of the thyroid to concentrate I-131.
MATERIALS AND METHODS

In all experiments, male rats of Holtzman strain, weighing 160 to 180 grams, were used, in groups of at least four animals each. As the animals were received they were placed on a low iodine diet (Nutritional Biochemical Company) and distilled water for one week prior to irradiation in an effort to increase the uptake of I-131 in the thyroid.

A. Irradiation Procedure

Radiation was administered under nembutal anesthesia (32 mg/kg body weight) with a G.E. Maxitron x-ray machine operated at 30 ma, 250 kvp, with a filtration of 0.5 mm copper and 1 mm aluminum. Half-value layer was 1.2 mm copper.

The animals were placed in a Lucite holder (Plate I) and positioned on a platform which consisted of one-fourth inch lead with a five-eighth inch hole in the area of the thyroid allowing a small localized beam of x-rays (Plate II).

Dosimetry was as follows: A phantom of approximately the same size and shape as the rat was made of tissue-equivalent Mix D wax. A hole was drilled in the phantom to hold a 250-r Victoreen chamber (Plate III), with the active portion in the same position as the rat thyroid. (This position was confirmed by an x-ray of the phantom, Plate IV.) The phantom containing the chamber was positioned on the lead shield in the identical position of the rat and direct measurement of the dose was made (Plate V). Dose rate at this position was found to be approximately 1550 r/min. This dose rate varied from one
irradiation to another, but was measured and corrected each time. Dose rate was also measured at several points corresponding to various parts of the rats' bodies. At a point 7.0 to 7.5 cm from the center of the x-ray beam the dose rate was 0.8 r/min. In the approximate location of the pituitary the dose rate was found to be 12.0 r/min, or a total dose to the pituitary of approximately 77 rads during the total irradiation time.

In addition to measurements made with the Victoreen chamber, isoresponse curves were run with an automatic constant signal plotter (54). These curves, illustrated in Plate VI, show the rapid falloff of the x-ray dose rate at a distance from the exit.

B. Thyroid Uptake Measurements

I-131 was administered intraperitoneally (0.5 µc) at various times following irradiation. Twenty-four hours following the injection of I-131 the animals were sacrificed by an overdose of nembutal. By means of heart puncture, blood was drawn into a syringe containing a small amount of heparin. Thyroids were removed with trachea intact and immediately placed in 10% formalin solution. Another section of the trachea was removed to serve as extrathyroidal tissue background for counting. Thyroids were counted in a well-type scintillation counter with pulse-height analyzer (base level...300 kev; window...100 kev). Counts were compared to a standard made from the injection material and expressed as a per cent of the amount injected.
C. Plasma I-131 Measurements

Blood was centrifuged at 2000 rpm for five minutes and the plasma removed. Plasma was counted in the well-type scintillation counter and then run through a special resin ion exchange column (Amberlite IR-400). Following this the columns were washed with three milliliters of normal saline and the filtrate counted. The columns remove the unbound iodine and allow the PB1 to flow through. A count of the total I-131 was obtained from the plasma and the count from the filtrate was the protein-bound I-131. The difference of these two counts gave the non-protein-bound I-131.

D. Whole-Body Counts

Total-body I-131 was determined in some of the experiments. A three-inch scintillation detector in conjunction with a pulse-height analyzer was used at a distance of 30 cm from rat to crystal (Plate VII). Standards were used to simulate the whole body as nearly as possible. Field size of the system was determined to be 80% at 10 cm. The pulse-height analyzer was set on the photopeak for I-131. Counts were made immediately following I-131 injection and at twenty-four hours.

E. Biological Half Life of Triiodothyronine

Animals that had received 10,000 rads to the thyroid ten days prior to the beginning of the study were used. The femoral vein was exposed surgically and the triiodothyronine labeled with I-131 was
injected intravenously. The specific activity of the T-3 was approximately 30 mc I-131/mg of T-3. Five microcuries of I-131 or approximately 0.2 μg T-3 was injected into each animal. Groups of four irradiated and four controls were used. Samples of blood were taken from the tail (0.1 cc) at five minutes after injection and this was considered as 100%. Samples were taken at 5, 10, 15, 20, 24, 40, 50, 70, and 120 hours after injection. Blood samples were brought to a volume of 1.0 ml with water (which hemolyzed the blood, giving a more even distribution of activity) and counted at one time in a well-type scintillation counter with pulse-height analysis. Counts were normalized to the five-minute sample and log count plotted against time after injection.

F. Electrophoresis

Electrophoresis studies of the serum from control and irradiated rats were made on the rats which had received 10,000 rads to the thyroid twenty-five days prior to sacrifice. Barbiturate buffer (Spinco B-2) of pH 8.4 was used. Samples were run at a constant voltage of 70 volts and 0 to 15 ma for sixteen hours. A hydrostatic head of 1.0 cm was placed on one side of the system to avoid the backflow of serum from the origin. Two strips were run for each animal. One strip was stained with bromophenol blue and ammonia and the protein components determined by use of the Spinco Analytrol Model RA. The second strip was cut into 1-cm lengths and these counted in the well-type scintillation counter. Net counts/min were plotted for the 1-cm strips on the graph obtained from the Analytrol.
G. Parabiotics

The sides of two rats were shaved and an incision made from above the forearm to the hind leg on the left side of one and on the right side of the other. Incisions of approximately 2 to 3 cm in length were made in the body walls of each. The body walls were sutured together and a suture placed through the scapula of each of the animals to prevent them tearing apart. The animals remained in this condition for fourteen days following surgery before the injection of $I^{-131}$ was made.
RESULTS

A. Comparative Study of Radiation Dose Versus Thyroid Uptake of I-131

In order to obtain an optimal radiation dose for use in further experiments, two preliminary studies were undertaken to determine the effects of radiation at various levels upon the twenty-four hour uptake value for I-131 in the thyroid. Total doses ranging from 1000 to 10,000 rads were given as summarized in Tables I and II. The animals were studied two, seven, and forty-three days after irradiation. As might be expected, uptakes taken forty-three days after irradiation showed that depression of I-131 uptake increased with the dose. The ability of external radiation to suppress uptake seven days after irradiation, however, could not be correlated with the dose. This phenomenon will be dealt with in later studies as attributable to a cyclic effect of irradiation on thyroid function during the first ten days after the gland receives the x-ray dose. As a result of these experiments, a standard dose of 10,000 rads was used in all subsequent studies.

B. Progressive Changes in I-131 Uptake after Irradiation with 10,000 Rads

The second group of experiments was concerned with studying the changes in the amount of I-131 in the thyroid gland twenty-four hours after injection when administered at various times following irradiation. In the first of these experiments thyroid uptake determinations were made on days 1, 3, 5, and 25, the results of which are summarized in Table III. The high statistical errors made it impossible to draw any
definite conclusions from this study but the suggestion of a "cyclic" effect of depression and recovery was noted, indicating that further investigations were necessary.

A second group of animals was irradiated, this time using a Lucite holder for positioning the animals more accurately during the irradiation procedure. In addition to the group of animals which were given x-irradiation to the thyroid, a second group was irradiated through the chest region with 10,000 rads and these animals were considered as "irradiated controls". I-131 uptake measurements, made daily for the first eight days following the irradiation and on days 17 and 38, are tabulated in Table IV. The pattern of depression and recovery was again seen (Figure I) during the first six days, with a gradual decrease in I-131 uptake thereafter. However, in the third experiment (Table V) the decrease in uptake on day 3 was not found. This apparent inconsistency may be due to animals reaching states of depression and recovery at different times. This will be discussed later.

C. Effect of Radiation (10,000 Rads) on Protein-Bound I-131 Distribution in Plasma

With the decreased amount of I-131 found in the glands of irradiated rats, one might expect to find a decrease in the amount of circulating protein-bound I-131, since the level of PBI-131 normally varies with thyroid uptake. However, this was not found to be the case in the irradiated animals. The circulating I-131 followed the pattern of the thyroid uptake during the first six to eight days of testing, but a
marked increase over the controls was found in the later stages (Figure II). Several studies on the plasma were made in an effort to elucidate the reason for this phenomenon. Electrophoretic studies showed no significant differences in the position of the labeled protein in control or experimental animals during the first six days after irradiation. However, by day 25, there was a definite shift of the radioactivity from the alpha globulin fraction to the albumin (Figure III) in the irradiated group.

Pooled plasma from irradiated animals (thirty-eight days after irradiation) and from control animals was incubated with I-131 in the form of sodium iodide to ascertain if the increase noted could be due to intravascular binding of the I-131 iodide to plasma proteins. It was found that no significant amount of I-131 was bound in either the control or irradiated plasma.

D. Histology of the Irradiated Thyroid Gland

The morphology of the irradiated gland was studied by a pathologist at M. D. Anderson Hospital, and the following changes were reported. There was a slight increase of fibrous connective tissue in some areas of the capsule. No apparent changes were found in the vascular wall of the blood vessels or in the nerves.

The follicles of the irradiated glands showed extreme irregularity in size, most of them being approximately one-fifth the size of the control. Almost no colloid was present within the follicles. The cytoplasm of the cells seemed to have been unchanged, possessing the same staining characteristics as the controls.
Considerable variations in size and form of the nuclei were noted in the irradiated gland. Moderate numbers showed early degenerative changes (pyknosis). These variations were noted as early as five days post-irradiation.

E. The Rate of Uptake by Thyroids of Irradiated Rats

The discrepancy between thyroid uptake determination and levels of circulating FBI-131 might be explained if the result of irradiation of the thyroid was an increase in the rate at which radiiodide was incorporated into thyroid hormone and released into blood. Thus, animals which had been irradiated thirty days earlier were injected with I-131 and uptake measurements made at one, three, six, and twenty-four hours. The results of this experiment are shown graphically in Figure IV and tabulated in Table VII.

The amount of I-131 in the glands of experimental animals was lower at all times than that in the control glands. However, since the irradiated glands contained, for the most part, follicles devoid or low in colloid, these studies do not rule out the possibility of an increased rate of uptake of I-131, synthesis of hormone, and secretion of this into the blood.

The rate of appearance of PBI-131 was studied at the same time as the rate of uptake. Here it was found that the ratio of PBI-131 in the irradiated animals to PBI-131 in the controls was lower at one hour after the injection but slightly higher at the other three periods tested.
F. **The Biological Half Life of Exogenous T-3 in Irradiated Rats**

The blood concentration of a hormone is, of course, dependent not only on the rate it is added at its source, but on the rate it is removed, either by metabolic processes destroying the hormone, or by its utilization at the tissue level. It was necessary, therefore, to explore the possibility that irradiation had produced systemic changes which would change the rate of removal of thyroid hormone from plasma. For this purpose, exogenous triiodothyronine labeled with I-131 was injected intravenously and its disappearance from the blood stream studied. These data (Figure V) do not indicate that the radiation has produced any systemic changes in the animals causing a decreased utilization of exogenous T-3. There did appear to be some difference in the period of intra-extravascular mixing time, as illustrated by the initial portion of the curve, giving the curves slightly different positions on the graph but essentially the same slopes. The biological half life for exogenous T-3 was 20.5 hours in the irradiated animals as compared to 23 hours in the controls. The limitations of this type of experiment are discussed later.

G. **I-131 Uptake by the Thyroid in Parabiotic Irradiated and Control Rats**

Since the changes observed by other investigators have been attributed by some (44) to "systemic changes, probably mediated through the hypophysis", it was decided that a study of I-131 uptake in an irradiated animal joined symbiotically to an unirradiated animal might be of value. In this experiment, if the decrease in uptake of I-131 by the thyroid following the irradiation was due to deficiency of TSH by the pituitary,
one would expect the unirradiated animal to supply the TSE for both, and thus stimulate the underactive gland of the irradiated animal to return to normal.

In the two sets of experimental parabiotics the uptake of the irradiated rat was significantly lower than that of the unirradiated animal. From Table VI it can be seen that the amount of total I-131 found in the irradiated or control animal is approximately the same. The PBI-131/non-PBI-131 ratio for the control and irradiated animals in set #1 was 0.283 and 0.184, respectively, and for set #2 it was 0.122 and 0.105, respectively, indicating that more PBI-131 was present in the control animal plasma than in the plasma of the irradiated rat. Unfortunately, the set of control parabiotics died before the uptake studies were made, and therefore no comparison can be made to ascertain if there might have been an effect produced in the control animal by the irradiated one.
### TABLE I

**Time and X-Ray Dose Versus Uptake of I-131**

<table>
<thead>
<tr>
<th>Days after irradiation</th>
<th>Group (rads)</th>
<th>$%$ uptake $\pm$ S.D.* 24 hours after injection</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Control</td>
<td>13.9 $\pm$ 3.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>7,000</td>
<td>10.5 $\pm$ 1.4</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>19.1 $\pm$ 2.1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>19.2 $\pm$ 2.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4,000</td>
<td>13.4 $\pm$ 1.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>7,000</td>
<td>21.5 $\pm$ 3.6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See Appendix for formula.

### TABLE II

**Time and X-Ray Dose Versus Uptake of I-131**

<table>
<thead>
<tr>
<th>Days after irradiation</th>
<th>Group (rads)</th>
<th>$%$ uptake $\pm$ S.D. 24 hours after injection</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Control</td>
<td>31.7 $\pm$ 2.2*</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>29.3 $\pm$ 3.6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4,000</td>
<td>28.7 $\pm$ 4.7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>7,000</td>
<td>22.3 $\pm$ 4.0</td>
<td>4</td>
</tr>
<tr>
<td>43</td>
<td>Control</td>
<td>20.6 $\pm$ 4.6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>18.6 $\pm$ 1.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4,000</td>
<td>13.4 $\pm$ 1.7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>7,000</td>
<td>9.4 $\pm$ 1.0</td>
<td>3</td>
</tr>
</tbody>
</table>

* Difference in control values due to different diet. Animals were given sodium iodide periodically to prevent goiter.
### TABLE III

**Time Versus Uptake for 10,000 Rads**

<table>
<thead>
<tr>
<th>Days after irradiation</th>
<th>Group</th>
<th>% uptake ± S.D. 24 hours after injection</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>26.2 ± 5.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>23.4 ± 6.9</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>33.2 ± 3.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>24.6 ± 11.1</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>30.5 ± 5.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>33.2 ± 5.3</td>
<td>4</td>
</tr>
<tr>
<td>25</td>
<td>Control</td>
<td>25.3 ± 4.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>14.0 ± 1.0</td>
<td>2</td>
</tr>
</tbody>
</table>
### TABLE IV
Time Versus Uptake for 10,000 Rads

<table>
<thead>
<tr>
<th>Days after irradiation</th>
<th>Group</th>
<th>% uptake ± S.D. 24 hours after injection</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>27.6 ± 2.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>25.5 ± 4.0</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>24.5 ± 3.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>24.3 ± 5.0</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>22.2 ± 2.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>15.0 ± 3.0</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>20.5 ± 3.4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>10.3 ± 0.8</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>12.9* ± 0.3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>13.6 ± 3.1</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>12.9* ± 3.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>14.6 ± 3.2</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>8.0* ± 1.4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>5.8 ± 2.0</td>
<td>4</td>
</tr>
<tr>
<td>17</td>
<td>Control</td>
<td>8.8* ± 0.8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>5.5 ± 1.4</td>
<td>4</td>
</tr>
<tr>
<td>38</td>
<td>Control</td>
<td>34.7 ± 3.6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.-Control+</td>
<td>31.9 ± 4.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>20.6 ± 5.1</td>
<td>6</td>
</tr>
</tbody>
</table>

* Decrease in uptake of normals due to stable NaI being added to drinking water to prevent goiter. The concentration was too high.

+ A group of 4 animals were irradiated through the chest region (10,000 rads) to serve as irradiated controls.
## TABLE V

Time Versus Uptake and Plasma Levels of I-131 for 10,000 Rads
(24 hours after injection of I-131)

<table>
<thead>
<tr>
<th>Determination</th>
<th>Day 3-1/2</th>
<th>Day 6</th>
<th>Day 25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Irrad.</td>
<td>Control</td>
</tr>
<tr>
<td>% dose in total body</td>
<td>29.2</td>
<td>61.5</td>
<td>43.4</td>
</tr>
<tr>
<td>% dose in thyroid</td>
<td>20.6</td>
<td>42.1</td>
<td>25.6</td>
</tr>
<tr>
<td>Total I-131 in plasma</td>
<td>0.169</td>
<td>0.360</td>
<td>0.399</td>
</tr>
<tr>
<td>(% dose/ml x 1% body wt.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total PBI-I-131 in plasma</td>
<td>0.091</td>
<td>0.237</td>
<td>0.176</td>
</tr>
<tr>
<td>(% dose/ml x 1% body wt.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-PBI-I-131 in plasma</td>
<td>0.076</td>
<td>0.122</td>
<td>0.224</td>
</tr>
<tr>
<td>(% dose/ml x 1% body wt.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrathyroidal I-131</td>
<td>9.2</td>
<td>19.4</td>
<td>17.8</td>
</tr>
<tr>
<td>(% dose)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average body weight (gm)</td>
<td>187</td>
<td>176</td>
<td>198</td>
</tr>
</tbody>
</table>
TABLE VI

Parabiotics Study

Set #1: Control animal + irradiated animal. Control animal injected with the I-131.

<table>
<thead>
<tr>
<th>Animal</th>
<th>% dose in thyroid</th>
<th>Plasma I-131 cpm/2 ml</th>
<th>Plasma PBI-I31 cpm/2 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.30</td>
<td>440</td>
<td>97</td>
</tr>
<tr>
<td>Irradiated</td>
<td>0.45</td>
<td>477</td>
<td>74</td>
</tr>
</tbody>
</table>

Set #2: Control animal + irradiated animal. Irradiated animal injected with the I-131.

<table>
<thead>
<tr>
<th>Animal</th>
<th>% dose in thyroid</th>
<th>Plasma I-131 cpm/2 ml</th>
<th>Plasma PBI-I31 cpm/2 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.11</td>
<td>651</td>
<td>71</td>
</tr>
<tr>
<td>Irradiated</td>
<td>1.00</td>
<td>629</td>
<td>60</td>
</tr>
</tbody>
</table>


TABLE VII

Rate of Uptake Study

(30 days after 10,000 rads to the thyroid)

<table>
<thead>
<tr>
<th>Hours after I-131 injection</th>
<th>Group</th>
<th>Average body weight</th>
<th>Thyroid uptake ± S.D.</th>
<th>PBI-131 irrad.</th>
<th>PBI-131 control</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>218</td>
<td>19.9 ± 2.4</td>
<td>0.944</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>206</td>
<td>11.9 ± 3.4</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>235</td>
<td>28.0 ± 3.8</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>176</td>
<td>20.4 ± 10.6*</td>
<td>1.127</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>231</td>
<td>52.4 ± 9.8</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>203</td>
<td>39.7 ± 8.5</td>
<td>1.777</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>24</td>
<td>Control</td>
<td>242</td>
<td>50.3 ± 12.4</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Irrad.</td>
<td>176</td>
<td>23.4 ± 6.3</td>
<td>1.040</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

* Animals had been on low iodide diet for approximately one month. This was found to cause more variation in the uptakes from one animal to another and, therefore, the high standard deviations.
FIGURE I

Uptake of I-131 versus time after irradiation.

Control animals' uptake was taken as 100 and irradiated animals normalized to this figure.
FIGURE I

THE EFFECT OF RADIATION

ON THE UPTAKE OF I-131 BY THE THYROID

TIME AFTER RADIATION (DAYS)
FIGURE II

The ratio of protein-bound I-131 to non-protein-bound I-131 versus time after the irradiation.

Control animals' ratio was taken as 100 and the irradiated animals normalized to this value.
FIGURE II

THE EFFECT OF RADIATION
ON PLASMA I-131 LEVELS

CONTROL
FIGURE III

Copies of the electrophoresis pattern of control and irradiated animals. Relative counts per minute per 1-cm strips is represented by the stippled area of the graphs.
FIGURE IV

Rate of uptake of I-131 by the thyroid in irradiated animals and control animals. Per cent uptake of I-131 is plotted versus time after the injection of I-131.
Figure IV
Rate of uptake of I-131 by the thyroid

- Control
- Irradiated

Percent Uptake

Time after I-131 Injection (Hours)
FIGURE V

Plot of the log of the counts per sample versus the time after the injection of I-131-labeled triiodothyronine. Flat portion of the curves are extrapolated to zero and the half-time calculated. Half life of exogenous T-3 was found to be twenty hours in the irradiated animals and twenty-three hours in the controls.
FIGURE V

BIOLOGICAL HALF-LIFE
OF TRIIODOTHYRONINE

- CONTROL
- IRRADIATED

TIME AFTER INJECTION (HOURS)
DISCUSSION

It was the purpose of the experiments presented in the preceding sections to determine if external irradiation to the thyroid gland of rats would produce changes in the functioning of the gland. In some routine clinical studies of thyroid function performed on patients who were receiving external beam radiation therapy it had been noted that areas of the thyroid which were included in the field of radiation showed less concentration of I-131 than they had shown prior to treatment. The work presented here substantiates this observation, demonstrating that relatively early following irradiation, there were measurable changes in the functioning of the thyroid gland.

The minimum dose used, that of 1000 rads, failed to produce changes which could be detected by the techniques used in these experiments. This would bear out statements made by other investigators (41) suggesting that thyroid tissue is relatively resistant to radiation. For a dose of 4000 rads, no detectable difference in function was noted until forty-three days after irradiation. At this time I-131 "uptake" by the irradiated gland was depressed 30%. When the dose was increased to 7000 rads a reduction in I-131 uptake was noted within seven days after irradiation. This relationship of the dose to the time at which radiation effects are obvious is typical of radiation damage.

As explained earlier, most of the experiments utilized a dose of 10,000 rads, delivered at the rate of 1550 r/min. Effects on the ability of the thyroid to concentrate I-131 with this dose were seen within forty-eight hours. The irregularity of results during the first eight
days suggested a cyclic function of thyroid tissue as the result of the
dose. Periods of depression of uptake appeared to be followed by periods
of overactivity of the gland resulting in uptakes higher than in non-
irradiated controls. This effect may be due to overcompensation by the
gland for early damage and a subsequent imbalance of the pituitary-
thyroid relationship. This imbalance could be further complicated by
the death of additional thyroid cells and possible injury to the others
resulting in their inability to multiply. After eight days there was a
gradual decrease in the I-131 uptake values. Complete radiation thyroid-
ectomy was not produced, however, during the period studied, and it is
assumed that an LD_{100} was not delivered to the thyroid tissue. One
might suggest that this value was approached, however, since there was
an approximate 60% depression in thyroid uptake.

It must be remembered that the term "uptake" of I-131 by the
thyroid, as commonly used, actually represents the net amount of radio-
activity in the gland at a specific time after injection and is the
resultant of the amount of iodide removed from the circulation minus
the amount of tagged hormone released back into the blood. The data
presented here indicated that the "uptake" was less in the experimental
glands at all intervals after injection of the radiiodine when measure-
ments were made over a twenty-four hour period in animals which had been
irradiated thirty days earlier. To determine whether this was the
result of a diminished glandular activity, it is necessary to compare
these changes with the appearance and quantity of protein-bound iodine
in plasma. In these studies, FBI-131 levels in irradiated animals,
where tested, were equal to or exceeded those values for control animals
which were obtained simultaneously. In addition, electrophoretic patterns of plasma showed a shift in the distribution of bound I-131 in the experimental animals from the alpha globulins to the albumins. It has been shown by Freinkel, et al. (28) that, when serum is incubated in vitro with increasing concentrations of thyroxine, a shift of the hormone from the fraction with greater affinity but lesser capacity (the globulins) to the fractions of lesser affinity but greater avidity (the albumins) occurs. Indications were, therefore, that the lower "uptake" values of the irradiated thyroids were due in part to the faster transfer of radiiodine through the gland. It must also be remembered that histological examination of the glands showed a marked reduction in the amount of colloid remaining in the gland of the experimental animals, and also indicated that many of the cells were no longer functional or capable of multiplication. The irradiated animals, after thirty days, appeared to be hyperactive, though this was a subjective analysis and not tested experimentally.

While the evidence is as yet inconclusive, these studies suggest that the effect of high-level irradiation limited specifically to the thyroid area resulted in the progressive destruction of the gland. As a process of this destruction, the more viable tissue becomes temporarily overactive but loses its ability to store its hormone. As a consequence, the plasma becomes saturated with thyroid hormones. Uptake of radiiodide from plasma will be decreased because of the reduced amount of active thyroid tissue. That which is taken into the gland will be transferred more rapidly into the plasma in the form of FBI-131 because
of the small amount of residual colloid in the gland with which it can be diluted. On reaching the plasma, already saturated with hormone, it becomes attached primarily to the albumin fraction which, while showing less avidity, has the greatest capacity for the hormone. The animal itself tends to become hyperthyroid.

While much needs to be done to prove this hypothesis, other experiments presented here serve only to rule out other possibilities. For example, the study of the biological half life of exogenous T-3 failed to show any difference in the ability of controls or irradiated animals to metabolize this hormone. Also, the study with the parabiotic animals failed to implicate the pituitary as a causative factor in the changes noted. While one cannot rule out as yet the possibility that irradiation produces a change in the hormone secreted, or in its affinity for plasma proteins, the apparent hyperthyroid condition of the irradiated animals suggests that the thyroids of these animals are producing active hormone products.
Per cent depression of thyroid uptake of I-131 plotted against total x-ray dose for seven days and forty-three days following the irradiation.
FIGURE VII

Theoretical explanation for the difference in uptake of I-131 in animals receiving 7000 rads and 10,000 rads.

Note that curve shape can remain the same in both instances, but is shifted from one dose to another or from one animal to another.
Theoretical Pattern of Depression and Recovery at Different Dosages

Figure VII
SUMMARY

From the studies presented here it has been shown that local x-irradiation of the thyroid gland of rats produces changes in the functioning of the gland.

The effects of the radiation increase with increasing dosages and with an increase in time following the irradiation. There were no detectable effects produced by 1000 rads when studied forty-three days following the irradiation.

Noticeable effects on the ability of the irradiated gland to concentrate I-131 were found. This is due, at least in part, to cellular damage as noted histologically, and to the reduced volume of colloid found in the irradiated glands.

Increased levels of circulating PBI-131 in the experimental animals are probably the result of the progressive destruction of the thyroid tissue and its subsequent loss of ability to store the hormone(s) which are produced by the remaining viable cells.
APPENDIX

1. Formula used for calculation of standard deviation

\[ \text{S.D.} = \sqrt{\frac{\sum (x^2)}{n}} \]

\( n \) = number of animals

\( x \) = deviation from the arithmetic mean.

2. Radiation dose to thyroid from I-131 (58)

\[ D_{\beta} \text{ (infinite)} = 73.8 \times C \times E_{\beta} \times T \]

\[ D_{\beta} (t) = D_{\beta} (1 - \frac{1}{2^{t/T}}) \]

\( C \) = concentration in \( \mu \text{c/gm} \)

\( E_{\beta} \) = average beta-ray energy per disintegration in Mev

\( T \) = effective half life of I-131 in days

\( D_{\beta} (t) \) = that portion of the beta dose delivered at time \( t \) in rads.

3. Calculation of beta dose for amount of I-131 used in these experiments

\[ D_{\beta} \text{ (infinite)} = 73.8 \times C \times E_{\beta} \times T \]

\[ = 73.8 \times \frac{0.30 	imes 0.5}{0.02} \times 0.187 \times 8 \]

\[ = 827 \text{ rads} \]

\[ D_{\beta} (t) = D_{\beta} (1 - \frac{1}{2^{t/T}}) \]

\[ D_{\beta} (24 \text{ hours}) = 827 (1 - \frac{1}{2^{24/8}}) \]

\[ = 69.5 \text{ rads.} \]
Assumptions:

Thyroid weight = 0.02 grams

Thyroid uptake = 30%

Injected dose = 0.5 μc

Effective half life = 8 days.
BIBLIOGRAPHY


17. S. H. Ingbar and N. Freinkel, Surface Binding of Thyroxine by Thyroidal Proteins, Endocrinology 61, 398 (1957).


23. N. B. Myrant, Relation between the Biliary Clearance Rate of Thyroxine and the Binding of Thyroxine by the Serum Proteins, J. Physiol. 135, 426 (1957).


31. K. Brown-Grant, The "Feed-Back" Hypothesis of the Control of Thyroid Function, Ciba Foundation Colloquia on Endocrinology 10, 97 (1957).


33. G. W. Harris, Neuroendocrine Control of TSH Regulation, Comparative Endocrinology, A. Gorbman, ed. (John Wiley and Sons, New York, 1959), pp. 202-222.

34. W. P. VanderLaan and R. Caplan, Observations on Relationship between Total Thyroid Iodine Content and Iodide-Concentrating Mechanism of Thyroid Gland of Rat, Endocrinology 54, 437 (1954).


48. T. Oyama, Effect of Di-ethyl Ether Anesthesia on Thyroid Function of Rat, Endocrinology 65, 56 (1959).


PLATE I

Lacite holder for holding rats during the irradiation.

PLATE II

Holder positioned on lead shield on x-ray machine.
PLATE III

250-r Victoreen chamber. Active portion is less than one-fourth inch.
PLATE IV

Copy of x-ray of wax rat phantom showing the position of the Victoreen chamber.
PLATE V

Wax rat phantom in position with Victoreen in place.
PLATE VI

Isoresponse curves run with the automatic constant signal plotter. Numbers represent the per cent of dose.
ISORESPONSE CURVES

FILTER

SHIELD

X-RAYS
Three-inch scintillation detector used for determining the whole-body I-131 in rats.