# THYROID FUNCTION

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IN THE

SALAMANDER AMPHIUMA MEANS

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SALAMANDER

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# A Thesis

Submitted to the Faculty of Graduate Studies in Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

April 1961

MASTER OF SCIENCE (1961) (Biology)

#### McMASTER UNIVERSITY Hamilton, Ontario

TITLE: Thyroid Function in the Salamander <u>Amphiuma means</u> AUTHOR: John William Moule, B.S. (University of Michigan) SUPERVISOR: Professor Paul Foley Nace

NUMBER OF PAGES: vii, 70

SCOPE AND CONTENTS: The metabolism of iodide was investigated in the adult amphibian, <u>Amphiuma means</u> using an adaption of a standard clinical radioiodine technique. The thyroid uptake of  $I^{131}$  after intraperitoneal injection of a tracer dose was linear on arithmetic plotting for seven days. The uptake then increased to a pronounced peak at seven days.

The thyroid iodide concentrating mechanism was examined in <u>Amphiuma</u> in which the organification processes were blocked by a high level of iodide (0.5% potassium iodide environment). These animals after intraperitoneal injection showed a small constant thyroid radiolodine uptake, prolonged residual activity in the abdomen, and an excretion curve that resembled the radiolodine uptake by the normal thyroids.

The autoradiographic examination of the unblocked thyroids indicated that the radioactivity was bound to a molecule soluble in the cellosolvebenzene histological reagents but insoluble in butyl alcohol.

The treatment by potassium iodide and by radiation from absorbed I<sup>131</sup> produced pronounced pathological thyroid lesions. The glands showed changes in the connective tissue and invasion by white blood cells.

(ii)

#### SUMMARY

Thirty <u>Amphiuma means</u> were used for <u>in vivo</u> thyroid radioiodine measurements in normal and potassium iodide treated animals. Histological and radioautographic examinations were made of the thyroid glands from these animals.

Ten normal <u>Amphiuma</u> were used for the determinations of the uptake of  $I^{131}$  after intraperitoneal injections of NaI<sup>131</sup>. The results, on arithmetic plottings, showed the rate of uptake to be a straight line for seven days. The greatest concentration of radioiodine by these thyroids and the maximum excretion by the kidneys occurred at nine days.

Two <u>Amphiuma</u> were treated by placing them in a 0.5% solution of KI to inhibit organification of the radioiodine. These thyroids had a reduced I<sup>131</sup> uptake after intraperitoneal injection of more than five times that of the normals. Radioactivity remained in large quantities in their peritonea in a dialyzable form for as long as twelve days.

Microscopic examination of the thyroids showed deviations from the expected histological pattern. The thyroids from the control animals had changes in the cellular detail resembling that due to Thyroid Stimulating Hormone stimulation. Those from the untreated I<sup>131</sup> experimental <u>Amphiuma</u> were atrophied and fibrosesed. The <u>Amphiuma</u> placed in the KI solution had thyroids that showed an inflammatory reaction with hyalinization of stromal elements. The glands from the KI treated I<sup>131</sup> experimental animals were very severely damaged with an intense inflammatory reaction, mitosis and loss of normal thyroid cellular elements.

(iii)

Autoradiography showed that the activity in the colloid was attached to a molecule, soluble in the tissue reagents, cellosolve and benzene and insoluble in butyl alcohol.

The theoretical aspects of these findings were discussed in respect to iodide metabolism and thyroid function in the <u>Amphiuma</u>, and to the larger problems of human thyroid disease and the role of this gland in vertebrates.

#### ACKNOWLEDGMENTS

The author wishes to acknowledge the encouragement and help of a number of people. Miss Margaret Moule gave invaluable assistance to the author in the writing of the thesis and the organization of the numerous drafts and revisions. Mr. C.E. Moule and Mrs. T. Garwood-Jones were responsible for the typing and reproduction of the manuscript.

The greatest debt is owed to Dr. P.F. Nace. Dr. Nace saw the author through all the trials that can befall a graduate student, an investigator and a writer. He was responsible for financial assistance for the academic year of 1956 - 1957 and for the summers of 1958 and 1959.

To these and to numerous others the author wishes to take this opportunity to say - "Thank You".

This work was supported by the J.P. Bickell Foundation Grant T-93 of the National Research Council and Grant A-1129 of the National Institute for Arthritis and Metabolic Diseases, U.S. Public Health Service.

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#### INTRODUCTION

The investigation was initiated to accomplish two objectives: the provision of quantative data in an area almost free of information, and the consideration of the value of measurements of thyroid function in lower vertebrates in our understanding of the role played by the thyroid and its hormones in the economy of the animal body.

Our present knowledge began with the descriptions of the human thyroid by the great Renaissance anatomist, Vesalius, in 1543. The name, thyroid, meaning shield-like, was suggested by Wharton, 93 years later. At that time theories of its function were proposed on the basis of its gross appearance and anatomical position. It was thought to be a lymphatic organ, a lubricating gland for the larynx, or, due to its vascularity, an escape shunt to protect the brain from sudden increases in blood pressure (30).

In the 19th century the accepted function for the thyroid, that of the production of an internal secretion or hormone, was shrewdly deduced from the recognition of human disease due to thyroid malfunction and the reproduction of these conditions in simple animal experiments. Support of the theory was provided in 1891 when Murray successfully treated a hypothyroid woman with lightly cooked sheep's thyroid. The patient continued on this therapy in good health for 28 years and died at 75, of pneumonia. The present explanation for the action of the thyroid hormones is based on the observation by Mognushevy in 1895 that the hypothyroid individual had a very low oxygen consumption. It is

thought that cellular respiration at basal conditions is controlled by the circulating levels of thyroid hormones.

From the turn of the century to the present, investigations have centred on the substance secreted by the mammalian thyroid. In 1895, Braumann showed that the thyroid contained a very high concentration of iodine. Very shortly after, Ostwald discovered that the iodine was found in the protein thyroglobulin, the homogenous material filling the follicles of the gland. Kendall, in 1915, isolated the hormone thyroxine from thyroglobulin. Harrington, in 1926, elucidated thyroxine's chemical formula; an amino acid thyronine and four iodine atoms. Recently, other thyronines containing only three iodine atoms have been discovered to be produced by the mammalian thyroid (25).

The introduction of artificial radioactive isotopes in the late thirty's gave new impetus to the study of iodine metabolism and thyroid physiology. The use of  $I^{131}$  in particular has led to a good understanding of iodide metabolism and the cycle within the thyroid of the synthesis of the hormones. It is now usually agreed (25, 30) that the hormones wre produced as outlined in the following four steps:

- Uptake of iodide and tyrosine from the plasma by the thyroid parenchymal cells;
- Oxidation of iodide, organification of iodine with tyrosine and linking of two iodinated tyrosine molecules to form thyroxine;
- 3. Storage of thyroxine in thyroglobulin;
- 4. Release of thyroxine by proteolytic enzymes from thyroglobulin and its secretion to circulation.

Almost all investigations of thyroid physiology have employed mammals and it is usually thought that other vertebrate thyroids are similar. The poikilotherm thyroid, generally speaking, has been overlooked and the amphibian in particular has been neglected. Lynn and Wachowski (18), who reviewed the literature up to 1950, made no mention of specific studies of the function of the amphibian thyroid. There are only two reports of such experiments in Gorbman's review of 1958 (8). The one notable exception has been the observation, in 1912, by Gudernatch that feeding minced thyroids to tadpoles caused a premature metamorphosis. (10). This led to a large body of literature on a fascinating, but still unresolved, puzzle.

As this thesis demonstrates, the amphibian thyroid cannot be dismissed as being a replica of the mammalian gland. While the gross and microscopic appearances of the glands are almost identical in different classes, there are significant functional differences. The amphibian thyroid does not show the same avidity for iodine. Berg (2) reported that both <u>Necturus</u> and <u>Amphiuma</u> have very low levels of radioiodine uptake, with a maximum of 1.5 and less than 1 percent of the dose respectively. In contrast, normal uptake by the human gland is from 20 to 45 percent (21, 30). There are other conspicuous differences between <u>Amphiuma</u> and mammalian thyroids, as will be discussed later.

The primary purpose of these experiments was to obtain some data on the relative rate of function of the <u>Amphiuma</u> thyroid. There are a number of ways of examining thyroid function. For example, the serum level of protein-bound iodide can be determined using a very sensitive chemical technique. This procedure in mammals gives the level of

circulating hormone. However, the method demands a fairly large volume of blood and this precludes serial sampling in any small animal. Thyroid function can also be examined by measuring some physiological parameter, such as oxygen consumption, which is affected by the level of thyroid hormones. This is the classical determination of the basal metabolic rate which has been used for fifty years in clinical medicine, but has been displaced by more direct tests because it is cumbersome and subject to numerous errors. The method chosen for these experiments was the external measurement of the uptake of radioiodine, which avoids these difficulties.

The introduction of radioiodine by Hamilton and Soley in 1939 as a tool in investigation of the thyroid was a major achievement (12). It enabled studies of the gland to be made using physiological or tracer doses of the radioactive element. This substance can be detected both <u>in vitro</u> and <u>in vivo</u> by radiation sensitive devices.

The metabolism of iodide through the use of tracer doses of I<sup>131</sup> has been widely studied. It has been demonstrated that although a number of glands, such as the salivary and gastric, and also the kidney, can clear the plasma of iodide, only the thyroid can maintain a high thyroid to plasma iodide ratio. Furthermore, the thyroid iodide concentrating mechanism is under pituitary control and when stimulated is capable of absorbing the iodide from the plasma when the level of the ion is very low (11).

It has been shown by radioautography that the iodide is localized not in the epithelium, but in the colloid (15). Recently, other radioautographic experiments using tritium labelled leucine have shown this

amino acid to behave in a similar fashion. It has been postulated that the thyroglobulin fraction of the colloid is built up of amino acids including leucine within the parenchymal cell and then it is secreted into the follicular lumen where iodination occurs (23). Some investigators have even pronounced that the rate limiting reaction in the hormone synthesis cycle is the uptake of iodide (7).

The second consideration of this thesis was the unknown tissue action of the thyroid hormones. The evolution of the gland indicates that iodinated amino acids and thyroxine in particular must play some important and fundamental role in the animal body. Iodinated amino acids have been found in many invertebrates and the thyroid has apparently evolved to produce this substance in the vertebrates (7). Unless it is postulated that the role of these substances has evolved within the vertebrate class, it must be assumed that the action of the thyroid hormones on a cellular level is the same for all animals possessing a thyroid.

The classical theory is that the effect of thyroxine is to increase cell respiration. This theory originated in the demonstration in 1895 that oxygen consumption was related to the state of the human thyroid. It was later shown that oxygen consumption at rest was constant and this is thought to reflect the constant temperature of the human body. The oxygen consumption at rest is known as the basal metabolic rate (B.M.R.) and it is thought to be under thyroid control.

A great many investigators have attempted to show that thyroxine has an effect <u>in vitro</u> on the biochemical reactions of cellular respiration. They have had little success (25). No one has clearly shown the fundamental property of the thyroid hormones' actions. If the role of the thyroid has not evolved within the vertebrates and if the B.M.R. in mammals is directly related to level of thyroid hormones, what explanation can be given for animals which do not possess a basal metabolic rate? These are the poikilotherms whose metabolism is dependent on the external environmental temperature. Their oxygen consumption is not dependent on internal regulatory mechanisms.

Hoar's review on the thyroid of fish emphasizes the difficulty of applying memmal-derived theories to other animals (14). Injections in fish of endocrine substances, such as the sex hormones, will produce a demonstrable increase in oxygen consumption (13). This effect cannot be clearly shown with thyroxine. Similarly, in trout when body growth rate and metabolism are the lowest, the rate of thyroid function has been said to be the greatest (26).

The best approach to the investigation of thyroid hormone action was thought to be to evaluate the thyroidal function of an adult amphibian. Such animals have an internal environment which is similar to that of mammals. They possess anatomically discrete thyroids, which on microscopic examination resemble mammalian glands. However, their thyroid hormone apparently does not influence their metabolic rate. If they do not have a thyroidregulated basal metabolic rate and assuming the classical theory of thyroid hormone action, why a thyroid?

This paradox stimulated curiosity, and curiosity, investigation.

#### MATERIALS AND METHODS

#### CHOICE OF ANIMAL

<u>Amphiuma</u><sup>\*</sup>were chosen for these investigations for a number of reasons. They were readily available from the biological supply houses and they proved to be easy to handle and maintain. The <u>Amphiuma</u> had several characteristics particularly suitable for the radioiodine tracer experiments. Their size was ideal for the equipment used in the counting procedures, as the head was large enough to fit over the area open to the scintillating crystal without exposing the rest of the animal. Thyroid tissue was present only in two glands in the neck so all the radioactivity in the thyroid was included in a count on the head.

Amphiuma possess other experimental advantages. They have the largest cells of any vertebrate. The entire thyroid structure is composed of very large units. The follicles could clearly be seen with the naked eye. In comparison with a mammal cell size the thyroid parenchymal cells were very large. This increased the ease and accuracy of microscopy and radioautography.

#### EXPERIMENTAL PROCEDURE

The experiments to be described were performed on three occasions; the summer of 1957, the winter of 1957 and the summer of 1958. The 1957 experiments were head-tail counts on intraperitoneally injected normal <u>Amphiuma</u>, groups B-1 and B-2 respectively. The rest of the experiments were done during the summer of 1958.

Thirty <u>Amphiuma means</u>, weighing from 300 to 650 grams, were obtained from the Caroline Biological Supply Company, Elon College, North Carolina. These were shipped to Hamilton in dozen lots over the course of the experiments. They were placed on arrival in a large aquarium, in fresh spring water at room temperature. During this time they were fed with live frogs. No animals died while being kept, even for several months, and they were particularly free of fungus infections.

The control <u>Amphiuma</u> were taken from the aquarium, sacrificed and their thyroids excised for histological preparation and microscopic examination. The animals were killed by decapitation, the head being severed by a single snip of a pair of bone shears. The thyroids were then dissected from the separated head. In urodele salamanders, the thyroid is not a conspicuous organ (4). The arch of the first basibranchial cartilage was exposed and grasped with a pair of hemostats. The entire thyroid arch was dissected free. Using the hemostats in the original position as a vice, the thyroids were dissected from the first ceratobranchial bone. A number of glands were first measured for length and width in millimeters before removing.

The experimental <u>Amphiuma</u> were kept in the radiation laboratory during the experimental period. There, they were placed in large plastic 10 pound flour jars, obtained from the Tri-State Plastic Company, Henderson, Kentucky. The jars were prepared for the animals by drilling quarter inch holes in the lids with a wood twist drill.

The metabolism of  $I^{131}$  was examined in experiments performed on four groups of <u>Amphiuma</u> - B-1, B-2, B-3 and E-2 (See Table I). The first two experiments, as previously mentioned, consisted of head minus tail

counts over short periods. The other two procedures were more detailed experiments over longer periods. In these, measurements were made on the head and tail regions, on the containers and on the abdomen. With the exception of the abdominal counts, the experiments were carried out over 19 days. For these investigations four normal <u>Amphiuma</u> were used from B-3, and two KI treated animals from E-2.

The pathological changes observed in the thyroids of the experimental <u>Amphiuma</u> were investigated in a series of experiments (Table I). The three variables examined were radiation from radioiodine, a high level of environmental iodide and a combination of both radiation and an iodide environment. There were two variations on each of the treatments, a total of six groups of animals.

The groups receiving  $I^{131}$  (B & C) were placed in two litres of distilled water for periods from a few hours to overnight before receiving the radiolodine. Either they were given the  $I^{131}$  intraperitoneally (B), or by adding the  $I^{131}$  to the water (C). Two groups D & G) were exposed to a high iodide environment. They were placed in two litres of 0.5% solution of either KI (D) or NaI (G). For both groups the length of exposure was 22 days.

Groups E and F were exposed to an environment containing a high level of iodide. They were also given either intraperitoneal injections of  $I^{131}$  (E), or had the radioiodine added to the iodide solution (F). At the end of the experimental periods, the animals were sacrificed as previously described.

The water or iodide solution was changed whenever it became foul. However, this was not done in cases where the radioiodide was added to

the solution or counts were made on the solution, i.e. container and contents. Counts on the solution and on the abdomen were made on four normal animals in B-3 and on the two animals receiving KI and  $I^{131}$  (E-2).

The material for dialysis was taken from an Amphiuma (from Group E-1), exposed to a high KI environment and then given I<sup>131</sup> intraperitoneally. The organs were excised four days after injection. The radioactivity in the kidneys, liver, cloaca and the fluid aspirated from the abdominal cavity was recorded. The organs and the fluid were placed in separate dialysis bags and agitated in two litres of distilled water overnight. The cloaca and peritoneal fluid were again counted.

#### HISTOLOGICAL TECHNIQUE

The thyroids were fixed in Bouin's or Gilson's solutions (9). Gilson's gave the best fixation, reduced the artifacts, preserved better cytoplasmic detail and gave more brilliant staining. The Gilson's fixed material was washed in Lugol's solution. The fixed thyroids were placed in a Lipshaw wire basket and cage, agitated in the following agents: 2 baths of cellosolve for one and a half hours each; 1 of benzene for 1 hour and 20 minutes; 2 tissuemat baths at 60 to 63 degrees Comfor 20 minutes. They were finally embedded in 60 degree Tissuemat. Two thyroid glands from B-3 were dehydrated through a series of six water-butyl alcohol baths and three butyl alcohol - paraffin baths before embedding. The sections were cut at ten microns and stained with hemoxylin and triosin and also with the periodic acid-Schiff procedure.

The microscopic examination of the finished slides was performed with as little prejudice as possible. The animals (and the corresponding slides) were numbered from one to thirty randomly and not by groups. The examination was made without consulting the list of treatments.

#### RADIOAUTOGRAPHIC TECHNIQUE

The distribution of radioactivity in the tissues of the thyroid glands was examined by means of the autoradiographic technique (6). Kodak NTB-2 nuclear track plates were employed in the method used in these experiments. These are 1 x 3 inch glass microscope slides coated on one side by a thin, even layer of the radio-sensitive NTB-2 emulsion. By placing tissue sections on this emulsion and exposing them, a record of the activity was made. The Tissuemat ribbons containing the tissues were spread on a water bath at 50°C. They were transferred on the surface of a clean glass slide to the surface of a distilled water bath at room temperature. The white light was turned off and the rest of the procedure was done by the light of Series Number 2,10 watt, G.E. Red Safelight. The NTB plates were removed from the package and the number of the thyroid scratched on emulsion with carborundum marking pencil. The floating sections were removed by dipping the NTB slide. emulsion side up, under the sections. The slides were then placed in a light-tight black slide box (Clay Adams A-1604B) with a little anhydrous calcium sulphate. The edges of the box were sealed with electrician's black plastic friction tape and the box placed in the refrigerator at 10°C. After one week, one of the plates was removed. It was dewaxed in xylene, run up the alcohol series and developed in D 11, five minutes at 68°F. The plate was fixed in a standard hypo solution for 10 minutes and washed overnight. The slide was stained by placing the plate in very dilute hemotoxylin for 4 hours and then washed overnight. The slide was finally run up the series to xylene and coverslip-mounted in H.S.R.

In this experiment, Kodak nuclear track plates NTB-2, emulsion number 512, 555-20 were used. The cellosolve-benzene and butyl alcohol embedded thyroid were sectioned at 7 microns.

#### RADIOACTIVITY MEASUREMENT IN VIVO (COUNTING PROCEDURE)

Radioactivity was detected by a scintillation counter consisting of a shielded crystal detector; a linear amplifier, pulse height discriminator and a counting rate meter or Esterline-Angus pen recorder (27). The detector was a shielded 1.5 in NaI crystal mounted in front of photomultiplier tube. The cylindrical lead shield projected 2 inches beyond the face of the crystal. The amplifier and pulse discriminator were combined in the Nuclear Chicago Radiation Analyser model 1810. It was operated at Base 250, window width 10 and gain of 1. The scaler unit was a Nuclear Chicago model 165.

The  $I^{131}$  was obtained from Charles E. Frosst Limited, Montreal, as carried free NaI<sup>131</sup>. This solution was diluted with distilled water, until the specific activity was about five microcuries per milliliter. The desired dose was calculated in milliliters, drawn into a hypodermic syringe and counted at a distance of one meter from the crystal for five minutes. After injection of the  $I^{131}$ , the empty syringe was oounted at one meter for five minutes. The difference between these two counts was considered to be the administered dose. The quantity of  $I^{131}$  represented by this value was determined by comparing it with a count, again at one meter for five minutes, of a standard of  $I^{131}$  of known strength.

A long slender bag was made by sealing one end of a piece of 2 inch diameter DuPont polyethylene tube. The only reliable method of sealing was to enclose the end of the tubing in an aluminium "button hole" jig and to melt the enclosed plastic with an oxygen-gas flame.

The Amphiuma were slipped, head first, into this bag. They were then carried into the counting room. The polyethylene bag had two functions: it prevented contamination of the counting area, and helped subdue the occasional restless animal - thus no anesthesia was necessary. While enclosed in the tubing, the salamanders did not show any signs of respiratory distress.

The animals were counted in the polyethylene bags by holding the head in front of the crystal for 3 minutes with the scaler or until the graph of the pen recorder showed a definite plateau of fluctuations. The tail was similarly counted. The abdominal count was obtained by drawing the salamander slowly past the counter face. These counts were done only with the pen recorder and the abdominal count was considered to be the highest level reached during this manoeuvre. The organs counted in the dialysis experiment were placed in separate polyethylene bags and were measured at the counter face. In those experiments where the activity was detectable in the water, the counts were made by placing the jar with the solution in front of the counter face. Although this did not include all the fluid in the container, an equal portion of it was counted each time.

#### METHOD OF CALCULATING COUNTING DATA

The rate of emission of particles from a radioactive source depends on the amount of unstable material and the rate of breakdown. This decay is a physical process independent of factors which influence chemical reactions, such as temperature, pH, light or catalysts. In the animal body, radioactive decay continues at its uniform rate, producing

a uniform decrease in emission rate. Deviations from this decrease are due to changes in the amount of radioactive material through biochemical displacement of tagged atoms.

A practical method of determining the quantity of radioactive material present in an animal is to measure the amount of external radiation. Detecting devices, such as the scintillation counter, express the measurement in counts per minute. The unit, counts per minute (c/m), has meaning only with reference to counter instrumentation. It is a measurement of the number of radioactive rays reaching and being detected by the counting device. Counts per minute, per se, are relative, not absolute. To produce data in absolute units with any degree of accuracy would require too much time and instrumentation to be practical and would be limited in value by the inherent variability of the experimental animals. For this reason, the individual measurements were not made with high degree of accuracy so that numerous determinations could be made, thereby minimizing the biological variability.

To simplify analysis and presentation, activity above background was used. In the case of the graph, all readings including background were recorded on one long piece of paper. A baseline was drawn through the background across the bottom of the paper. Each count was recorded by drawing a line through the plateau of fluctuations by sight. The count without background was measured as the distance between these two lines. The scaler unit gave the count directly. The thyroid count was the difference between head and tail counts. All readings were corrected for decay and plotted on ordinary graph paper.

A number of thyroids were measured <u>in situ</u> before being dissected free from the underlying cartilage. For each gland, the length and width

were recorded in millimeters. The lengths and widths were multiplied together individually and the resulting values for each of the two thyroids in each animal were added together to provide a simple numerical expression of thyroid size.

#### RESULTS

#### I. EXPERIMENTS MEASURING MOVEMENTS OF RADIOIODINE IN NORMAL AND POTASSIUM IODIDE TREATED AMPHIUMA

A number of technical points must be considered in presenting these results. There were numerous errors introduced in the method of measuring in vivo the quantity of radioiodine in the Amphiuma. The geometry between the radioactive material and the detecting device varied with each count, as it was impossible to place the head of the animal in exactly the same position each time. Also, the occasional movements of the animals inside the plastic bag had a similar effect. This affected the reproducibility of the repeated measurements of the radioactivity and the calculation of the relative quantity of radioiodine present. There was another factor to evaluate. The difference between the head and tail activity did not give the precise value for the thyroid activity for two reasons. The mass of the head was not equal to the mass of the tail but was slightly greater. The average difference was about 6 grams. The blood volume in the head was larger than in the tail and this would have affected measurements of activity that was present in the circulation. In consideration of the above factors, the thyroid measurements were recorded as head minus tail counts (head - tail).

The measurement of radiolodine in the containers after the Amphiuma were removed was fairly accurate. There was no difficulty

in positioning the flour jar in front of the scintillation counter. However, not all the contents fell within the cone surveyed by the radiosensitive crystal. This was not important in serial sampling of the relative quantity of activity as the same fraction was measured at each count.

#### (a) Measurements of radioactivity in normal animals.

A number of parameters of the normal radioiodine metabolism were measured. In all, ten <u>Amphiuma</u> were employed in these experiments. There were three animals used in August 1957 (B-1), three in December 1957 (B-2) and four in July 1958 (B-3). The first two experiments utilized animals from the same shipment received in July 1957. Each animal received tracer doses of  $I^{131}$  intraperitoneally. The quantity of  $I^{131}$  administered was usually between 10 and 20 microcuries. Two <u>Amphiuma</u> in B-3 were given larger doses. They received about 50 microcuries.

The results of measurements of thyroid activity after correction for decay showed minimal scatter when plotted arithmetically on normal graph paper (Graphs I, II). The values for head - tail counts showed a straight line distribution for the short period of 5½ days. The linear relationship held in all three groups over this interval (Graph I). Examination of the straight lines of thyroid uptake revealed a number of interesting points. The slopes from B-1 and B-2 were identical on visual inspection. The slope from the third group (B-3) is somewhat greater. The projection of the lines back through the Y axis gives a Y intercept of approximately 10 c/m for both B-2 and B-3. The Y intercept from B-1 comes close to passing through the origin. Thus it is displaced below the other two lines.

The measurements of the activity in Group B-3 were more extensive and were carried out over a longer period. For 19 days the "thyroid", tail, abdomen, and the contents of the containers were counted and the calculated corrected activity was plotted arithmetically (Table III, Graph II). The results of this experiment showed that the straight line of Graph I continued for only seven days. On the eighth day, the head tail count was much greater than the value on the projected line. The next day, the head count was so great that it could not be recorded with the equipment at the original adjustments. The head activity remained off scale for the next three days. On the twelfth day the activity dropped to a level where it could again be recorded and the head - tail corrected value was 23,000 counts per minute. For the rest of the experiment, the head - tail value oscillated at an average value of slightly more than 24,000 c/m.

Radioactivity was recorded over the tail area for one week. After this time, it had declined so low in some animals as to be indistinguishable from background. The plot of the tail activity for one week after injection showed a plateau of approximately 2,000 counts per minute gradually decreasing beginning in the third day.

The results of monitoring the abdominal cavities of the <u>Amphiuma</u> in Group B-3 (Table VI) were dependent on the amount of radioiodine administered. The animals which received more than 25-30 microcuries showed a greater residual radioactivity than those which had received less. The highest value in the abdomen was 13,000 c/m recorded in one of these <u>Amphiuma</u> on the fourth day. The average activity decreased rapidly from 10,000 c/m (third day; first measurement) to 5,000 c/m by the seventh day.

No more readings were attempted after the seventh day as it was too difficult to distinguish the recorded abdominal peak from large fluctuations in the background.

The amount of radioactivity detected in the containers was also dependent on the administered dose. In those <u>Amphiuma</u> given small doses, the highest reading was 1,500 c/m recorded on the third day and after the twelfth day little activity could be observed. The other animals which had received injections of larger quantites of radioiodine showed more abdominal activity and their readings are plotted on Graph II. The corrected counts rose to a level of more than 3,000 c/m by the second day where they stayed for five days. On the eighth day, a peak of 8,200 c/m was recorded followed by a return to the previous level.

The head - tail counts on the <u>Amphiuma</u> kept in the radioiodine solution were low (Table V). However, they rose gradually until the tenth day when the value was 12,000 c/m and the counting was terminated.

#### (b) Measurement of radioactivity in KI treated animals.

Both <u>Amphiuma</u> in Group E-2 were injected with I<sup>131</sup> intraperitoneally ten days after being placed in the KI solution. One animal received a large tracer dose (more than 50 microcuries) and the other less than 25. The results of the head - tail, abdomen and container counts are in Tables IV and VI and Graph III.

The average corrected head minus tail activity curve was very low. It began, as did the curve for the normal animals, with the first count close to 2,000 c/m and the values remained around 2,000 c/m with the highest reading of 2,400 c/m on the fifth day. There was a drop to 900 c/m the next day and after four days of oscillation, the activity declined until it was indistinguishable from background by the tenth day.

The activity in the tail exceeded that of the head - tail with the exception of one day (Table IV, Graph III). It was detectable for 15 days. The maximum reading of 2,800 c/m was not a pronounced peak being only 200 c/m more than the previous day.

On the first two days that the abdomens were scanned (Days 3 and 4), there was so much activity in one as to make it unrecordable with the available equipment. This animal received a tracer dose of more than 50 microcuries and corresponded to those in Group B with the largest doses. There was a residual activity in the abdomen on the twelfth day of 24,000 c/m.

The plot of activity in the KI solution (i.e. container) resembled that of the normal thyroid throughout the entire experimental period. After the second day, the values showed a straight line distribution with the same slope and duration. The maximum corrected count was 28,700 recorded on the ninth day, roughly corresponding to the normal thyroid peak. By the twelfth day the values dropped to 13,700 c/m and over the next four days decreased slightly. The experiment was then terminated because the solution had become too foul. The <u>Amphiuma</u> sloughed their skins in large quantities and these accumulated in the container, polluting the solution.

The uptake of radioiodine from the KI solution to which I<sup>131</sup> had been added was minimal (Table V); however, it could be detected. The tail count did not exceed background radiation.

The results of the dialysis experiments from one of the KI and I<sup>131</sup> treated animals is shown in Table VIII. The radioactivity in the liver was twice that shown in one kidney and that in the three milliliters

of peritoneal fluid was double that in the cloaca. After the dialysis, the cloaca showed a decrease in activity of four times while the peritoneal fluid dropped more than twenty times.

# TABLE I

		Sub	Number of	Tr	eatment Time	in Days
Group	Group Treatment	Groups	Animals	Total	Before I <sup>131</sup>	After I <sup>131</sup>
A	Control		4			
В	1 <sup>131</sup> 1.P.	B-1* B-2 <sup>/</sup> B-3 <sup>x</sup>	3 3 5	6 6 19		
С	I <sup>131</sup> Environ.		2	23		
D	KI Environ.		3	22		
E	KI Environ. I <sup>131</sup> I.P.	E-1 E-2 E-3	2 2 2	12 26 28	8 10 23	4 16 5
F	KI and I <sup>131</sup> Environ.	F-1 F-2	1 1	25 25	2 7	23 18
G	Nal Environ.		1	22		
		TOTAL	29 <sup>0</sup>			

#### TABLE OF TREATMENT

\*Summer 1957 /Winter 1957 \*Summer 1958 ØFast spimal was injected wi

<sup>0</sup>Last animal was injected with 10 ml. of 2% NaCl solution, but died in 4 days before any observations were made.

## TABLE II

#### AVERAGE VALUES FOR RADIOACTIVITY MEASUREMENT IN NORMAL AMPHIUMA.

# HEAD - TAIL VALUES FOR 6 DAYS Nal<sup>131</sup> INJECTED 1.P.

В·	-1	B-2		B	-3
Time	Value	Time	Value	Time	Value
2*	3.2	2*	3.3		
3*	11.4				
6*	22.0				
9*	15.8				
12*	15.0	12*	8.5		
30*	39.5			14	40
36*	39.0	36*	27.1		
21/2+	58.0	234	52.0	2 <sup>4</sup>	73
312+	86.0	33/2*	62.0	3+	90
4124	80.0	45+	85.0	4 <sup>4</sup>	120
5支4	118.0	5-24	104.0	54	140
				64	170

\*Time in Hours

<sup>4</sup>Time in Days

Values in 100 counts per minute.

## TABLE III

#### AVERAGE VALUES FOR RADIOACTIVITY MEASUREMENTS IN NORMAL AMPHIUMA

# COUNTS FOR 19 DAYS - GROUP B-3 Nal<sup>131</sup> INJECTED I.P.

Mine da Dava	in Days Head-Tail Tail	m-41		Container and Contents	
Time in Days		Abdomen	*	+	
1	4.0	2.0		.4	1.3
2	7:3	2.2		.6	3.3
3	9:0	1.9	10	1.5	3.4
4	12.0	1.8	8	1.0	3.2
5	14.6	1.8	6	.8	3.4
6	17.1	1.4	7	.6	3.1
7	21.5	1.1	5	.6	5.5
8	33:0	1.1		.6	8.2
9			4		7.9
10					
11				.3	4.0
12	28.8				2.7
13	22.5				2.5
14	23.3		50000000000		3.2
15	23.4	2 Martine Contraction	Contract of the second s	Sector Sector	2.9
16	27.1				3.0
17	23.9		and the second se		2.7
18	24.0				2.4
19	26.4	in the la			

\*Small tracer dose I<sup>131</sup> injected. <sup>4</sup>Larger tracer dose I<sup>131</sup> injected. Values in 1,000 counts per minute.

## TABLE IV

# AVERAGE VALUES FOR RADIOACTIVITY MEASUREMENTS IN KI TREATED AMPHIUMA NaI<sup>131</sup> INJECTED I.P.

Time in Days	Head-Tail	Tail	Abdomen	KI Soln.
1	1.9	2.3		2.4
2	2.0	2.4		3.2
3	2.1	2.6		5.1
4	1.8	2.8		8.2
5	2.4	2.3	13	10.5
6	.9	2.5	8.5	12.2
7	.5	2.2	13	17.0
8	1.5	1.6	11	25.3
9	.7	1.6		28.7
10	.6	1.8		
11				13.7
12		.8		12.6
13		.2		12.1
14		.2		11.0
15		.1		
			A CONTRACTOR OF A CONTRACTOR	

GROUP E-2

# TABLE V

# AVERAGE HEAD - TAIL VALUES FOR NORMAL AND KI TREATED ANIMALS

Nal<sup>131</sup> ADDED TO WATER OR TO KI SOLUTION

Time in Days	Normal (C) Head-Tail	KI Treated (F) Head-Tail		
1		2,3		
2	5.0	2.0		
3				
4	2.8	1.8		
5	2.9	.8		
6	3,0	.5		
7	5,2	.2		
8	5.2	.1		
9	6.5	Trace		
10	12.0	Trace		

GROUPS C AND F

Values in 1,000 counts/minute

# TABLE VI

# VALUES FOR RADIOACTIVITY MEASURED IN ABDOMENS NORMAL AND KI TREATED ANIMALS 1<sup>131</sup> I.P.

# GROUPS B AND E

Time	NORMAL (B)			K	I TREATE	D (E)		
Days	Low	Dose	High	Dose	Average	Low	High	Average
1								
2								
3		8		12	10	13	20+	
4	4	7	13	10	8	10	20+	
5	5	6			6	6	20	13
6	3	5	12		7	5	12	8.5
7	2	4	8	6	5	4	22	13
8						6	16	11
9				4	4			
10								
11								
12							24	

Values in 1,000 counts/minute

# TABLE VII

#### ORGAN COUNTS BEFORE DIALYSIS AND AFTER

Organ	Before	After
Kidney	1.0	*
Liver	3.2	*
Cloaca	4.0	1.0
Peritoneal Fluid	8.4	0.4

\*No count made

In 1,000 counts/minute
#### **II. AUTOPSY RESULTS**

(a) Gross Examination

On gross examination the thyroids of the normal control salamanders were full, white in colour and elliptical in shape. They could be removed from the underlying cartilage without too much difficulty. Their average size was 55 units (Table VIII).

With the exception of the NaI (Group G), the treated animals had markedly atrophied glands. They were yellowish in colour, flattened ovals in shape and usually somewhat shrunken into the slight depression on the basibranchial cartilage. It was often difficult to remove them. They varied in average size from 22 to 27 units.

The NaI treated animals' thyroids resembled the normal controls on gross inspection and measurement. They were 50 units in size.

#### (b) Microscopic Examination

Microscopic examination, an integral part of any autopsy, was performed on twenty four animals with the results from the thyroid glands being tabulated in Table IX. The histological changes occurred in the supporting stroma, in the colloid and in the thyroid epithelium (parenchymal cells). The stroma showed two changes, an increase in the quantity of fibrous material and hyalinization of this substance (Plate II, Fig. 3 & 4). Similarly, the colloid underwent two changes; one, becoming granular and losing its normal staining properties (Plate III, Fig. 8), and two, becoming the site of infiltration by white blood cells (Plate I, Fig. 2 & 3; Plate IV, Fig. 9, 10, 11). The epithelium showed three changes; one, it became invaginated and hyperplastic (Plate III, Fig. 6 & 7),

# TABLE VIII

## GROSS SIZE OF THYROID GLANDS

Group	# Animals Measured	Size	
A	4	55	
В	2	22	
C	2	40	
D	2	36	
E	2	37	
F	2	22	
G	1	50	

Cross section of both thyroid glands measured in arbitrary units.

Experimental Group		STROMA		THYROID EPITHELIUM			COLLOID			
		Fibrosis	Hyalinizatio	Hyperplasia	Invagination	Hurthle Cells	Granular Colloid	Inflammation	Type of Cell	
Control	A		+	+++++	+	+			den en en angelen og men er en	
	B-1	++		++	++ + +			+	polys	
1 <sup>131</sup> I.P.	B-3	* ++ ++ + +	*	+++ ++ ++ +	++ ++ +- +	+++ + + +	++	++	polys polys	hemorrhage
1 <sup>131</sup> Environ.	C	+	+++	+++++	+++ ++	+		+++	lymphs polys	
KI Environ.	D		++ ++ ++	++++	++ + ++	+++	++	+	polys, lymphs	
KI Environ. I <sup>131</sup> I.P.	E-1		+++	2		9	+++	++++ ++	polys, lymphs polys, lymphs	
	E-2 E-3		++	+	+		+	+++ ++	lymphs, macros, lymphs, macros,	mitosis mitosis
KI I <sup>131</sup> Environ.	F	+	++	+	+	+++++	+++++++++++++++++++++++++++++++++++++++	++	lymphs, macros, lymphs, macros	hemorrhage
Nal Environ.	G		+++	+	+	+++			*JMPHO, MULLOO	

Degree of change by subjective assessment

+ Mild ++ Moderate +++ Strong ++++ Intense Abbreviation of cell types:

polys - polymorphonuclearcytes lymphs - lymphocytes macros - macrocytes CHANGES OBSERVED ON MICROSCOPIC EXAMINATION OF THE THYROIDS

TABLE IX

two, the parenchymal cells were sometimes increased in size, a condition accompanied by mitotic figures in the nuclei (Plate IV, Fig. 10 and 11), and three, there appeared in the epithelium large aberrent brightly, acidophillic cells (Plate V, Fig. 12 and 13).

The normal stroma (Plate I, Fig. 1) surrounding the follicles and the vascular structures was thin, acidophilic and fibrous. When the quantity of the acidophilic material increased, the follicles were spaced further apart. Similarly, the arrangement of the follicles was changed by hyalinization. This material was an amorphous, basophilic substance which often had a mottled appearance. It was associated with an increased number of thin angular hyperchromic nuclei. Both fibrosis and hyalinization appeared together in some glands. The hyaline then appeared as flattened globules within the stroma.

The colloid in normal thyroids, a clear amorphous substance, was acidophilic and stained with periodic-acid Schiff (P.A.S.). The lumens of some follicles were filled with a granular chromophobic or basophilic and P.A.S. negative material. The lumens of these and normal appearing follicles were invaded by cells of the peripheral white blood series. There were three patterns of these invasions, depending on type of cell, polymorphonuclear, polymorphonuclear-lymphocytic, and lymphocytic macrocytic. Occasionally, the macrocytes of the latter infiltration were filled with droplets of acidophilic, P.A.S. positive material. Erythrocytes were found in the lumen of a few follicles. In one instance, disintegration of the nuclei of these cells was observed.

There were a number of deviations in the appearance of the parenchymal thyroid cells which are normally cuboidal with a round basal

nucleus. (See arrow, Fig. 1). Invagination and hyperplasia of these cells usually appeared together, especially in the periphery of the glands. Invagination was an epithelial unfolding. Such changes were often so pronounced as to greatly distort the normal spherical shape of the follicles. Hyperplasia, an abnormal increase in the number of the parenchymal cells, resulted in a crowded columnar epithelium cells with basal oval nuclei and bulging apical cytoplasm. The hyperplasia was often very noticeable especially in the invaginated epithelial folds.

Under certain conditions, there were variations in the dimensions of the parenchymal cells; in nuclear diameter and in cytoplasmic volume. For instance, the cells were occasionally thin and squamous while in the same gland, other epithelial cells appeared large and columnar. Numerous mitotic figures were seen scattered in the epithelia of two thyroids which showed these changes. The most striking observation was the appearance in the epithelia of large polyhedral cells. These cells had an abundance of cytoplasm which was filled with brilliantly staining granules, acidophilic in nature and usually P.A.S. positive. The nucleus was apical rather than basal in this case and frequently pyknotic. In mammals, cells of similar appearance have been called Hurthle cells (1).

The intact <u>Amphiuma</u> were subjected to three types of stress, radiation from  $I^{131}$  (B, C), a high electrolytic environment of KI (D) or of NaI (G) and of both radiation and KI environment (E, F). The greatest degree of pathological changes was found in Groups E and F where the <u>Amphiuma</u> were subjected to the combined effects of radiation from  $I^{131}$ and from a high concentration of KI in the environment.

Control: The control animals appeared to have normally functional thyroids. However, there was some evidence of cellular change in these glands. The normal <u>Amphiuma</u> thyroid should present a sound follicle lined with cuboidal cells on a thin acidophilic stroma (1). The epithelia of some of these control thyroids were mildly hyperplastic, invaginated and contained the odd Hurthle cell.

Radiation: On microscopical examination, the thyroids from animals receiving this treatment appeared functional. The stroma had thickened to varying degrees, a change described by Lindsay (17) as perifollicular fibrosis. A slight deposition of hyalin was also observed in the stroma of two of the thyroids. Epithelial changes were quite pronounced with hyperplasia and invagination, often accompanied by large numbers of Hurthle cells. The colloid was occasionally invaded by polymorphonuclearcytes. In one case, there were granular colloids observed and in others there was intrafollicular hemorrhage.

The <u>Amphiuma</u> which were subjected to radiation were divided into three smaller groups, B-1, B-3 and C. Those in B received intraperitoneal injections of  $I^{131}$ ; animals in B-1 being sacrificed 6 days after injection, in B-3 after 19 days. The three groups had the same histological appearance. There was a minimal variation between individuals but no pronounced deviation from the expected pattern.

KI Environment: The three <u>Amphiuma</u> in this group were subjected to a KI environment for 22 days. The thyroids from these animals were slightly distorted. There appeared to be considerable hyalinization of the stroma. The epithelia were mildly hyperplasic and slightly more invag-

-inated than those of the controls. One thyroid showed both granular colloids and a mild subacute inflammation. Another had a large number of Hurthle cells.

KI and Radiation: This treatment produced the most extensive thyroid lesions. In some glands, the normal structure was completely obliterated by an intense chronic inflammatory reaction. The stroma, when could be visualized, contained varying degrees of hyalin. Accompanying the inflammation, there was a disappearance of the normal cuboidal epithelium which was replaced by **aell** types ranging from squamous to columnar. The tissue also showed mitotic activity, necrosis, and in one follicle, hemorrhage.

The lesions described above applied to most of Groups E and F. These animals had a total treatment time of from 26 - 28 days with the interval at which the  $I^{131}$  was administered varying from 5 - 23 days (Table I). The one exception was the thyroids from the sub-group E-1 which did not exhibit the extremes of pathological changes observed in the rest of E and F. In these glands the normal thyroid architecture was retained in portions of the gland. The total treatment time for the sub-group was 12 days with an interval of 8 days in KI before giving  $I^{131}$ .

NaI Environment: The single Amphiuma placed in an NaI environment for 22 days appeared surprisingly normal. There was considerable stromal hyalinization. The epithelium was no more hyperplastic or invaginated than the controls were. However, numerous Hurthle cells were observed.

#### III. AUTORADIOGRAPHIC EXAMINATION

The NTB-2 slides were examined microscopically under low power for the presence of black grains of silver in the developed emulsion. Throughout all the NTB-2 plates there was a small random scattering of these particles indicative of background radiation. Beneath the tissue sections processed through the usual histological reagents, only the background level of the grains was found. The thyroids which had been dehydrated and embedded via a butyl alcohol series produced an intense reaction in the emulsion. The greatest concentration of the grains was under the follicles. Here the emulsion was completely blackened (Plate VI, Fig. 14). The intensity decreased peripherially and below the areas of tissue distant from the follicles only a background level was observed.

#### DISCUSSION

#### I. MEASUREMENTS OF RADIOIODIDE IN NORMAL AMPHIUMA

The fate of iodide from ingestion to excretion has been extensively studied through the use of radioiodide **as** a tracer (21, 30). After the administration of a small quantity of  $I^{131}$ , the radioactive ion quickly becomes distributed through the extra-cellular fluids (25). The iodide also diffuses into most of the fluid cavities. Inman, for example, it has been shown that the activity in ascitic fluid reaches 75% of the maximum level in as short a time as 30 minutes after an intravenous injection of  $I^{131}$  (21).

The thyroidal utilization of iodide cannot be considered as being isolated from the general systemic metabolism of iodide. The iodide is progressively cleared from the circulating plasma by a number of exocrine glands, by the thyroid and by the kidney. The glands of the gastrointestinal tract are very active in this respect and it is interesting to note that the thyroid is derived embryonically from a gut analogue (11). The iodide concentrated by the gut is re-absorbed back into the body in the small intestine (24), so that the only permanent loss of iodide from the plasma is to the thyroid and to the kidney.

The technique of measuring thyroid radiolodine uptake must take into account the tissue distribution of radiolodide. One of the most widely accepted clinical methods has been the neck-thigh count. This test

has been adopted because it is simple, quick and reasonably accurate. Two counts of radioactivity are made, one on the neck and the other on the thigh. Since the circumference of the thigh, a few inches above the knee is approximately equal to that of the neck, the quantity of radioactivity in the thigh is taken as being approximately equal to that of the non-thyroidal tissues of the neck. Therefore, the neck minus the thigh count is considered to be equal to the activity in the thyroid gland.

Studies, using this method to determine the radioiodine uptake of the human thyroid, have shown it to rise rapidly during the first few hours after the administration of the dose. The maximum uptake occurs within 1 - 2 days and then the curve plateaus or drops slowly (20, 30).

The quantity of radioactivity in the thyroid must not be taken to be the result of a single process, but rather the result of several reactions. For example, there is the uptake of radioiodine by the thyroid cells followed by the incorporation of the radioiodide into the protein complexes of the colloid. Furthermore, the uptake of iodide is not a unidirectional reaction. Studies in the rat have shown that there is a simultaneous uptake and loss of the iodide ion between the circulating plasma and the thyroid (22). Careful measurements of several thyroid parameters followed by detailed calculations have demonstrated that the rate of production of thyroxine is <u>not</u> equal to the rate of iodide uptake.

The practical details of the counting procedure should also be considered. As has been pointed out, a number of technical factors introduced a degree of error. This error, however, was constant. The final corrected value for the head - tail count was not an absolute value

but one relative to values determined at other times. From these data a relative thyroid iodine uptake curve was drawn with some assurance. The activity detected in the contents of the containers of the I.P. injected animals represented the excretion of radioiodine. The curves plotted from these data were an indication of the changes of iodide excretory rate. Again the measurements were relative rather than absolute due to inherent errors in the technique. Also when comparisons were drawn between the excretory rate and the rate of thyroid uptake a further error was introduced as each procedure did not measure the same percent of total activity. Thus the curves differed by a constant factor. The abdominal counts were made in a very haphazard fashion and these results can only be interpreted qualitatively.

Three conclusions were made from the results of the various experiments using normal Amphiuma. They are as follows:

- 1. The thyroids of Amphiuma definitely showed an uptake of I<sup>131</sup> whether the radioiodine was injected intraperitoneally (Table 2, 3) or placed in the water in the containers (Table 5). In this respect the <u>Amphiuma</u> resemble the rest of the vertebrates that have been studied (8). The uptake, however, was not as rapid as that of the mammalian thyroid. The peak of thyroid activity occurred at 9 - 10 days rather than 24 - 48 hours. The straight line for 6 days, on arithmetic plotting, was an interesting observation.
- 2. There was a rapid increase in the rates of both the thyroid uptake and of the excretion to a peak at 9 days (Graph II).

3. There was a considerable residual activity in both the abdomen and in the tail over a considerable period of time (Table 3). The tail activity could possibly have represented organically bound radioiodine. However, this would not have explained the presence of radioactivity in the abdomen. There are two bits of evidence from the literature to indicate that the non-thyroid activity was probably the radioiodide ion. Several days after injection of thyroxine (to lower circulating thyroid stimulating hormone), Berg (2) gave Amphiuma a tracer dose of 1<sup>131</sup>. He found that no more than 1% was taken up by the thyroid in 2 to 4 days. Presumably the rest would have remained as free radioiodine. Similarly, Broberg (3) attempted to make chromatographs and measure the quantity of labelled thyroxine in the various tissue of Necturus after I.P. 1<sup>131</sup> injections. She made Butanol extractions (in which thyroxine is soluble) of her sample. However, no labelled thyroxine could be found even after intervals up to three days. Only radioiodide could be demonstrated in the chromatographs. These two experiments support the hypothesis that the tail activity was due to free radioiodide.

II. MEASUREMENTS OF RADIOIODIDE IN THE POTASSIUM IODIDE TREATED AMPHIUMA

The study of iodide metabolism is a fairly complex problem as was briefly outlined in the preceding section. There are several pathways that can be taken by the plasma iodide and each of them involves several mechanisms. When all of the biochemical reactions are occurring simultaneously, it is impossible to measure the overall response and then to decide the role played by any individual mechanism.

Among the methods available of attacking the dilemma was the one chosen in this experiment. It is possible to isolate the process of iodide concentration by using  $I^{131}$  as a tracer and by blocking the organification of iodine within the thyroid gland. The technique has been employed on mammalian thyroids and has demonstrated that this gland can maintain an iodide concentration several hundred times that of the plasma (29). The method used to create the block was the maintenance of a high level of iodide. The excess iodide in some fashion completely inhibits the biochemical mechanisms by which iodide would normally be linked to an amino-acid moeity (5).

The uptake curve of radioiodide by the thyroid, after the administration of the blocking agent, represents the concentration of only the iodide ion. There is no masking by other thyroid reactions involving radioactivity. This reduces the quantity of the tracer dose that enters the thyroid, leaving the renal pathways as the major route for the movement of the radioiodine. The renal mechanisms are not affected by the iodide used to block the thyroid cycle. The kidney then becomes the major route for the metabolism of iodide and, in fact, block-

-ing the thyroid is one means of studying the renal iodide excretion rate.

The use of an excess of iodide as in this experiment precludes quantitative radioiodide measurements. The radioactive iodine is diluted in the much larger quantity of stable iodine. Since no measurements were made of the quantity of the stable isotope, no statements can be made regarding the ratio of radioactive to stable iodine or the total amount of iodide concentrated by the gland. This experiment simply indicated whether or not the thyroid was capable of concentrating the iodide ion. If this could be shown to occur, then the first portion of the thyroid cycle would be similar to that in mammals.

In these experiments, the high level of iodide was produced by placing the <u>Amphiuma</u> in a 0.5% solution of KI. KI was used as it happened to be in the laboratory in quantity. It must be pointed out that no control was made on this and that the uptake results obtained could, in part, be due to the high levels of potassium.

Three conclusions were drawn from the results of the KI treated Amphiuma. They were:

1. Despite the dilution of radioiodine by the stable iodide and the destructive lesions in the thyroids, there was a minimal thyroid uptake from the I.P. injected animals. This indicated that the <u>Amphiuma</u> thyroid was capable of concentrating and retaining the iodide ion. The fact the thyroid was subject to the effect of the iodide in solution was demonstrated by the traces of activity which could be detected in the thyroid after I<sup>131</sup> was added to the KI solution.

- 2. The counts on the containers represented the renal excretion of iodide into the environment. The iodide excretion curve showed a striking resemblance to the normal thyroid uptake curve. This was an observation which will be discussed later.
- 3. There were large amounts of residual activity in the abdomen. The dialysis of the peritoneal fluid indicated that this radioactive material in the KI treated animal was a small permeable molecule, presumably the iodide ion. The presence of such a small molecule in the abdomen for long periods was another interesting observation. The iodide ion should have diffused into the general circulation fairly rapidly considering the speed with which it is known to enter the ascitic fluid in man.

# III. THE EFFECTS OF 1<sup>131</sup> RADIATION AND/OR POTASSIUM IODIDE ENVIRONMENT ON THE GROSS AND MICROSCOPIC APPEARANCE OF THE AMPHIUMA THYROID

The thyroid lesions that have been described were first observed quite unexpectedly on the routine autopsy of the <u>Amphiuma</u> used in the counting experiments. These observations were followed by the detailed procedure which was designed to investigate these intriguing changes more fully. By comparing the histological picture of the thyroids from <u>Amphiuma</u> given various combinations of two stresses, I<sup>131</sup> radiation and the KI environment, it was hoped that some understanding of the observations might be reached.

It should be pointed out before attempting interpretations of

these changes that this discussion rests primarily on the evidence derived from the procedure of Table I. The procedure did not provide definitive answers. The number of animals limited the variety of treatments and the size of each group. Although the size of the group, or statistical sample was very small, the observations were interpreted under the assumption that this was not relevant and the results were not due to chance.

The conclusions that were drawn from the results of the microscopic examination are summarized in the following points:

- The thyroids from the controls showed epithelial changes compatible with those due to a slight stimulation by T.S.H. The appearance of normal glands was used as the basis for comparison with those from the experimental animals.
- Atrophy was a non-specific change resulting from the treatments.
- Fibrosis of the stromal elements was a radiation induced change.
- 4. Hyalinization of stromal elements resulted from the increased level of iodide ion in the environment.
- 5. The inflammatory-glandular colloid reaction was produced by the high concentration of environmental potassium.
- 6. The chronic inflammatory reaction with almost total destruction of the gland was due to the presence of KI and radiation. The degree of change was dependent on the length of time the animal was in the KI solution.

A correlation between the treatment the <u>Amphiuma</u> received (Table I) and the resulting microscopic appearance of the thyroid can be made by examining Table IX, in which the results of the microscopic examination have been grouped according to the previous treatment as in Table I. A quick appraisal of the tabulation shows that the variation in the histological picture was qualitatively dependent on the previous stresses to which the animal had been subjected. The type of change was related to the type of stress. Quantitatively the results varied with the duration of treatment and the other factors which will be discussed later.

The gross examinations of the experimental thyroids showed decreased size (i.e. atrophy) and an increased quantity of fibrous tissue (fibrosis). The crude method of determining size of the glands <u>in situ</u> was effective enough to demonstrate that all the treatments, with the possible exception of NaI, caused atrophy. The difficulty with which the thyroids were dissected out was due to fibrosis. These two findings were described by Lindsay (17) in human thyroids subjected to high doses of  $I^{131}$ . The finding of atrophy in thyroids subjected to KI, but no radiation suggests that this may not be a specific reaction to radiation. The NaI treated <u>Amphiuma</u> did not exhibit these changes.

Control: The thyroids of the control animals showed certain cellular changes. The epithelia were hyperplastic and invaginated; the classic picture produced by increased levels thyroid stimulating hormone (T.S.H.). There were also Hurthle cells present. Lindsay claims the presence of these cells is also due to high levels of circulating T.S.H.

and not to proliferative or radiation changes. The reasons for this increased T.S.H.in the control animals is not clear. Marine found hyperplasia and invagination in thyroids of Great Lakesfishes. He postulated that this was due to the low levels of iodine in the waters of this area (19). The low environmental quantity of iodine reduced the hormone output which in turn stimulated the pituitary to more active secretion of T.S.H. The increased amount of T.S.H. produced the cellular changes which are thought to raise the efficiency of the iodide trapping mechanism of the thyroid and thereby compensate for the decreased iodine supply.

I<sup>131</sup> Radiation: The stromal elements of the <u>Amphiuma</u> which were used to measure the uptake of I<sup>131</sup> showed fibrosis. Also, as previously mentioned, these thyroids were atrophied. Atrophy and perifollicular fibrosis were changes found by Lindsay (17) in radioiodine treated human thyroids. Such fibrosis was not observed in the other groups suggesting that this may be a specific change due to radiation.

These glands also had a few polymorphonuclear leucocytes in the lumen of the follicles. The presence of these cells is indicative of some non-specific irritation. This was a mild acute inflammatory response or thyroiditis. The follicles of one of the thyroids contained granular colloid and another thyroid showed intrafollicular hemorrhage. Lindsay did not mention hemorrhage or inflammation as occurring in his material. However, his thyroids had received larger doses of I<sup>131</sup> over a longer period of time.

The microscopic appearance of the thyroids from <u>Amphiuma</u> in Group B (I.P. injected) did not differ from those in C (I<sup>131</sup> in the water environment). Thus the method of administration was not important.

There was no difference in the pathology of those from B-1 and B-3. Since these varied considerably with respect to length of treatment but not to dose, it suggests that within the range of the experiments, the length of time the <u>Amphiuma</u> were exposed to radiation was not a critical factor.

KI Environment: The comparison of the thyroids from these KI treated <u>Amphiuma</u> (Group D) with the controls (A) showed the stroma of experimental animals was more hyalinized, hyperplasic, invaginated and there were more Hurthle cells present in the epithelia. One thyroid had many granular colloids and a mild subacute inflammatory reaction. These changes were the result of a high level of potassium and iodide ion in the solution. It is difficult to draw conclusions from the data because no measure was made of the serum levels of potassium iodide in these animals. There was the possibility that high levels of iodide may have had effects other than merely blocking the normal function of the thyroids.

If there were increased levels of serum potassium, this would have disrupted the normal electrolyte balance, as potassium is normally an intracellular ion (1). By using the NaI (Group G) as a control, an attempt was made to differentiate the effects of potassium from those of the iodide ion. It was postulated that the effect of the potassium on the thyroid would cause those changes that did not occur in an NaI environment. These included atrophy, a high degree of hyperplasia, invagination and granular colloids associated with the mild subacute inflammatory response. Similarly, it was assumed that changes induced by the iodide were those common to both (provided that sodium, a normal extracellular

ion, had a negligible effect). There were two changes observed to be common to both KI and NaI, hyalinization of the stroma and the presence of Hurthle cells. If the assumptions and postulates were correct, by excluding the T.S.H. effects, it may be inferred that the inflammatorygranular colloid changes were due to potassium and the stromal hyalinization due to iodide.

Ki Environment and Radiation: The comparison between these thyroids and those of the controls (A) revealed that all pathological changes were more pronounced except hyperplasia, invagination and Hurthle cells. The degree of inflammation was maximal and, in some cases, the destruction was so severe as to completely distort the normal architecture of the thyroid tissue. The striking feature of such thyroids was the intense chronic thyroiditis.

The difference between the above changes and the ones occurring from KI alone (Group D) showed that although there was an inflammatory response in D it was mild and subacute rather than intense and chronic. Radioiodine alone (B) did not produce the same type or degree of change. Therefore, it was the combination of KI and radiation that produced the lesions.

The time at which the I<sup>131</sup> was given the animals after they were subjected to KI varied in E-2, E-3, F-1, F-2 from the second of 25 days to the twenty-third of 28 days. Surprisingly, the pathology was uniform throughout these groups. This suggested that, within the limits of the experiment, the time interval for the KI and radiation together was not a decisive factor. However, the microscopic appearance of Group E-1 was

different from the rest of E and F. This group was in KI for a short time, 12 days, as compared to the 25 - 28 days during which the rest of the animals received the treatment. The E-1 thyroids had a subacute inflammation, a less advanced type of inflammation, while the remaining showed chronic inflammatory response. The KI controls (D) did not demonstrate this response, and they had a total treatment time of 22 days. Therefore, the total time in KI with the addition of radiation (i.e.  $I^{131}$ ) given sometime during this period governed the type of inflammatory change.

The investigation of chronic inflammatory degenerations of the thyroid has received new impetus recently. It has been shown that the thyroid can become involved in an auto-immunization reaction. Witowski (31) has shown that the mammalian body can develop an immune response to its own thyroglobulin. The thyroglobulin is then attacked as a foreign substance by the macrophages, the body's defense mechanism. The observation was made, in these thyroids, of the macrophages actively phagocytosing the colloid. The KI and radiation treatment may have caused sufficient damage as to allow some of the colloid to escape into the circulation where it produced an immune reaction and the resulting response by the macrocytes. If this be the case, then this observation could be of theoretical interest in the understanding of human thyroid disease. This connection will be discussed in the following section.

An interesting comparison could be drawn between the thyroid epithelium from animals in Groups E and F and those from the rest of the experimental groups. There was almost no hyperplasia, invagination or Hurthb calle present, instead, the epithelia were often squamous. The cells also showed considerable variation with respect to the cytoplasmic

and nuclear volume. This could be interpreted as a lowering of T.S.H. levels or as a loss of the normal response by the epithelia to the hormone. There was one more very pertinent observation, that of numerous mitoses of the parenchymal cells. This is unusual. Leblond after detailed statistical studies, stated that mitosis is infrequent in the normal rat thyroid (16). All of these observations of the epithelial changes including those indicative of a loss of normal cellular response to controlling substances are criteria for the microscopic recognition of cancer. A conclusive pathological diagnosis of cancer, however, demands the demonstration gross or microscopic of new, abnormal and inappropriate cellular growth. This condition was not shown on examining these thyroids. Therefore, although it cannot be said that these <u>Amphiuma</u> developed cancerous lesions, the possibility exists.

### IV FUNCTIONAL CONSIDERATIONS

The quantity of radioiodide present after I<sup>131</sup> administration will depend on both the systemic and the thyroidal iodide metabolism, as has been previously discussed. Before either of these pathways was taken, the tracer dose should have distributed itself in the iodide space. There was considerable evidence that this did not occur as quickly as was expected. It was assumed that if the iodide can diffuse into the peritoneal cavity within a few hours in man, then the even distribution of radioiodide throughout the <u>Amphiuma</u>, after intraperitoneal injection, would occur over a similar period.

The radioactivity remained in the abdomen for several days and did not diffuse rapidly throughout the extracellular fluid. This was especially

to agree with mammalian data in these experiments. The iodide concentrating mechanism of the thyroid is independent of the rest of the cycle. This was demonstrated when the blocking of the organification by KI did not reduce the uptake of I<sup>131</sup> completely. The next steps, the formation of the hormones and their storage in the colloid, were not directly documented in these experiments. If the rate of uptake of I<sup>131</sup> is proportional to the rate of hormone production in the Amphiuma, then this is a very slow process. This conclusion would agree with Berg (2) who has said that these thyroids "form thyroxine very slowly, if at all". However, it must be recalled that it has been shown that the uptake of iodide is not directly related to the rate of synthesis of thyroxine by the rat thyroid, as the iodide is also lost to the plasma without undergoing organification and also because the iodide concentrating reaction is functionally independent of the rest of the hormone synthesis cycle. Since there was a greater uptake of I<sup>131</sup> in the thyroids of Amphiuma not subjected to KI, and there was also evidence of activity on radioautography after butyl alcohol dehydration, there is an indication of processes beyond that of concentration of iodide. The last and most critical in respect to general body function is the secretion of the stored hormone to the circulation and this was not examined in these experiments. The level of circulation hormone is maintained by this process. Measurements of this parameter in the Amphiuma must await further investigation.

The radioautographic experiments, although not extensive, produced results of theoretical significance in the understanding of amphibian thyroid physiology. Thyroxine, the familiar thyroid hormone,

true in the KI treated animals and yet dialysis of the residual ascitic activity showed it to be in a permeable form, presumably the iodide ion. This observation agrees with the results that Broberg (3) found on wellcounted tissues of Necturus after I.P. injections of I<sup>131</sup>. After two days, she found that muscle gave a value of 14 c/m/mg, thyroid 100 c/m/mg and that the plasma was too active to count. The delay in the movement of the I<sup>131</sup> into the circulation is reflected in the appearance of the peak of the thyroid and of the excretion curves at nine days. The delayed peak in the thyroid uptake could be explained as due to the slow incorporation of iodide into the colloid complexes. The kidney, on the other hand, does not have any similar mechanism. The peak in the rate of excretion must reflect an increased plasma iodide level. The thyroid peak would then have been caused in the same way, since it has been shown that a constant volume of plasma is cleared of iodide by both the mammalian thyroid and the kidney, where the rate of thyroid uptake and the rate of renal excretion form a constant ratio (20). It was concluded, therefore, that the movement of the I<sup>131</sup> from the peritoneal cavity was a slow process requiring several days. If this is a defect in active transport, it may be related to the low environmental iodine of the Mississippi Basin. This, in turn, may be related to the limited distribution of Amphiuma. If this form has a competitive advantage in low iodine environment, it may have evolved in such an environment and must be a recent form, phylogenetically.

The synthesis of the Amphiuma thyroid hormones <u>in vivo</u> was examined for correlation with that occurring in the mammal which is better documented. The first step, that of the uptake of the precursors, was shown

to agree with mammalian data in these experiments. The iodide concentrating mechanism of the thyroid is independent of the rest of the cycle. This was demonstrated when the blocking of the organification by KI did not reduce the uptake of I<sup>131</sup> completely. The next steps, the formation of the hormones and their storage in the colloid, were not directly documented in these experiments. If the rate of uptake of I<sup>131</sup> is proportional to the rate of hormone production in the Amphiuma, then this is a very slow process. This conclusion would agree with Berg (2) who has said that these thyroids "form thyroxine very slowly, if at all". However, it must be recalled that it has been shown that the uptake of iodide is not directly related to the rate of synthesis of thyroxine by the rat thyroid, as the iodide is also lost to the plasma without undergoing organification and also because the iodide concentrating reaction is functionally independent of the rest of the hormone synthesis cycle. Since there was a greater uptake of I<sup>131</sup> in the thyroids of Amphiuma not subjected to KI, and there was also evidence of activity on radioautography after butyl alcohol dehydration, there is an indication of processes beyond that of concentration of iodide. The last and most critical in respect to general body function is the secretion of the stored hormone to the circulation and this was not examined in these experiments. The level of circulation hormone is maintained by this process. Measurements of this parameter in the Amphiuma must await further investigation.

The radioautographic experiments, although not extensive, produced results of theoretical significance in the understanding of amphibian thyroid physiology. Thyroxine, the familiar thyroid hormone,

is soluble in butyl alcohol but not in the cellosolve-benzene tissue preparation solutions. The radioautographs prepared from I<sup>131</sup> injected Amphiuma showed that the activity was in a form insoluble in butyl alcohol and soluble in the tissue reagents. This discovery coincides with the results that puzzled Broberg (3). She made butyl alcohol extractions of sodium hydroxide and of trypsin-treated homogenates of Necturus thyroids, 1, 2 and 3 days after I.P. injections of I<sup>131</sup>. With one questionable exception, she could not demonstrate the presence of active thyroxine or other hormones. Only radioiodide was present. In contrast, she was able to extract active thyroxine from the glands of I.P. injected frogs. These data very strongly suggest that thyroxine and thyroglobulin, as found in most vertebrates, are not present in Amphiuma and Necturus thyroid. Nevertheless, the substance produced by the thyroids is known to be an active material because transplantation of Necturus thyroids into frog tadpoles causes metamorphosis (3). The identity of the urodele thyroid hormones is an interesting question.

It was postulated that the circulating thyroid hormones facilitate the movements of electrolytes and other charged molecules. This effect is quantitative and is reflected in changes in the rates of ionic movements, at specific loci, such as membranes. This new theory would aid in explaining the slow movements of radioiodide from the peritoneal cavity and also the pathogenesis of the thyroid lesions observed in the experimental animals.

The <u>Amphiuma</u> appears to be an exception to the observation that the diffusion of radioiodide through the animal body is rapid and complete. The movement of this ion involves its penetration of the various cellular

membranes separating the body fluid spaces. The delay in the thyroid uptake and in the renal excretion was probably due to an inhibition in the movement of iodide through the membranes separating the peritoneum from the lumen of the blood vessels. The reduced rate of iodide movement is coincidental with a histological picture which was thought to result from a lowered level of circulating thyroid hormones. If these two observations are correlated, then the reduced effect of the hormones on the membranes would be the best explanation for the phenomena.

The proposed hormone action was incorporated into an explanation for the lesions produced by the combination of potassium iodide and radiation. The KI produced indirect cellular evidence of a very low level of circulating thyroid hormones as it caused a pronounced hyperplasia and invagination of the thyroid epithelia. It can be postulated that the cell membranes were affected by this reduced quantity of the hormone and that structures were therefore more susceptible to radiation damage. One of the effects observed due to radiation was a hemorrhage into the follicle. This implies a loss in the integrity of the blood vessels surrounding the follicles. It can be assumed that the colloid could also have leaked into the circulation at the same time. The lesions in the KI plus radiation may be explained by this sequence of events. The resulting auto-immunity would cause a phagocytosis of the colloid by the animal's macrocytes.

Inflammatory reactions with other cell types were observed in the radiation and in the KI exposed animals. Could these glands, in time, have shown the macrocytic response? The loss of cell membrane integrity due to changes in electrolytes and/or levels of circulating thyroid

hormone would lead to an inflammatory response, to the loss of normal thyroid tissue and to mxyedema. If this were so, it would imply an interesting correlation between Hashimoto's disease and spontaneous mxyedema, in man. At present, the cause of both these conditions is unknown.

The tentative explanations in this section cannot be considered as anything more than speculations. There was not enough data to draw firm conclusions. On the other hand, to ignore the glaring contradictions in the classical theory would have been inexcusable. This work made three points: one, that the <u>Amphiuma</u> is a suitable experimental animal for I<sup>131</sup> thyroid studies; two, that the results of such experiments do not always duplicate mammalian data; and three, that radiation and/or a potassium iodide environment produce very interesting thyroid lesions. A fourth and final point can be inferred, that further exploration of the physiology of the <u>Amphiuma</u> thyroid may result in the modification of theories about the thyroid hormones in such a manner that they will explain the presence of such hormones throughout the entire vertebrate class.



GRAPHS I AND II - I<sup>131</sup> INTRAPERITONEALLY





GRAPH III - KI ENVIRONMENT AND 1131 INTRAPERITONEALLY



Graph III - Radioactivity in Thyroid, Tissues of Tail and in Containers for 14 Days

DESCRIPTION OF PLATES

PLATE I - EXAMPLES OF PATHOLOGICAL CHANGES

Fig. 1 Normal Control (A)

This is the microscopic picture of the control <u>Amphiuma</u> thyroid. It is assumed that this is the normal appearance of the gland. The stroma is inconspicuous. The colloid is homogenous and evenly stained. The ripples are a fixation and sectioning artefact. The normal epithelium is clearly shown. The arrow points to a typical thyroid parenchymal cell.

Fig. 2 1<sup>131</sup> Injected Intraperitoneally (B)

This is a section from one of the more severely radiation damaged thyroids. It demonstrates the mild acute inflammatory reaction. There is also hyperplasia, fibrosis (stroma at upper left) and colloid changes. The arrow points to a polymorphonuclearcyte.

Fig. 3 KI + 1<sup>131</sup> I.P. (E)

AL

The almost complete obliteration of the normal thyroid tissue is an example of the lesions produced by the KI and radiation. The arrows point to two remnants of thyroid epithelium. The rest of the

PLATE I



Fig. 1

ж90





Fig. 3

x90

structure is a mixture of destroyed follicles and invading inflammatory cells.

PLATE II - STROMAL CHANGES

Fig. 4 Fibrosis - I<sup>131</sup> (B)

This is an intense perifollicular fibrosis. The follicles are widly displaced by this material. There are a few nuclei within the fibrous substance.

Fig. 5 Hyalinization - KI Control (D)

In contrast to Fig. 5, the perifollicular hyalinization is anamorphous and basophilic material. The staining differences (fibrosis is acidophilic) do not show in these photographs. There are many angular nuclei throughout the stroma.

PLATE III - PATHOLOGICAL CHANGES IN EPITHELIUM AND COLLOID Fig. 6 Hyperplasia - 1<sup>131</sup> I.P. (B)

> Hyperplasia is an increase in the number of epithelial cells. The parenchymal cells have changed from cuboidal (Fig. 1) to columnar. The nuclei are oval and crowded into a pseudostratified arrangement (i.e. no longer on one plane). The epithelium is deeper due to the elongated shape of the cells.

> > 2. 0,0





**x18**0





Fig. 6

z180



Fig. 7

x180



Fig. 8

x180

Fig. 7 Invagination I<sup>131</sup> I.P. (B)

The epithelium has become involved and folds into the lumen of follicle. On this invaginated ridge, the cells are clearly hyperplastic.

- Fig. 8 Granular Colloid KI + I<sup>131</sup> (E) The follicular lumen is filled with a granular chromophobic substance. This does not have the usually amorphous appearance of colloid. Note that the epithelium lining in this follicle is squamous.
- PLATE IV INFLAMMATORY REACTIONS
  - Fig. 9 Mild Acute Inflammatory Reaction (B) There are two polymorphonuclearcytes in this colloid. In one, the characteristic lobated nuclei can be seen. The epithelium is normal except for a small degree of hyperplasia.
  - Fig. 10 (PAS stained) Intense chronic Inflammatory Reaction (E) The follicular lumen is filled with lymphocytes and macrophoges. These cells are often referred to as round cells (note outline of the nuclei). The larger of two are the macrophages which have a crescent, moon-shaped nuclei. A number of these can be seen to have phagocytosed droplets of PAS positive material (colloid?). There are




Fig. 9

x180







two mitotic figures in the epithelium. The parenchymal cells also show considerable variation in the size of their nuclei.

Fig. 11 (PAS stained) Subacute Inflammatory Reaction (E) Both polymorphonuclearcytes and round cells can be seen in this follicle. At 12 o'clock in the photograph there is a macrophage which has engulfed a polymorph. The thyroid epithelium is thin and squamous. This microphotograph and that of Fig. 10 were taken from the same tissue section and they demonstrate the great variability in the number and size of the parenchymal cells in this thyroid.

## PLATE V - HURTHLE CELLS

Fig. 12 Normal and Hurthle Cells (G) This shows the differences between the normal epithelium (lower) and the Hurthle cells (upper). The Hurthle cells are larger with granulated cytoplasm and apical often pynotic nuclei. The stroma shows a small amount of hyalinization.

Fig. 13 Normal and Hurthle Cells (D)

This is a cross section through a thyroid epithelium. The normal cells show only their nuclei. The increased quantity of cytoplasm and the polygonal shape of the Hurthle cells can be seen.



Fig. 12

x180



Fig. 13

**x18**0



Fig. 14

x90

## PLATE VI - RADIOAUTOGRAPHS

Fig. 14 Positive Radioautograph

The dense block silver granules are clearly shown as covering almost the entire photograph. On the lower right, there is a clear ring where an epithelium surrounds a colloid. This demonstrates that the activity is confined to the colloid.

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