A HISTOPHYSIOLOGICAL STUDY OF THE THYROID GLAND OF THE ALBINO RAT IN VARIOUS STAGES OF PRENATAL AND POSTNATAL DEVELOPMENT

By

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HISTOPHYSIOLOGY OF THE THYROID GLAND

ALLEN

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AN ABSTRACT OF THE THESIS OF

Delmas J. Allen for the M.S. degree in Biology

Title: A histophysiological study of the thyroid gland of the albino rat in various stages of prenatal and postnatal development

In this study, histometric measurements and radioiodine uptake and release determinations were made to relate structural aspects and functional activity of the thyroid gland. Various age groups were used to follow the changes in the functional status of the thyroid gland as related to changes in development.

The mean follicle size was found to increase from birth until about 60 days of age. Thereafter, little or no change was observed in follicle size. Epithelium percentage gradually decreased from birth until approximately two months of age, after which little change was observed. A comparison of the values of follicular size and epithelium percentage suggests an inverse relationship.

Radioiodine uptake values increased rapidly from birth to about 20 days. Thereafter, a sudden decrease was observed until approximately 40 days when a value comparable to that of the newly born was obtained. After 40 days only slight changes were observed among the age groups. A comparison of the quantitative histological measurements with radioiodine uptake data revealed a relationship or similarity only up to 20 days. Thereafter, no apparent relationship seemed to exist.

In the radioiodine turnover study, it was shown that the rate of iodine metabolism was much faster in younger, pre-weanling animals, known to have smaller follicles, than in older, mature animals with larger follicles. From this it was concluded that the rate of activity per unit weight thyroid tissue was dependent to a certain extent on follicular size.

The fetal study indicated that biosynthesis of the thyroid hormone had definitely proceeded beyond the iodide accumulation stage. On the 20th day of gestation, weak traces of monoiodotyrosine and diiodotyrosine were observed, but even in the newly born, no traces of triiodothyronine or tetraiodothyronine were noted.

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I. INTRODUCTION

The thyroid gland is an organ which is highly specialized both as to structure and to function. In addition to carrying out many of the general processes common to other secretory organs, it is specifically geared to meet the needs of the body for two specialized substances, triiodothyronine and thyroxine. In order to accomplish this end effectively and efficiently, its structure and function seem to be closely related and integrated. This complex of structure and function has received considerable attention in the literature since the early 1930's when Junkman and Schoeller (1932) classified the histological changes which the thyroid gland manifested under the effect of thyrotropin (TSH).

Several methods have been devised for measurement of the functional activity of the thyroid gland, and these are based on the rate of release of its hormones into the blood stream. Many of these methods have taken into consideration structural or histological dimensions. An early and a greatly favored method was the use of histological preparations of the thyroid for the measurement of the height of epithelial secretory cells. Here it was assumed that an increased height of the cells was indicative of greater activity. Such an assumption had its justification in greater cell height being shown to be associated with greater activity resulting from stimulation by thyrotropin. However, due

to the lack of precise knowledge concerning the physiology of the thyroid epithelial cells and the gland as a whole, early investigators were not sure that cell height was always related in such a clear-cut fashion to activity.

Epithelial cell height measurements were begun in 1936 (Starr and Rawson, 1936) and used as an assay technique for the thyrotrophic hormone and antithyroid drugs. For lack of a better and more objective method, this procedure was used until recently in both experimental and clinical investigations with full awareness of its limitations and subjectivity. The importance and usefulness of this method as a tool in assay work and in pathological studies is recognized and unquestioned, but in the normal untreated thyroid, minor but possibly important fluctuations in activity may go completely unnoticed with this method.

The failure to elucidate many of the problems of histophysiology of the thyroid gland was due to the physiological complexity of the epithelial cell itself, and to the inadequacy of the methods available to detect the histological and physiological changes of the gland. The early literature reveals various controversies concerning the histophysiological changes of the thyroid and the questions related to these changes. The various opinions concerning the physiological mechanisms of the epithelial cells and the identification and the actual existence

of many of the elements in the colloid and the epithelial cells are documented in the reviews of the 1920's and 1930's on the histophysiology of the thyroid gland by Cowdry (1922), Cramer and Ludford (1926), and Maximow and Bloom (1939).

Disagreement in the literature and the lack of an entirely satisfactory histological method for studying the histophysiology of the thyroid gland plague us even today, but the relatively recent advances in thyroid biochemistry since the discovery of radioactive iodine have done much to facilitate thyroid investigation by providing a new procedure and an additional dimension for measuring the activity of the thyroid gland.

This new technique is much simpler and has now replaced the histometric method of assaying the thyroid response to TSH and to agents known to block the metabolism of iodine (Means et al., 1963). In addition, it has proven to be quite useful in studying thyroid activity in normal as well as diseased thyroids. Since its introduction, a combination and comparison of physiological (biochemical) and histological procedures have furnished new insight into the relationship between structural components and functional activities. In this connection the most frequently used test of thyroid function is the measurement of the fraction of radio-iodine accumulated in the thyroid gland after administration of a small tracer dose.

Despite the existence of an extensive literature dealing with histological and physiological methods for determining the functional activity of the thyroid gland, there seems to be little or no information concerning the general pattern of activity of the thyroid gland in normal animals[1] of various ages. The purpose of this study is to compare histometric measurements and radioiodine uptake and release in order to relate structural aspects and functional activity of the thyroid gland and at the same time investigate the changes in the functional status of the thyroid gland as related to the various changes in development.

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1. Controls for previous investigators who have manipulated thyroid function in some way.

II. REVIEW OF LITERATURE

Since this study applies the two most objective methods known to date for studying thyroid activity to the study of normal animals, the literature review covers the development and validity of these techniques.

Histological Studies

Several histological procedures have been devised for measuring the principal components of the thyroid gland as indicators of activity. As early as 1936, Starr and Rawson systematically measured 200 follicles from paraffin sections of the thyroid glands of guinea pigs under oil immersion with the aid of an ocular micrometer. For each follicle the most representative cell was chosen and its height measured. From these measurements the mean cell height was determined. Griesbach and Purves (1943) found that this microhistometric technique used by Starr and Rawson provided the necessary sensitivity and accuracy while retaining the advantage of being an objective method. However, they discovered that this method did not lend itself from a practical standpoint due to the time required in taking measurements of large numbers of epithelial cells. They presented a method by which the necessary measurements of epithelial cell height could be more readily obtained. In their study, sections

stained by Heidenhain's azan method were projected and measurements of well defined follicles were made up and down a strip parallel to the long diameter of the section using a celluloid scale. The measurements of 50 follicles from a single section of one of the thyroid lobes were taken for each animal.

Lever (1948 a, b) was unique in his approach in that he used a mathematical method to determine the state of activity of the thyroid gland. All of his work was based on the assumption that the follicles were the internally secreting parts of the gland, and consequently only methods based on changes in the follicles could be used to reflect its activity. First the thyrotrophic hormone and antithyroid drugs were used to change the activity of the thyroid gland for tracing the phases of histological variations. Then these variations were used both as a bioassay for the active substances and to describe the normal differences in thyroid activity.

The aim of his investigation was to find a method which considered the three characteristics of the follicle, i.e., epithelium height, colloid content, and total number of cells and the laws governing variations in them. He presumed that in a thyroid follicle each epithelial cell was of the same size and since follicles were circular, he concluded that there must be a relation between the diameter of the follicle and the number of cells. He considered this relationship to be true for all

follicles since he maintained that the follicles of a given thyroid were all in the same functional state.

Lever used three micra sections from three inactive thyroids of control cockerels and from three thyroids of cockerels treated with anti-thyroid drugs to count the number of epithelial cells of four follicles and to measure their outer and inner diameters. From these measurements, he prepared graphs of regression lines in which the outer and inner diameters of the thyroid follicles were plotted against cell numbers. From these two sets of graphs, he used the perpendicular distances between the almost parallel regression lines, the inclination of the regression lines, and the distance between the bottom regression line and the abscissa to show simultaneously the three principal histological facts concerning the activity of the thyroid follicle.

Uotila and Kannas (1952) presented a method, the main principle of which was invented in 1939 by Uotila, to determine the percentile proportions of epithelium, colloid, and stroma in the thyroid gland. Their modified technique was tested on guinea pigs treated with graded doses of thyrotropin and also used in pathological studies of goiter. In both cases they reported results which appeared promising with regard to usefulness and precision.

Essentially two methods were employed. In one method a longitudinal 50 per cent section was cut from the central part of each thyroid lobe, and measurements were made at three to five

points. In the other, serial sections were cut from the thyroid, and one section from the medial quarter, another from the middle, and a third from the lateral quarter were used. Three to five measurements were made in each section in this latter method.

These sections were projected vertically on a white paper with either two straight lines at right angles or four to five parallel lines drawn within the field of vision. Then the segment of the line covered by the entire projected image was measured in millimeters. Similarly, the lengths of the segments of the line covered by epithelium, colloid, and stroma were determined, and the percentages of these three components were calculated by dividing the total segments covered by each component by the whole length of the line.

Uotila and Kannas verified the reliability of their method by first drawing on paper the outlines of epithelium, colloid, and stroma from projected thyroid sections. These three components were then cut out and weighed separately, and the proportions of each component were determined in percentages. When these percentages were compared with the results obtained by their method, the difference was found to be 0.5 per cent or less.

As a result of this particular investigation, the authors set forth the following conclusions: First, the method of weighing gave the most accurate results, but nevertheless they emphasized that their method was not as laborious and gave results fully

comparable to those obtained by the weighing method, both in homogeneous guinea pig material and in heterogeneous human material. Secondly, they found that the percentile proportion of epithelium gave a more accurate indication of thyroid activity than that of either colloid or stroma.

Another approach was followed by Tala (1952, 1953) in which he made a comparative study of epithelium percentage and nuclear volume of the cells as indicators of thyroid activity following stimulation by thyrotropin. Here the percentage epithelium was determined by the quantitative linear histological method of Uotila and Kannas, while measurements of the cell nuclei were made following the principles presented by Jakobj in 1931. These latter measurements were made at a magnification of 2,600X with the aid of a Zeiss-Abbe drafting apparatus to trace the circumference of the nuclei. In order to arrive at a single figure for comparison, the average mean nuclear volume for each group of animals was calculated and compared with the average change in the percentage of epithelium in the same group.

Using the linear measurement and the nuclear volume measurement method, Tala was able to demonstrate the activity of the minute amount of 1.0×10^{-5} unit of TSH, and obtained almost identical curves for increasing doses in both measurements. Even though these two methods gave practically analogous results, Tala considered the epithelium percentage method preferable because of

its rapidity and objectivity.

Isotalo, Lofgren and Uotila (1954) described two special types of rulers used by them to facilitate and expedite measurements using the histoquantitative linear method. A ruler of acrylic resin was devised in such a way that the parts of the components to be measured could be summed on it geometrically by drawing, and the final result could be read directly from the ruler either as an absolute or percentile value. Another ruler of white diatex which served as a picture surface itself was devised to permit the simultaneous measurement of all three components of the thyroid gland directly from the image projected. The measuring accuracy with both rulers was reported to be fully sufficient, and considering that all three components could be measured simultaneously, the ruler system was preferred by the authors in view of its facility.

Lever and Vlijm (1955) compared the sensitivity of four methods based respectively on thyroid weight, colloid percentage, the relationship between the inner diameter and number of cells in median sections of the follicles (d/n method of Lever), and the epithelium percentage for determining the activity of thyroid glands. In this investigation the antithyroid activity of tetramethyl thiourea and 4-methyl-2-thiouracil in cockerels was compared by means of these methods. In case of lower thyroid activities, all of these methods were reported to show essentially identical results with about the same sensitivity, but with higher

activities only the d/n method was sensitive enough to be useful.

An unusually large number of histological observations on control Wistar rat and guinea pig thyroid glands was made by Cheymol, Delsol and Perrin (1955) in which they utilized both the follicle diameter and the epithelial cell height methods. In the latter method, the epithelia of 50 follicles found along an imaginary line traced by a micrometer were measured. From the results the authors concluded that the measurement of diameters of 30 follicles, as was previously reported to be adequate, was not enough to make conclusions on the status of the thyroid. Of the two methods, epithelium height was reported to be a much more reliable and precise criterion of the histologic status of the thyroid gland.

The most sensitive methods for determining the true histophysiological state of activity of the thyroid gland prior to the methods of Uotila and Uotila and Kannas were perhaps cytological. Although many of these studies utilized criteria such as the outgrowth of mitochondria into slender filaments (Cramer and Ludford, 1926), the absorption and release of colloid (De Robertis, 1941), colloid content (Stern, 1940), and the number of colloid droplets in the epithelium (Dvoskin, 1947), the majority of them were also based on measuring the height of cells selected by different considerations. Consequently, almost all methods, especially those since 1948 (Lever), recognize the

measurement of epithelium height as being the fastest and the most accurate indicator of thyroid activity.

Linear measurements of follicular cell height bring together epithelial cells of small and large follicles, shown in the next section to vary in their degree of activity.

Radioiodine Studies

Since the introduction of the radioactive isotope of iodine in 1938 and the later development of reliable techniques for measuring the isotope and separating iodinated compounds, a number of procedures have been devised in which radioactive iodine accumulation and release were used to reflect thyroid activity. As seen in the following paragraphs, these procedures provide new and perhaps more reliable dimensions in which the physiological activity of the thyroid is directly related to the course of hormonal biosynthesis.

Gorbman and Evans (1943) used the radioautograph method to determine the beginning of thyroid function in the fetal rat. These investigators found no iodine accumulation in fetuses less than 19 days old. When histological preparations of the thyroids of the fetuses were compared with these radioautographs, they discovered that definite histological features could be correlated with the beginning of function. This study is significant in that it was one of the first attempts to make use of radioactive iodine

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to draw a parallel between histological differentiation and physiological activity.

Gross and Leblond (1951) conducted an investigation in order to elaborate earlier findings concerning the iodinated compounds in the thyroid (Harrington, 1933; Fink and Fink, 1948; Gross et al., 1950). In this study they investigated the presence of relative amounts of labelled iodinated compounds in the thyroid gland and in plasma at various time intervals after the administration of radioactive iodide. Paper chromatograms of butanol extracts of both blood plasma and thyroid gland homogenates were exposed to x-ray film in order to detect radioactive spots. The radioautographs of animals receiving an adequate iodine intake, revealed only the presence of iodide after 15 minutes. After one hour, definite spots due to monoiodotyrosine, diiodotyrosine and iodide were found along with a weak spot due to thyroxine, while only iodide was demonstrated in the plasma for both time intervals. After three hours it was evident that all components including thyroxine had increased. As for the plasma, the intensity of iodide had increased and the presence of thyroxine was noted after three hours. In the thyroid extracts after 24 hours, a decrease was noted in the monoiodotyrosine, diiodotyrosine, and iodide components, while a noticeable increase in intensity was observed for the thyroxine spot with a noticeably fainter iodide spot. Neither monoiodotyrosine nor diiodotyrosine were ever detected in the plasma.

This work extended the earlier results obtained from radioautographs (Leblond and Gross, 1948; Dougherty et al., 1951), and allowed the authors to summarize the function of the thyroid gland as follows: First, the circulating iodide is collected by the epithelial cells, and then transformed into thyroglobulin at the apex of the thyroid cells. Afterwards, this thyroglobulin is deposited in the colloid of the follicle. In conclusion, a "steady state" was proposed to explain the presence of the free iodinated compounds and the continuous entry of iodine and the deposition of thyroglobulin over a 24 hour period.

The existence of such a "steady state" of thyroidal iodine in the thyroid gland was demonstrated by Dougherty, Gross, and Leblond (1951) by investigating iodine metabolism in rats at various time intervals over a 24 hour period. In order to establish this "steady state" hypothesis, the uptake of radioiodine by the thyroid gland and the rate of secretion of thyroid hormone from the gland into the circulating protein bound iodine (PBI) of the plasma were shown not to change significantly at these intervals which included definite physiological changes due to feeding and resting habits. After satisfactorily demonstrating a "steady state", these investigators concluded after referring to previously published arguments based on such an assumption (Leblond and Gross, 1948) that the function of the thyroid gland consisted of a series of activities in which all cells of all follicles were

continuously involved, and these activities occurred simultaneously and at a constant rate characteristic for each follicle.

A closely related investigation was conducted by Nadler, Leblond and Bogoroch (1954) in an attempt to relate size and functional activity of individual thyroid follicles. In this study the rates of iodine accumulation and release by individual follicles were determined by radioautography. From these results, it was observed that the smaller follicles accumulated radioiodine more actively, reached a higher peak, and then released the isotope at a much faster rate than the larger follicles.

When the results were expressed in terms of rate of accumulation of iodide per follicle, it was apparent that the total amount of iodine taken up by the smaller follicles was less than that taken up by the larger ones, but when the rate of accumulation per unit colloid volume was calculated in order to measure the efficiency of follicles of various sizes, the efficiency of the smaller follicles was found to be greater than that of the larger ones. In addition, the turnover of the radioactive isotope was found to be faster in smaller follicles as witnessed by the determination of the biological half-life of the colloid iodine for follicles of different diameters.

After analyzing these findings, the authors state that the rate of iodide accumulation by any thyroid follicle was proportional to the number of cells (total cell volume) in the follicle and to

the outer surface area (effective follicle surface) of the follicle. Therefore, they suggested that the ultimate factor controlling iodide accumulation and iodide metabolism in the thyroid follicle, as well as in the whole thyroid gland, was the total volume of epithelial cells.

Berson and Yalow (1955) investigated the iodide binding efficiency of the thyroid gland in treated and untreated animals. In normal, untreated animals their findings indicated that the fraction of trapped iodide that was returned to the plasma without being bound was so minute that it was generally undetectable. Iodide, antithyroid drugs and Tapazole were shown to inhibit binding. The latter was shown to reduce the binding efficiency of radioactive iodine to less than three per cent when the animals were treated with 25 to 30 mg. doses every six hours. From this study which included and offered for comparison cases of induced inhibition of binding, spontaneous physiological or pathological blocks to binding and normal (untreated) glands, they concluded that in the latter case thyroid clearance was constant for all practical purposes because of the extremely rapid rate of binding.

Ingbar and Freinkel (1956) studied the concentration gradients for radioiodide in unblocked thyroid gland and the effect of sodium perchlorate on the gradients. Low temperature dialysis was employed in human serum to separate inorganic from organically-bound radioiodine in excised thyroid glands of rats. Employing

this technique, concentration gradients for inorganic iodide-131 were demonstrated in thyroids of normal rats. These gradients were decreased after sodium perchlorate was injected. From this study they concluded that thyroidal organic-binding reactions proceeded at a finite rate. They also suggested a rate limiting role in hormonal synthesis by these reactions.

Nadler and Leblond (1958) calculated the rate of passage of iodine into and out of the thyroid gland in rats given radioiodine on the basis of plasma iodide activity and total radioactivity of the gland. This method (Nadler, 1958) was applied to series of adult rats fed graded doses of iodine and to young and adult rats fed on Purina Chow.

Contrary to results reported by Berson and Yalow (1955), this study revealed that in animals receiving an adequate iodine intake a substantial fraction of the labelled iodine entering the thyroid gland was returned to the circulation within two or three hours as iodide. After 20-26 hours a second release of labelled iodine was reported, but this time in the organically-bound form. They estimated that only 36-52 per cent of the iodide taken up by the thyroid was converted into the thyroid hormone, with as much as 50-60 per cent being discharged as iodide. An increase in the fraction of circulating iodide taken up by the thyroid was noted when the dietary iodine intake was reduced to a low, goitrogenic level, but no change was noted in the rate of organic binding

because the absolute rates of entrance and discharge decreased. From this they concluded that under steady state conditions, hormone production was generally independent of iodine intake.

In the same study, Nadler and Leblond reported an increase in the rate of organic binding in the thyroid gland when body weight increased without a change in the iodine content of the diet. This variation was found to be proportional to the total volume of thyroid epithelium and to the rate of oxygen consumption. From these observations, they suggested that body weight conditioned the volume of thyroid epithelium, which in turn controlled the rate of organic binding of iodine.

The site of iodination of thyroglobulin was investigated by Pitt-Rivers, Niven and Young (1964) after many conflicting reports on the subject (Leblond and Gross, 1948; Doniach and Pelc, 1949; Yamada et al., 1961; Wollman and Wodinsky, 1955). One group of investigators suggested that, in the normal thyroid gland, iodination of thyroglobulin occurred within the lumen of the follicles, while the other (Yamada et al., 1961) presented evidence which indicated that radioiodine was incorporated into organic combination in the thyroid epithelial cells. After reviewing the studies on the subject over the previous 15 years, these investigators suggested the possibility that iodination occurred in the epithelial cell, but the failure to detect iodinated protein in the epithelium was because it was secreted so rapidly into the

follicular lumen.

Their study was conducted to determine whether organic radioiodine could be detected in epithelial cells at short intervals after radioiodide injection. As a result of their radioautographic studies, they reported a number of thyroid follicles containing protein-bound radioiodine. Some follicles were shown to have even a higher concentration of organic radioiodine in the epithelial cells than in the colloid of the lumen.

III. MATERIALS AND METHODS

One hundred eighty male and 20 female albino rats of the Wistar strain were used in this investigation. The animals were kept under identical conditions of light and heat, and were fed on Purina Chow. The animals selected for the experiments were housed individually in 18X18X25 cm. mesh-bottom cages in a room with a window, at a temperature of 25 $\pm$ 2^{o} C. Four experiments were conducted in an attempt to determine the histophysiological state of activity of the thyroid gland in various age groups.

In the first experiment, the thyroid glands of newly born, 10, 20, 30, 40, 60, 140, 180, 270, and 900 day old male rats, respectively, were excised, and histological preparations were made in order to determine the proportions of the principal components of thyroid tissue. Only male animals were used here to avoid possible variation in iodine metabolism due to estrus cycles. Thyroid glands of five individual animals of each of the age groups were surgically extirpated under ether anesthesia and fixed "in-toto" in 10% formalin. According to Holmgren and Nilsonne (1948), this method of fixation does not cause any swelling or shrinkage of tissue. The thyroid glands were kept in the fixative for 24 hours, after which they were washed several times in distilled water before being dehydrated in increasing concentrations of ethanol. Xylol was used for clearing after the

dehydration process, and the tissues were infiltrated and embedded in 56-58°C paraffin. Sections 5-7 micra thick were cut using a Spencer rotary microtome and mounted serially using albumen fixative. The sections were stained with Delafield's hematoxylin and counterstained with eosin. This procedure has been reported most useful for thyroid studies by Uotila and Kannas (1952).

The histology of the thyroid was studied by two different methods. In order to measure the size of the follicles and the height of the epithelial cells, a modification of the histoquantitative linear method introduced in 1952 by Uotila and Kannas was employed. A Leitz (Prado 500) microprojector was used to project an image of the thyroid section vertically onto a white paper on which two straight lines had been drawn at right angles. The distance from the top of the table to the microprojector and the magnification (430X) were kept constant for the entire experiment. A stage micrometer was used to calibrate the field.

Every tenth section from the serial mounts of each left thyroid lobe was projected, and the inner and outer diameters of those follicles falling on the lines were measured using a ruler divided into 0.5 millimeter units. One field of vision was chosen from each section in order to include follicles from both the peripheral and the central portions of each lobe. For each lobe fifty follicles were thus measured. The percentage epithelium was obtained from these measurements by multiplying the ratio of the

epithelial cell height to the follicle size by 100. According to Uotila and Kannas, values obtained in this way are characteristic for the gland, and Uotila presented the mathematical foundation and a verification of the reliability of the method.

A second method was used to measure the epithelial height as a check on the above mentioned method because it was felt that the microprojector did not produce an image of sufficient clarity and resolution in several instances. In this second method previously used by Cheymol, Delsol and Perrin (1955), 50 epithelia were measured, taking the height of the epithelial cells of all follicles found along an imaginary line traced by a calibrated ocular micrometer at a magnification of 430X. A comparison of the values obtained with the two methods revealed a close correlation.

In the second experiment, thyroidal uptake of radioiodine was measured in five of each of newly born, 20, 30, 40, 60, 90, 130, 210, 270, and 900 day old male rats. Five animals of each age were injected subcutaneously with 10 microcuries of carrier-free sodium thiosulphate labelled with iodide-125^{1} in 0.5 ml. of 0.9 per cent saline solution. At the time of injection, five samples of the same dose used for injection were set aside to be

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1. Obtained from the Radiochemical Centre, Amersham, England.

used as standards. Twenty-four hours later the animals were sacrificed with ether and the thyroid glands removed, cleaned, and weighed on a precision torsion balance (Griffin & George Ltd.) with a capacity of 100 milligrams. Both lobes of each gland were digested for 24 hours at 54^{o} in four ml. of alcoholic potassium hydroxide (potassium hydroxide, 5 g; water, 50 ml.; ethanol, 100 ml.) as suggested by Cowan, Saghir and Salji (1966). Total thyroidal radioactivity was measured after digestion using a well-type crystal scintillation counter (Baird-Atomic, model 135) with a counting efficiency of approximately three per cent for this particular isotope.

The percentage uptake of iodine-125 was determined by comparing the counts obtained per mg. thyroid tissue with those values obtained from the standards counted at the same time as the thyroid digests.

In the third experiment, male rats of a pre-weanling (15 days old) and a fully mature group were injected subcutaneously with 10 microcuries of radioiodine, and the uptake and release of the labelled iodine was followed over a period of 200 hours. The pre-weanling group consisting of 35 animals and weighing 34-44 gm. was subdivided into seven groups of five animals each. These were sacrificed at 2.5, 14, 25, 50, 100, 160 and 200 hours, respectively. The mature group of 30 rats, weighing 320-400 gm., was subdivided into six groups and

sacrificed at 2.5, 25, 50, 100, 150, and 200 hours, respectively. For each time interval in the two groups, the procedure for the digestion of the thyroid glands and assay for total radioactivity was identical to that mentioned in the second experiment.

The fourth experiment was devised to confirm the time of beginning and the extent of function of the thyroid gland in the fetal rat. In this experiment a 25-microcurie dose of radioactive iodine was injected subcutaneously into rats in a known stage of pregnancy (Farris and Griffith, 1962). These pregnant females were sacrificed 48 hours after injection. The thyroid glands of the fetuses of a single female were removed, cleaned, pooled and processed for uptake and biosynthesis studies. Only 18, 19, 20, and 21 day old fetuses were used; Gorbman and Evans (1943) conclusively proved in their work employing radioautography that no iodine accumulation (uptake) occurred in fetuses before the 19th day of gestation.

Paper chromatography was used to determine to what extent the formation of the thyroid hormone had proceeded beyond the stage of accumulation or trapping of iodide. In order to detect biosynthetic activity, a procedure similar to the one used by Corradino and Parker (1962) was employed. The pooled glands were homogenized and placed in a 10 ml. tube containing 1.5 ml. of 0.5% pancreatin at pH 8.6 and incubated under toluene for 25 hours at 38^{o}C. Twenty-five and 50 microliters of each hydrolysate

were applied to Whatman No.4 filter paper strips (24 x 1.5 in.), respectively, and subjected to descending chromatography for 24 hours at room temperature in the upper layer of an n-butanol, dioxane, 2N NH_4OH (4:1:5) solvent. Standards of known amounts of tetraiodothyronine, triiodothyronine, diiodotyrosine, monoiodotyrosine, and iodide (NaI) were run simultaneously. The standards and the experimental chromatograms were developed with a chloroform solution of collidine (0.1%) and ninhydrin (0.1%); an autoradiogram of the chromatogram of the hydrolysate of the thyroid glands was made by placing the paper strips in direct contact with Kodak x-ray film (Royal Blue) for 20 days in a black, light-proof envelop (Gross et al., 1950). The amino acids present in the radioactive spots were identified by comparing the reference value of the blackened areas with the color areas developed with the known standards.

IV. RESULTS AND DISCUSSION

Follicle Size

The results of the measurements of linear follicular dimensions of the thyroid gland in 11 age groups are presented in Figure 1 and Table 1. The figure shows the average follicle size for five animals in each group, indicating also the range. The points representing the average values were connected by a smooth curve to suggest a pattern of change related to the growth of the thyroid.

From the Figure, as well as the Table, it is evident that the average follicle size has gradually increased from birth until about 60 days of age. Thereafter, a plateau indicates little or no increase. These findings are supported by the work of Leblond and Peet (1951) in which they suggested that the increase in mean follicular diameter before the age of two months was due to the addition of new cells to individual follicles by mitosis. These investigators reported that the number of follicles per gland remained constant at about 40,000 until around two months, but from thereon the number progressively increased to about 90,000 in eight to ten month old rats. They explained this increase by further suggesting that in the older animals many of the growing follicles reached a certain critical size and divided into two or more, thus increasing and eventually more than doubling the number of follicles. On this basis they concluded that the lack of an increase in the mean diameter after two months of age was due to the inclusion of the small, newly formed follicles in the average.

The range of variation in follicular size varied greatly among the different age groups until the age of 60 days. Compared with the

work of other investigators, the range difference within any particular age group during this period seemed to be well within the limits reported for rats and other mammals of comparable sizes. For example, Saadeh (1966) in his study on sexually mature animals reported a difference in the extreme values of 10.9 micra for the albino rat and 27.9 micra for the guinea pig. In this study the greatest difference noted was 8.3 micra, and this was only in the 20 and 140 day old rats. When the variance test[1] was applied, no statistically significant difference was found among the average values for the individual animals within a group, but among the groups a significant difference was obtained.

In terms of follicle size distribution within a thyroid lobe, it was quite evident that the larger follicles were located peripherally. Small follicles were often found near the periphery among the larger ones, but large follicles corresponding to the size of the more peripheral ones were seldom, if ever, found in the medial portions of the gland.

From these observations alone, one could speculate that the differences in average follicle size are associated with, and in some way responsible for, functional differences in the gland. These results may suggest a change in functional activity up to about 60 days, after which there was little change in functional status as suggested by the plateau. This relationship between follicle size and functional status will be discussed in more detail under radioiodine uptake studies.

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1. Statistical analyses were obtained from the IBM computer at the Computer Center, American University of Beirut.

FIG. 1. Average follicle size in various age groups of control albino rats

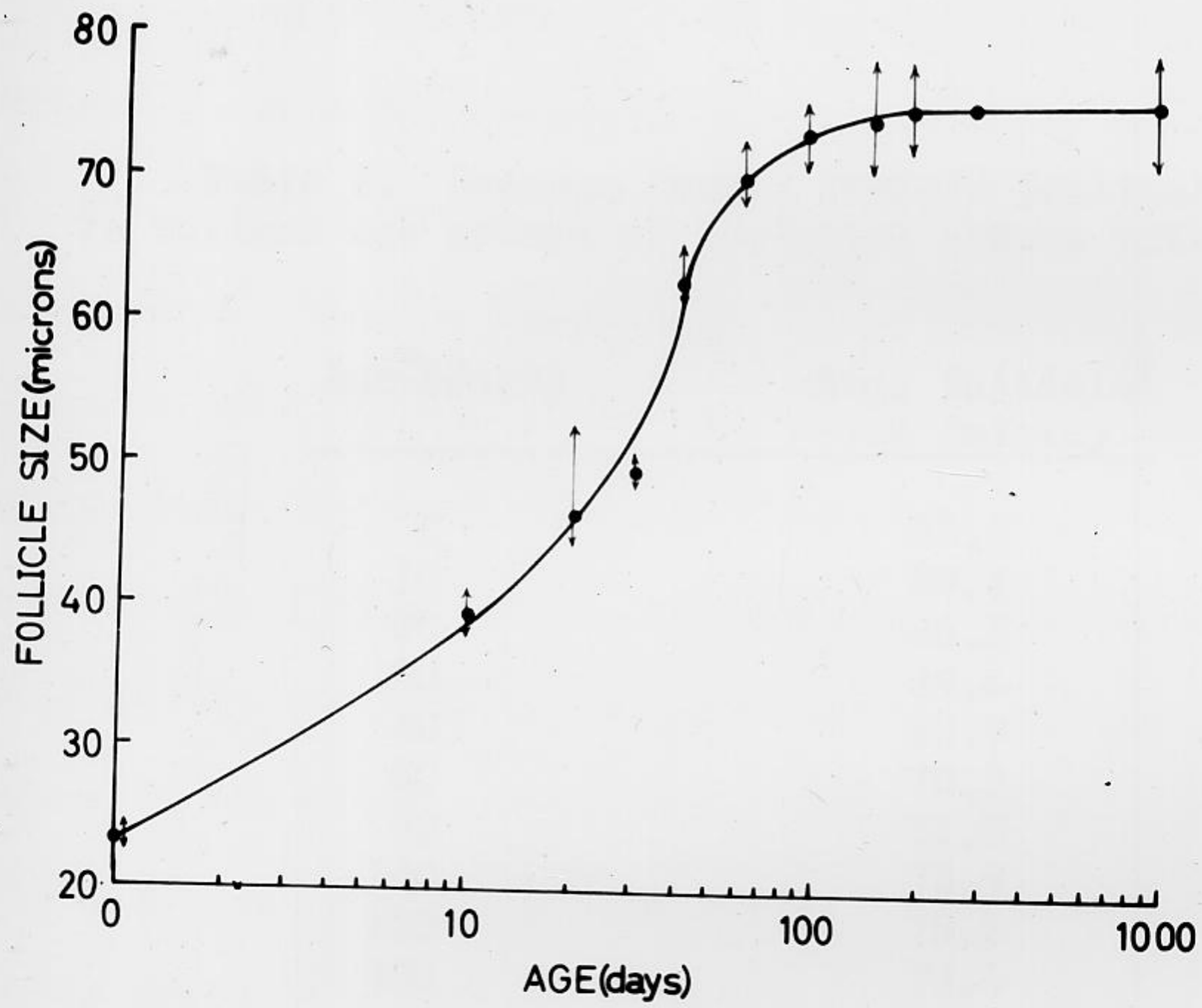

FIG. 2. Average epithelium percentage in various age groups of control albino rats

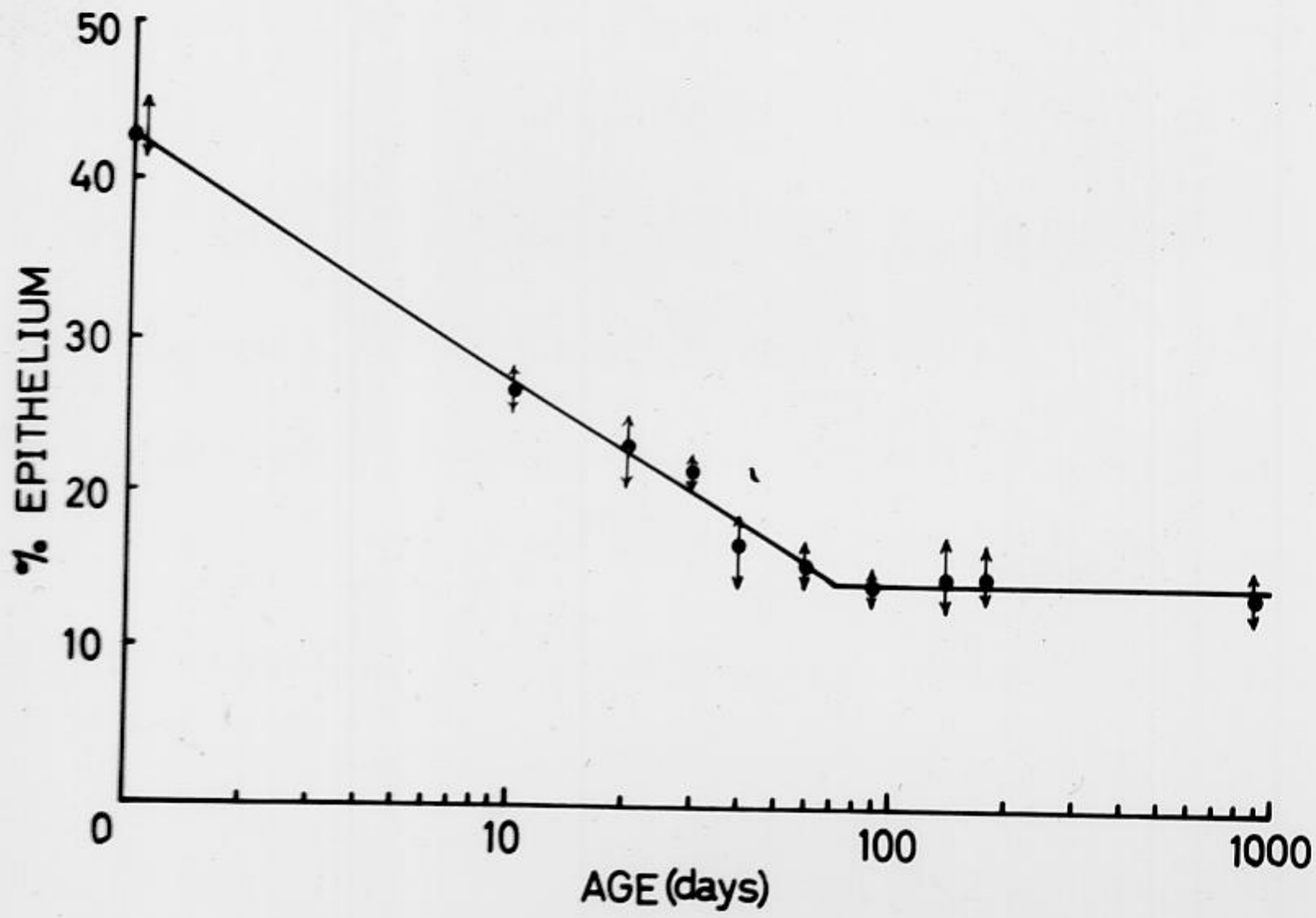

Table 1. Average (mean) thyroid follicular size in various age groups of postnatal albino rats.

Age (days)	Avg. follicle[1] size (micra)
0	23.3
10	39.4
20	46.5
30	49.6
40	62.9
60	70.3
90	71.8
140	73.9
180	75.0
900	74.6
LSD[2]	.011

1. Five animals/group; 50 follicles measured in each animal.

2. Least significant difference. (5%)

Epithelium Percentage

The results of the calculations of epithelium percentage from the measurements of the size of the follicles and the height of epithelial cells are presented in Figure 2 and Table 2. In the Figure, the average values for the five specimens are given along with the ranges for each age group. It is seen that the highest percentage epithelium was in the newly born group. Epithelium percentage gradually decreased until approximately two months of age, after which little change was observed. A comparison of the values of follicular size on the one hand and the epithelium percentage on the other, suggests an inverse relationship between follicle size and epithelium percentage.

The range of variation in epithelium percentage was not as great as that for follicle size, but in both cases there was no correlation between range and age. For epithelium percentage, the greatest range difference of 4.9 per cent was in the 20 day old animals. No statistically significant difference was noted within any group, but again between groups there was a significant difference (Table 2).

While making the histological measurements in any particular individual animal, it was noted that the height of the epithelial cells[1] seemed constant for every follicle, confirming Lever's

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1. A direct function of the difference between outer and inner follicular diameters.

Table 2. Average (mean) epithelium percentage in various age groups of postnatal albino rats.

Age (days)	Avg. epithelium[1] percentage
0	42.7
10	26.9
20	23.0
30	21.5
40	16.7
60	15.3
90	14.0
140	14.6
180	15.9
900	13.3
LSD[2]	.006

1. Five animals/group; epithelia of 50 follicles measured for each animal.

2. Least significant difference. (5%)

Table 3. Average epithelium height in micrometer units.[1]

Age (days)	Avg. epithelium[2] height
0	4.1
10	4.0
20	4.0
30	4.0
40	4.0
60	4.0
90	4.0
140	4.0
180	4.0
900	3.9
LSD[3]	0.0

1. (Outer diameter - Inner diameter) = Epithelium; multiplied by factor of 2.5 gives epithelium in micra.

2. Five animals/group; 50 measurements/animal.

3. Least significant difference. (5%)

hypothesis (1948a) that in every follicle the same relationship exists between out and inner diameter. This same relationship was noted in all of the animals in a particular age group and did not change with age as shown in Plates 1-5[1]. From the actual measurements, no statistically significant difference was noted within the groups, and at the 5% level there was also no significant difference among groups (Table 3). This general observation of a uniform cell height in the normal animal groups may lead to the conclusion that all of the thyroids were in the same state of activity. Such a conclusion is contrary to what is known concerning the important influences of the thyroid hormone on growth in immature animals and metabolic rate in general. Thus, the findings in this investigation seem to support Ludwig's (1951) conclusion that epithelial cell height alone in normal animals offers no exact criterion or indication of activity of the thyroid gland; such criteria have been used and shown to be useful, however, in pathological studies and in experimental animals when thyroid function was manipulated.

Radioiodine Uptake

Average radioiodine uptake values for 12 age groups are represented in Figure 3 and Table 4. The smooth curve was drawn

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1. A single thyroid section was selected randomly from one individual animal within each age group and photographed for demonstration purposes.

FIG. 3. PERCENTAGE IODINE-125 UPTAKE PER MG. THYROID TISSUE OVER A TWENTY-FOUR HOUR PERIOD IN VARIOUS AGE GROUPS OF ALBINO RATS

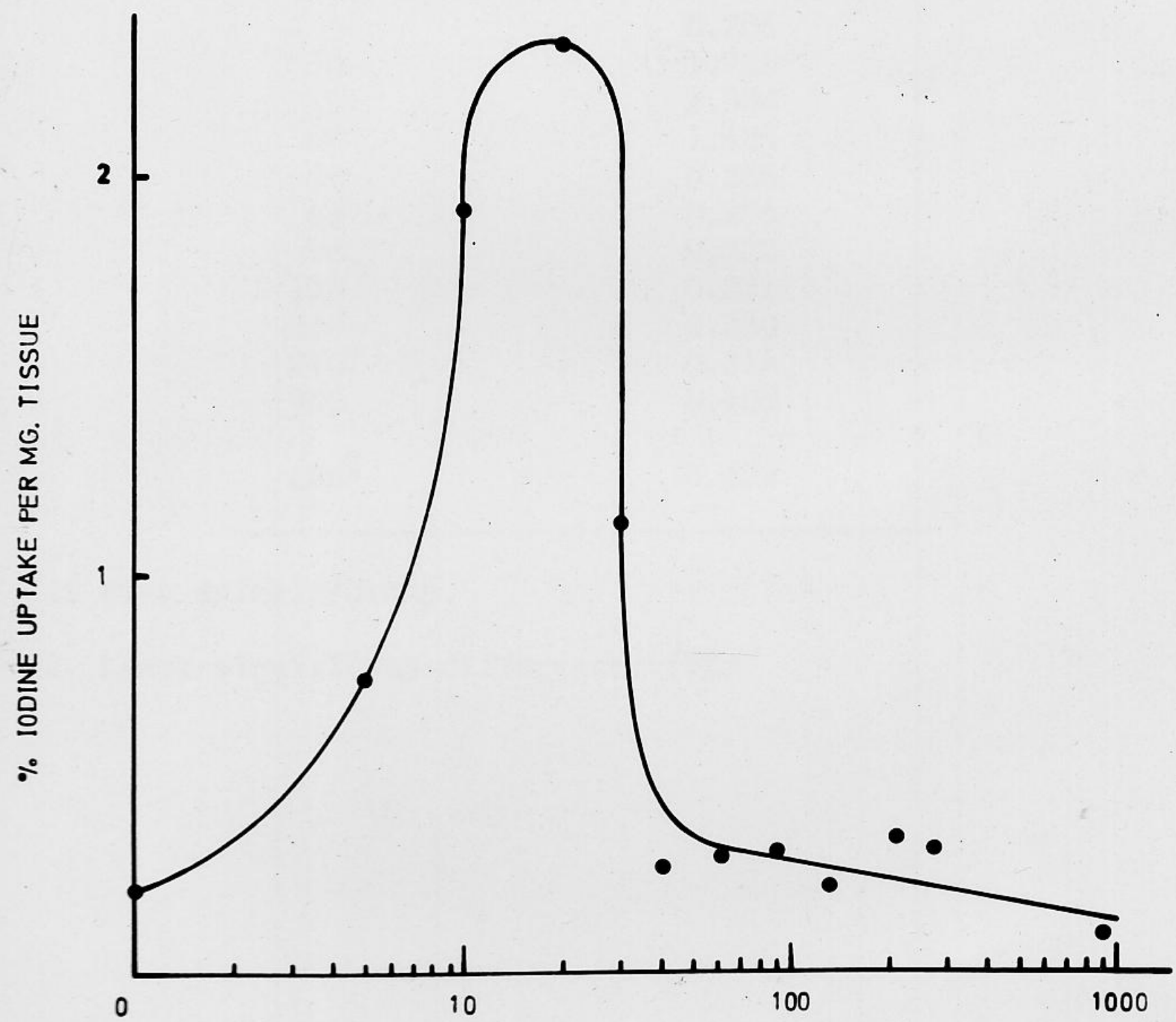

Table 4. Percentage iodide-125 uptake per mg. thyroid tissue over a 24 hr. period in various age groups of albino rats.

Age (days)	Avg. percentage[1] I-125 uptake
0	0.206
10	1.925
20	2.334
30	1.135
40	0.268
60	0.296
90	0.322
130	0.221
210	0.350
270	0.316
900	0.105
LSD[2]	0.122

1. Five animals/group.

2. Least significant difference. (5%)

through specific points only to suggest a pattern of functional activity of the thyroid gland from percentage uptake values determined from total thyroidal radioactivity. Uptake values increased rapidly from birth to about 20 days. Thereafter, a sudden decrease was observed until approximately 40 days when a value comparable to that of the newly born was obtained. After 40 days only slight changes were observed with the possible exception of the 900 day old animals which consistantly showed the lowest values. Within a single group, considerable variations were noted in uptake values, but these differences were found not to be significant when the "analysis of variance" was applied. Such variations are not surprising according to Barrington (1963) when one considers that the amount of iodine trapped and concentrated depends on the amount brought to the gland by circulating blood and body fluids which, in turn, are influenced by many factors effecting the rates of metabolic and excretory processes. A significant difference was noted between newly born and the 10, 20 and 30 day old groups, but there was no significant difference between the newly born and all of the other groups (Table 4).

The results up to 20 days, indicating more active concentration of iodine per mg. thyroid tissue in the younger age groups, were not surprising. This is evident since the thyroid hormone (thyroxine) is known to play an essential role in the growth of immature animals, and without iodine the hormone can not

be produced. However, the sharp decrease in percentage uptake after only 20 days of postnatal life was somewhat of a surprise. Since the albino rat does not reach sexual maturity until several weeks after this stage, the author anticipated higher uptake values until puberty when supposedly a functional peak was reached. A possible explanation for this became evident when body weight (Fig. 6), as will be discussed later, was taken into consideration. After 20 days, a sudden increase in body weight was noted. Along with this sharp increase in body weight, a corresponding decrease in metabolism would be expected because it is known that the metabolic rate of a larger animal (rate of chemical reaction per gram tissue) is lower than a smaller one (Harding, 1961). The greater concentration of iodine, per unit weight or volume, is indicative of a more rapid production and use of the hormone in the smaller, lighter animals. This rapid use of the hormone can be in turn ascribed to the part played by it in the maintenance of metabolic rate (Barrington, 1963).

The difference in the body weights of the 20 and the 30 day old groups are possibly large enough to account for the greater concentration of iodine in animals younger than 30 days of age. Because of their relatively larger surface area per unit volume, these younger (smaller) animals have a relatively higher heat production, weight for weight, for the maintenance of body temperature; this requires a more rapid uptake of iodine for the

production of the required thyroid hormone.

Another and perhaps a more plausible explanation for this sudden decrease in percentage iodide uptake after 20 days may be made on the basis of follicle size and efficiency of uptake of follicles of various sizes. It seems that after 20 days, a critical follicle size was possibly attained. As the average follicle size increased beyond this point, the rate of iodide uptake per unit weight or volume of thyroid tissue was greatly decreased. Experimental evidence in support of this explanation will be presented and discussed in the radioiodine "turnover" study. At about 40 days, a time when the average follicle size approached a plateau, possibly another critical point was reached where a constant rate of uptake was maintained as a direct result of the division of the larger follicles into two or more smaller ones.

Considering the report of Leblond and Peet (1951) indicating that the number of follicles per gland remained constant until about two months, this study suggests that the very small follicles in animals younger than 30 days were more efficient in accumulating iodide because of their comparatively greater exposed surface area per unit volume. Due to this greater surface area, they were able to concentrate iodide more actively as a result of their ability to carry out faster exchanges with the surrounding tissues and body fluids. As the size of follicles increased in animals older than 20 days, their ability to carry on these exchanges decreased

proportionately until a certain intermediate size range (around 40 days) was attained. Once the follicles reached this size, the percentage uptake was approximately the same for all follicle classes or age groups. Nadler, Leblond and Bogoroch (1954) reported similar results for intermediate follicle sizes in their radioautographic studies. They reported approximately the same rate of iodine accumulation in all but the very small and the very large follicles.

This attempt to relate radioiodine uptake to structural characteristics suggests that the rate of activity per unit weight and volume of the gland is dependent to a certain extent on follicle size. Such an explanation seems appropriate enough since the follicle is generally considered as the functional unit of the thyroid. The precise relationship between average follicle size and activity will become more evident in the study to be presented subsequently on radioiodine "turnover".

A comparison of the quantitative histological measurements in Figures 1 and 2 with the radioiodine uptake data in Figure 3 reveals a relationship or similarity only up to 20 days. Thereafter, no apparent relationship seems to exist. This does not mean that histological methods have no value in determining thyroid activity, but it does show how much less sensitive this method is than the one which employs radioiodine uptake of the gland. From these relationships, it seems safe to conclude that the activity of the

thyroid gland in normal animals is better reflected in its iodine uptake than by its morphological changes observed in histological preparation.

Radioiodine uptake and release ("turnover")

This experiment was designed to demonstrate conclusively that the thyroid glands of very young animals, known to contain small follicles, were more active in iodine uptake and release[1] than older animals with much larger follicles. Such an experiment would also indicate whether the uptake peaks occurred at the same time interval or were influenced markedly by different physiological states due to differences in age, weight, food intake, etc. The approach used to follow the uptake and release rates was to inject a standard dose of iodide-125 into a series of animals of two widely separated age groups and then measure the radioactivity of the thyroids of individual animals sacrificed at certain intervals.

The results of this investigation are presented in Figure 4 and Tables 5 and 6. No statistically significant difference was noted within any of the pre-weanling or mature groups; the "least significant difference" (LSD) among these respective groups are presented in the above mentioned tables. It is apparent when the two curves (Fig. 4) are compared that the pre-weanling group accumulated the isotope much more actively, reaching a peak concentration approximately five times greater over the same time

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1. An indication of the rate of iodine metabolism as suggested by Nadler, Leblond, and Bogoroch (1954).

Fig. 4. A pre-weanling and a mature group of albino rats were injected subcutaneously with 10 microcuries of radioiodine at the same time. The percentage of radioiodine concentrated per mg. thyroid tissue was determined at the indicated time intervals. The values presented are the average values for five individual animals in each group sacrificed at the time indicated after the initial injection.

FIG. 4. VARIATION IN CONCENTRATION OF IODINE-125 WITH TIME IN A PRE-WEANLING AND A MATURE GROUP OF ALBINO RATS

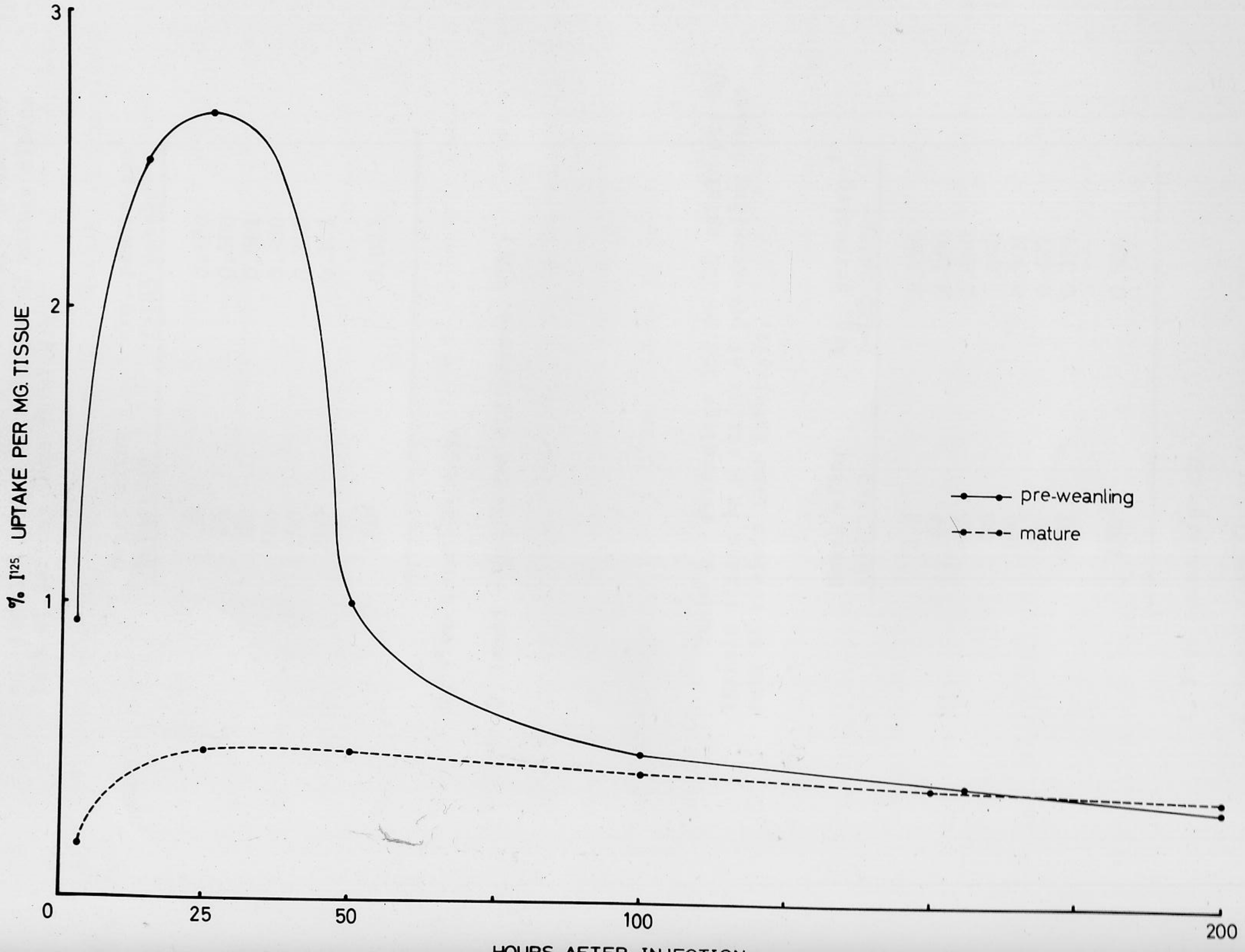


Table 5. Percentage iodide-125 uptake per mg. thyroid tissue in a series of mature albino rats at various time intervals.

Hours after injection	Avg. percentage[1] I-125 uptake
3	0.180
25	0.500
50	0.501
100	0.430
150	0.395
200	0.355
LSD[2]	0.022

1. Five animals/group.

2. Least significant difference. (5%)

Table 6. Percentage iodide-125 uptake per mg. thyroid tissue in a series of pre-weanling albino rats at various time intervals.

Hours after injection	Avg. percentage[1] I-125 uptake
2.5	0.936
14	2.500
25	2.660
50	1.043
100	0.502
156	0.427
200	0.329
LSD[2]	0.386

1. Five animals/group.

2. Least significant difference. (5%)

interval. The rate of release was so much faster in the younger group that by 200 hours the concentration was even less than in the mature group. These findings confirm those of Experiment II in which the thyroids of the younger age groups were more active in terms of iodide uptake; they also suggest that the rate of elaboration and release of the hormone ~~was~~ were much faster in the younger group than in the older group.

The pre-weanling group exhibited a definite peak around 25 hours, while in the older group no definite peak was established, but a maximum uptake value was noted at 25 hours. Since the maximum uptake values coincided closely for both groups at approximately 25 hours, the uptake values in Experiment III may be taken as reliable and characteristic for these two age groups. Any dramatic change due to diurnal habits or physiological changes due to age differences would have invalidated the experiment.

It would be worthwhile, however, to follow uptake and release in series of animals of each of the age groups to follow functional activity more closely. An extensive study of this nature may further the understanding of the relationship between follicle size and functional differences in the various age groups.

Body weight-thyroid weight

The average weight of the thyroid gland for each of the age groups is shown in Figure 5, along with the range differences.

FIG. 5. AVERAGE THYROID GLAND WEIGHT (SHOWING RANGE) IN VARIOUS AGE GROUPS OF CONTROL ALBINO RATS.

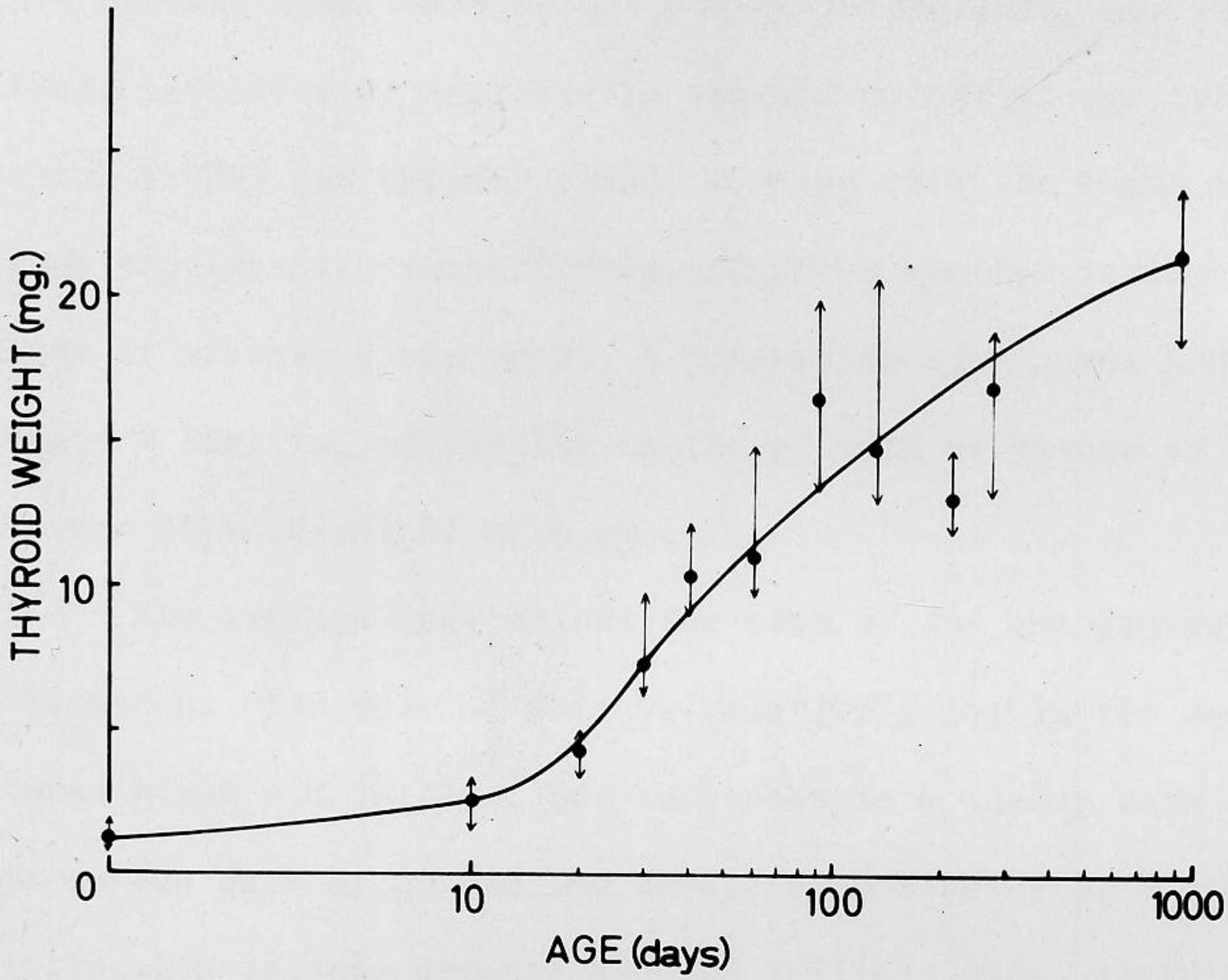
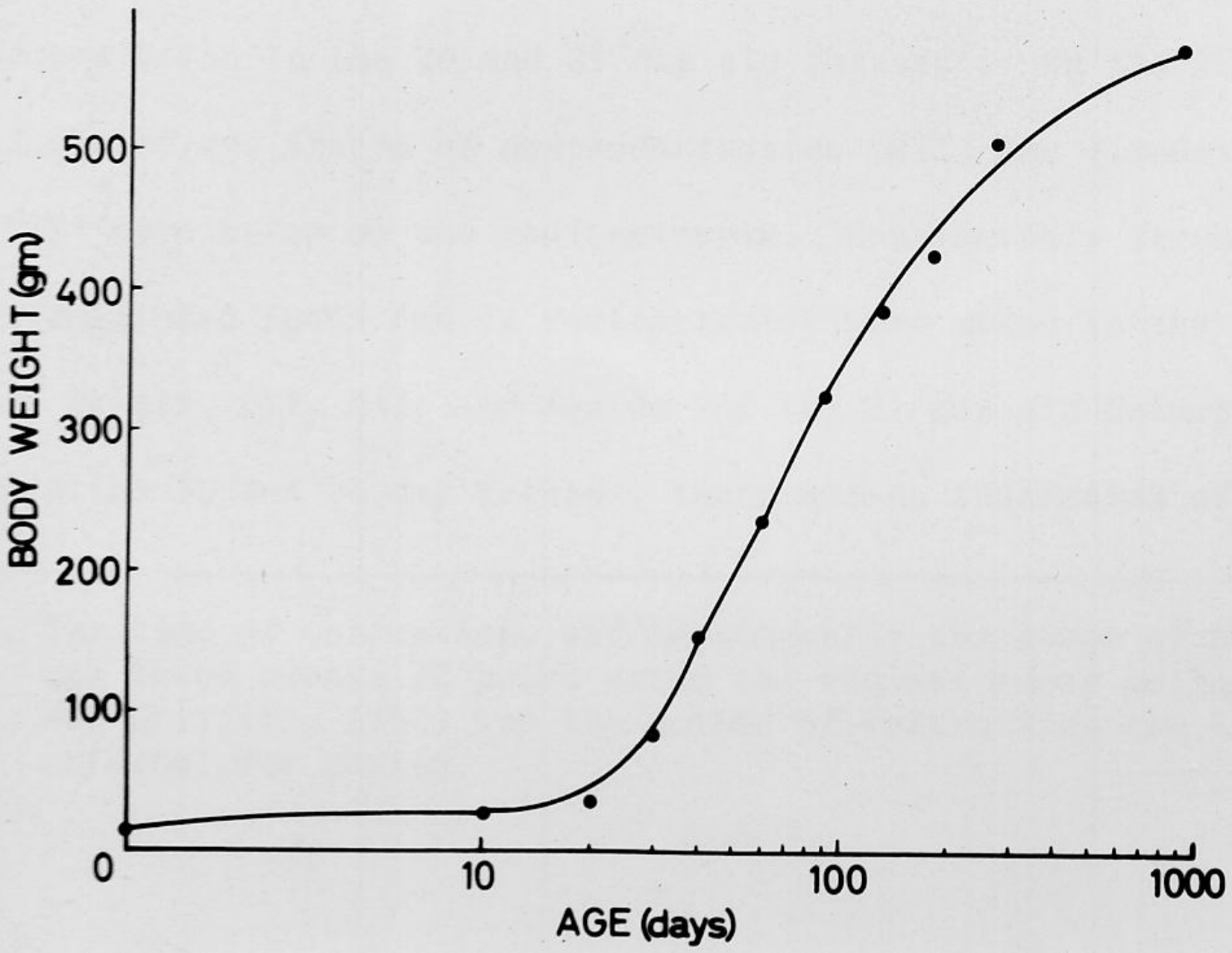


FIG. 6. AVERAGE BODY WEIGHT IN VARIOUS AGE GROUPS OF CONTROL ALBINO RATS.



It is evident that these values are quite variable, but the general pattern indicates an increase in thyroid weight as age increases. Freeman (1934) and Latimer (1951) working with the human and the guinea pig thyroids respectively, reported similar variations in weight at several given ages. A comparison of Figures 5 and 1 reveals a striking similarity in the pattern of change of follicle size and thyroid weight with age.

The average body weight for each of the age groups is given in Figure 6. The rate of gain is relatively low in the animals between birth and 20 days, but increases to a steady rate of gain between the ages of 20 and 100 days. From Figures 1, 5 and 6, a relationship is seen between age and follicle size, thyroid weight and body weight.

Fetal studies

A comparison of the chromatogram (Fig. 7) and the radioautogram (Fig. 8) indicated that biosynthesis of the thyroid hormone had definitely proceeded beyond the stage of iodide accumulation in the 20 and 21 day old fetuses[1]. On the 20th day of gestation, traces of monoiodotyrosine (MIT) and diiodotyrosine (DIT) were noted on the radioautogram. Considerably stronger or more intense spots due to radioactivity were noted in the areas of the origin, DIT, MIT, and iodide for the 21 day old fetuses. For both the 20 and 21 day fetuses, there was no indication of either

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1. The time of conception, and consequently the stage of pregnancy was known within 12 hours using the vaginal smear method (Farris and Griffith, 1962) and the method of controlling the time interval for mating.

Fig. 7. Photograph of paper-strip chromatograms of thyroid gland digests of fetal rats subjected to radioactive iodide and standards of monoiodotyrosine, diiodotyrosine, triiodothyronine, tetraiodothyronine (thyroxine) and sodium iodide. A collidine-ninhydrin mixture was used to develop the strips to show the positions of the standards and the amino acids in the thyroid digests.

FIG. 7. Paper chromatograph of standards and experimentals.

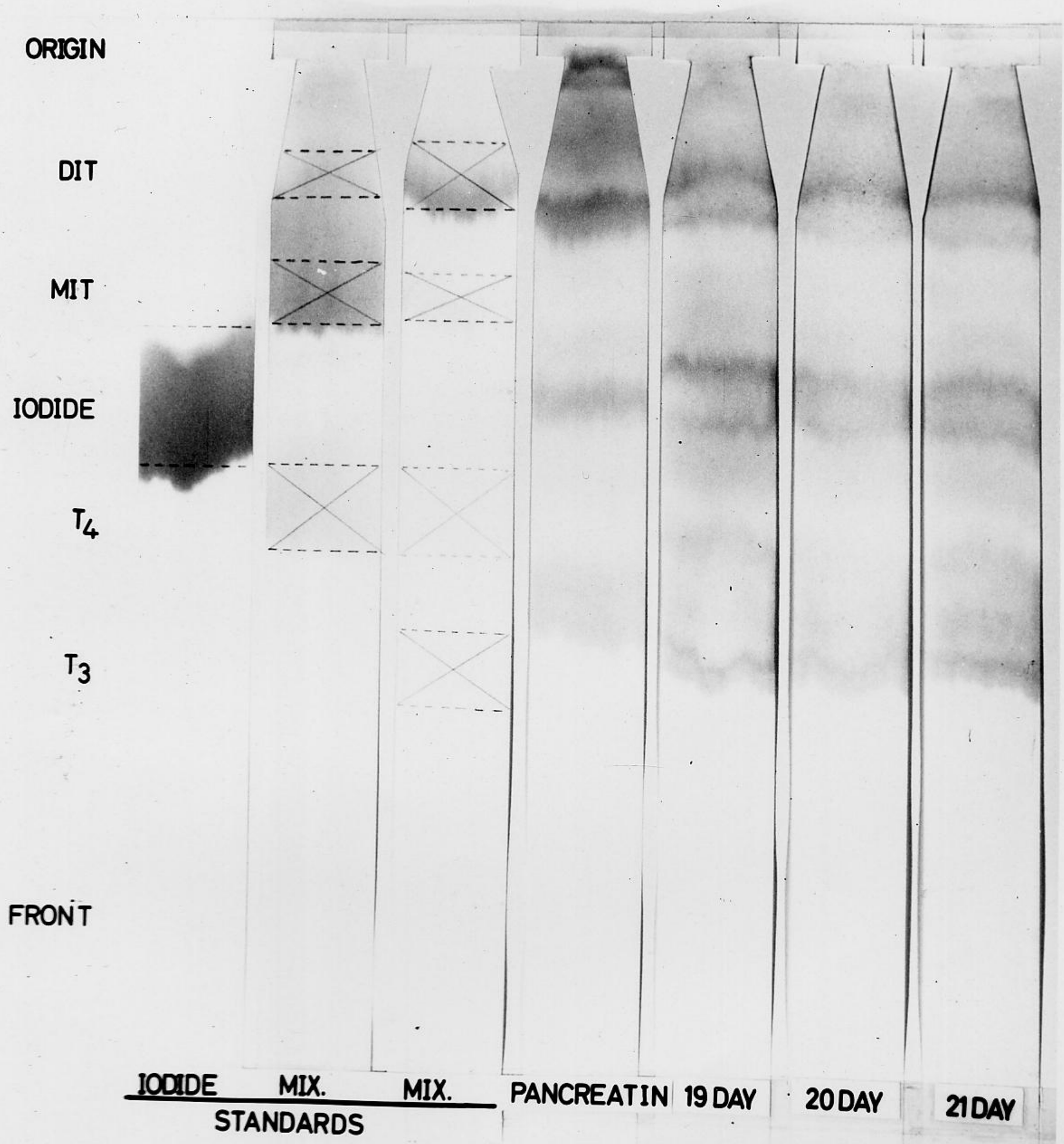
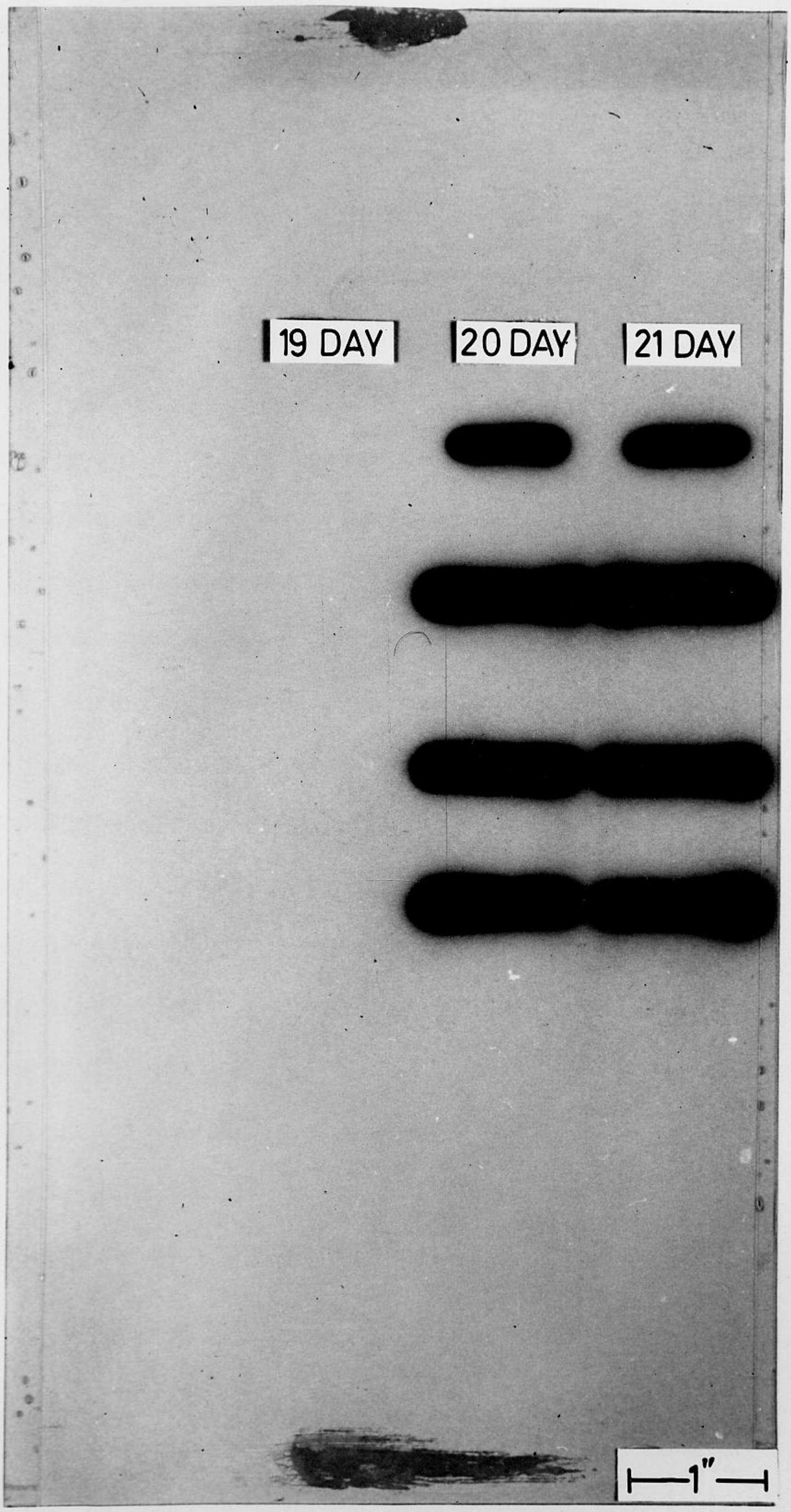


Fig. 8. Photograph of radioautogram of the paper chromatograms of the digests of the thyroid glands of fetal rats subjected to radioactive sodium iodide (Fig. 7). The black spots indicate the positions of the radioactive components in the extracts of the digests. The radioactive spots on the original radioautogram were too weak (light) to be photographed; for purposes of photography, a replicate of the original was made.

FIG. 8. Radioautograph of chromatograph



triiodothyronine (T_3) or tetraiodothyronine (T_4). No iodine accumulation was evident in either 18 or 19 day old fetuses. Because of the relative weakness of the radioactive areas on the radioautogram, it can not be concluded from this study that iodine accumulation does not occur before 20 days or that T_3 ~~or that T_3~~ and T_4 are not produced in the 20 and 21 day old fetuses. It is evident , however, that the amount of iodide accumulated before 20 days and the amount of T_3 and T_4 produced in the fetal rat is not detectable with this method.

Gorbman and Evans (1943) reported that functional accumulation of iodine was initiated in 18-19 day old fetuses. From histological sections used in their radioautographic study, they reported the thyroid as a solid mass of cords on the 18th day of gestation. In 19 day old fetuses, only follicles at the periphery possessed lumina, but at 20-21 days the peripheral follicles showed a considerable advance in differentiation.

This study indicated that thyroid function in the rat began late in intra-uterine life. Just before birth, iodination, the second step in the biosynthesis of the thyroid hormone, was initiated as shown by the presence of both MIT and DIT (Figure 8). The reason for low uptake values in the newly born rats is evident from this study. At birth the gland has not completed its development and is not completely functional.

V. SUMMARY AND CONCLUSIONS

1. Using the histometric method, it was shown that the average (mean) follicle size in "normal" albino rats increased from birth until about 60 days. Thereafter, a plateau was reached, indicating little or no increase.

2. From the histological measurements, the highest percentage epithelium was found in the newly born group. Epithelium percentage gradually decreased from birth until approximately two months of age, after which little change was observed.

3. A comparison of the values of follicle size and epithelium percentage suggests an inverse relationship.

4. The findings in this study seem to support Ludwig's conclusion that epithelial cell height alone in "normal" animals offers no exact criterion or indication of activity of the thyroid gland.

5. Radioiodine uptake values increased rapidly from birth to about 20 days. Thereafter, a sudden decrease was observed until approximately 40 days of age when a value comparable to that of the newly born was obtained. After 40 days, only slight changes were observed.

6. A comparison of the quantitative histological measurements with radioiodine uptake data revealed a relationship only up to 20 days of age. Thereafter, no apparent relationship seemed to exist.

7. This study suggests that the activity of the thyroid gland in "normal" animals is better reflected in iodine uptake than by the morphological changes observed in histological preparations.

8. The radioiodine "turnover" study clearly demonstrates that young, pre-weanling animals, known to possess smaller follicles, accumulated and released radioiodine more actively than older animals with larger follicles. Thus, it is concluded that the rate of activity per unit weight thyroid tissue is dependent to a certain extent on follicular size.

9. The fetal study confirmed that iodide accumulation in the thyroid was initiated in late intra-uterine life. In addition, it revealed that biosynthesis actually proceeded beyond the accumulation stage during the 20th and 21st days of gestation.

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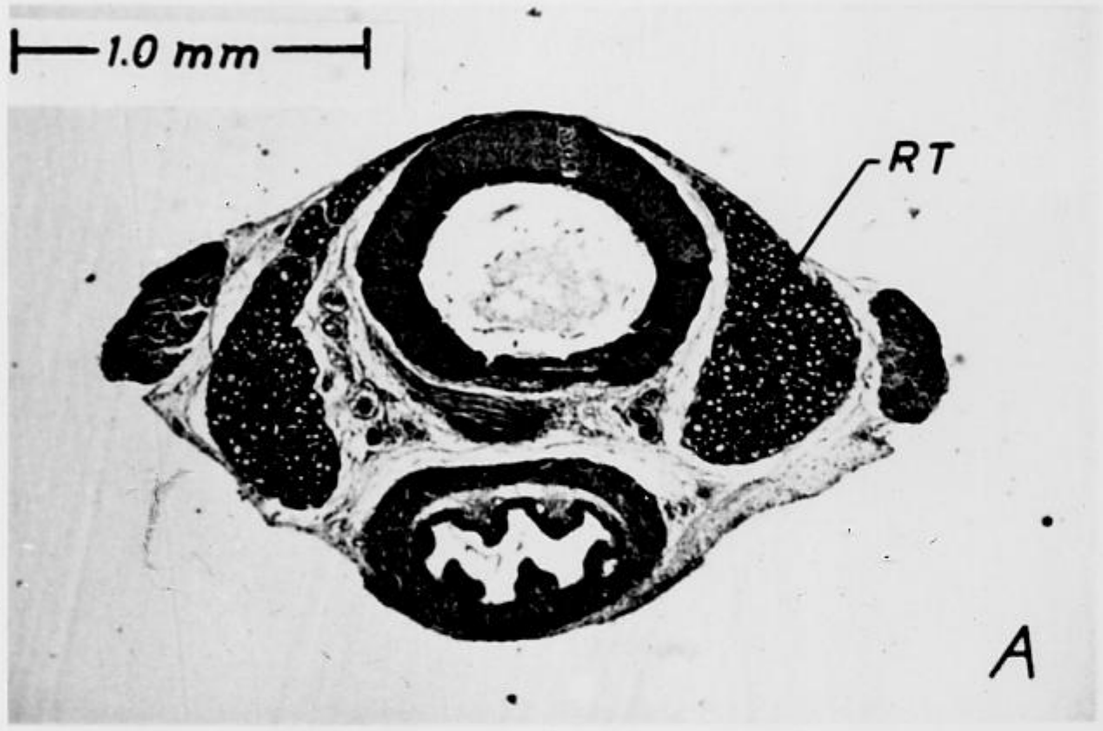
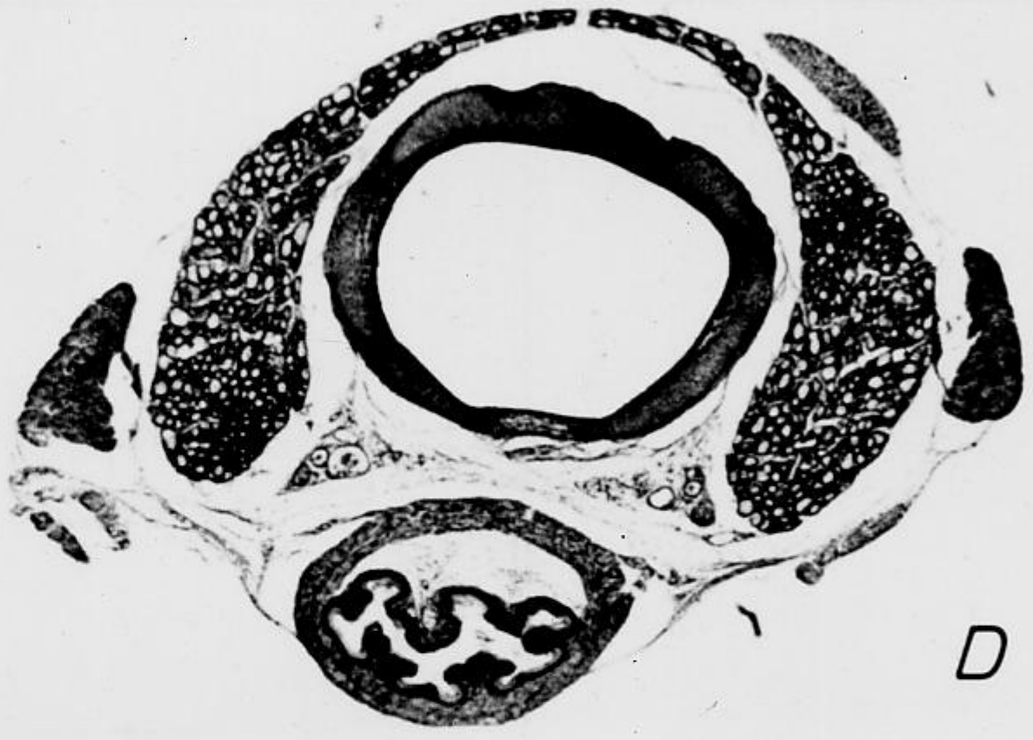
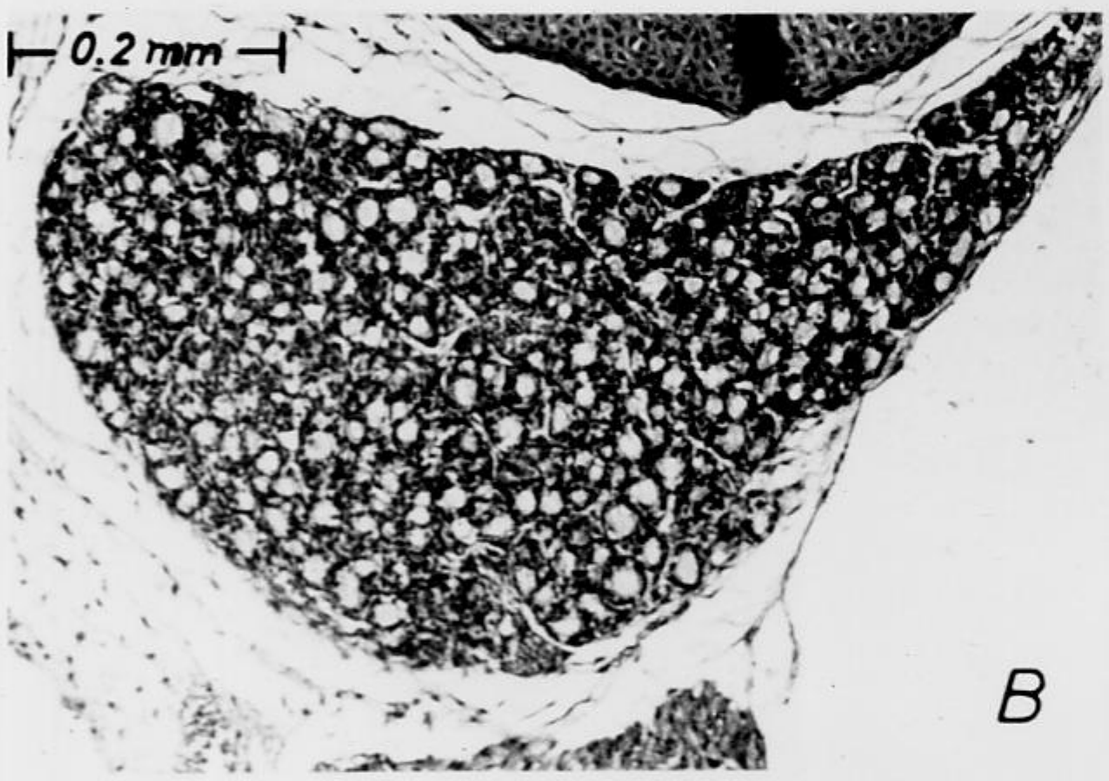
PLATE 1.

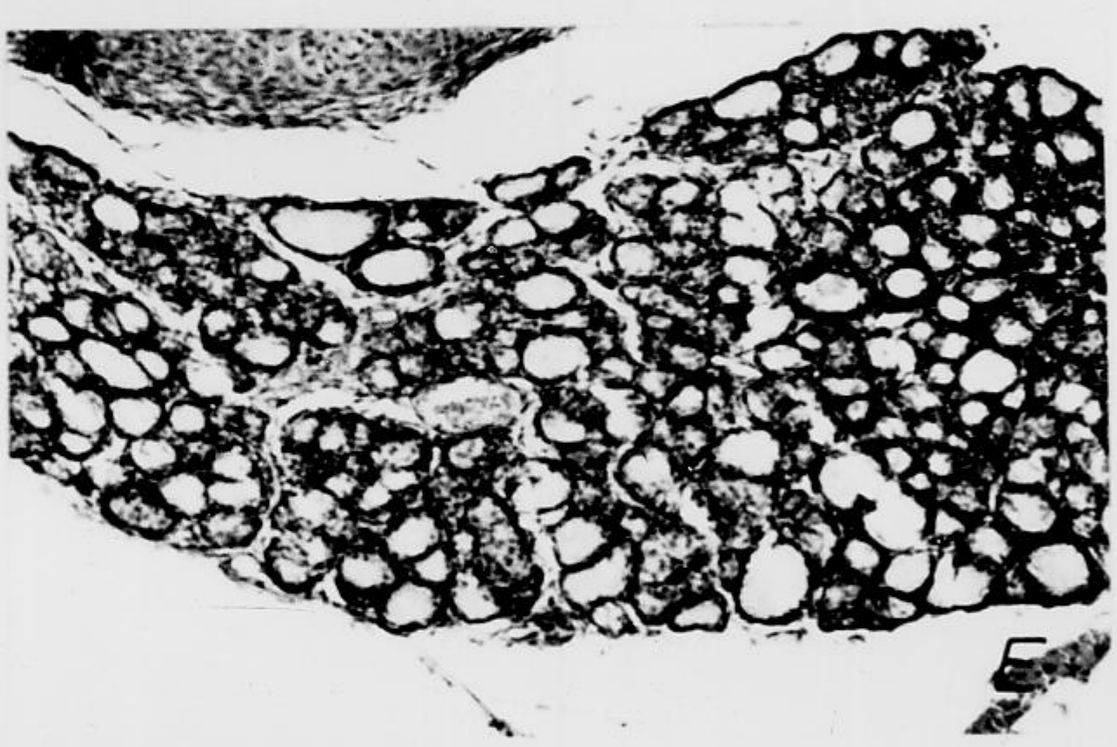
Fig. A. Thyroid gland (with trachae and esophagus) of newly born rat in C.S. (25X)
Fig. B. Left thyroid lobe of newly born rat (100X)
Fig. C. Left thyroid lobe of newly born rat (250X)
Fig. D. Thyroid gland of 10 day old rat in C.S. (25X)
Fig. E. Left thyroid lobe of 10 day old rat (100X)
Fig. F. Left thyroid lobe of 10 day old rat (250X)

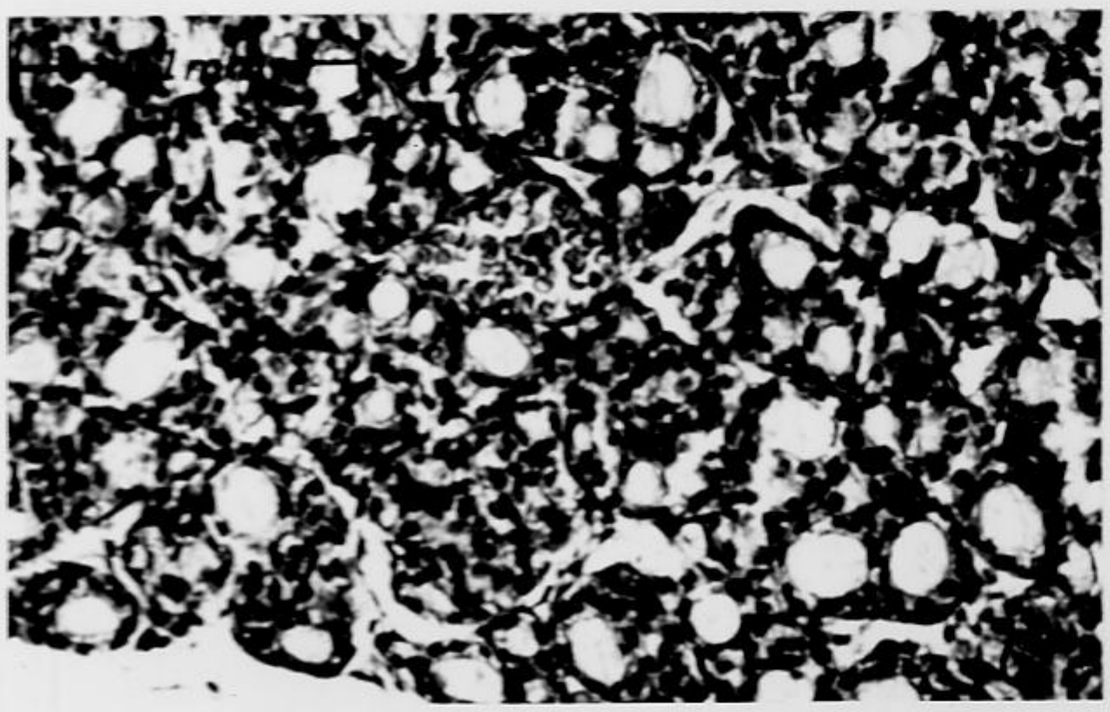
PLATE 1.











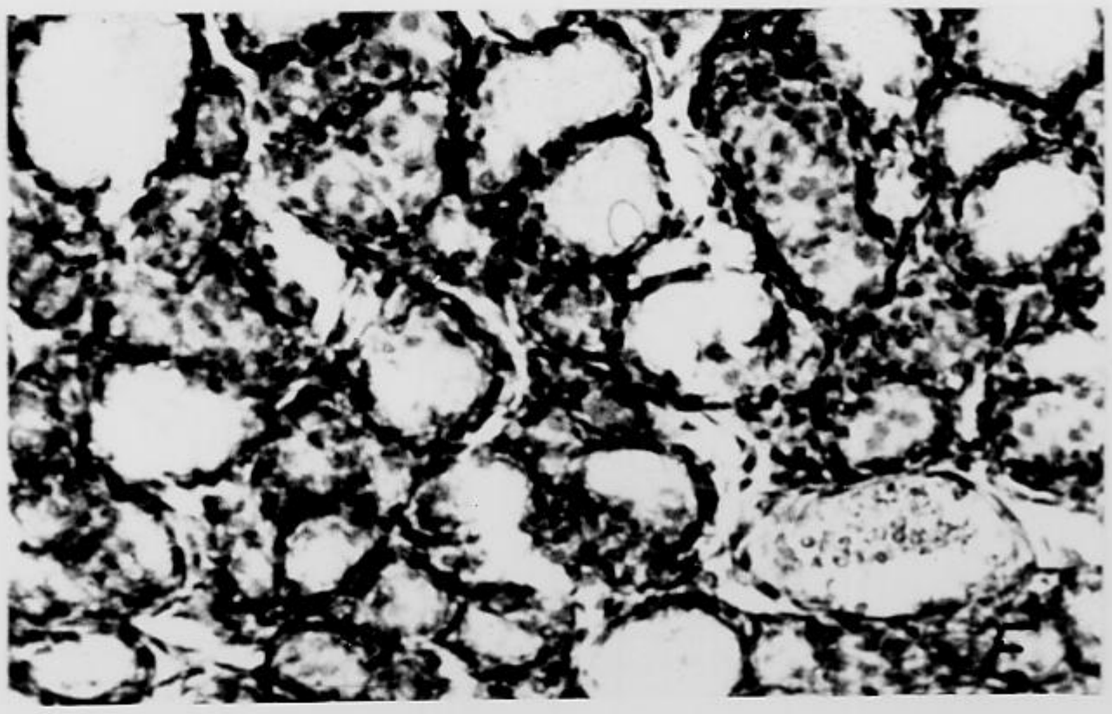
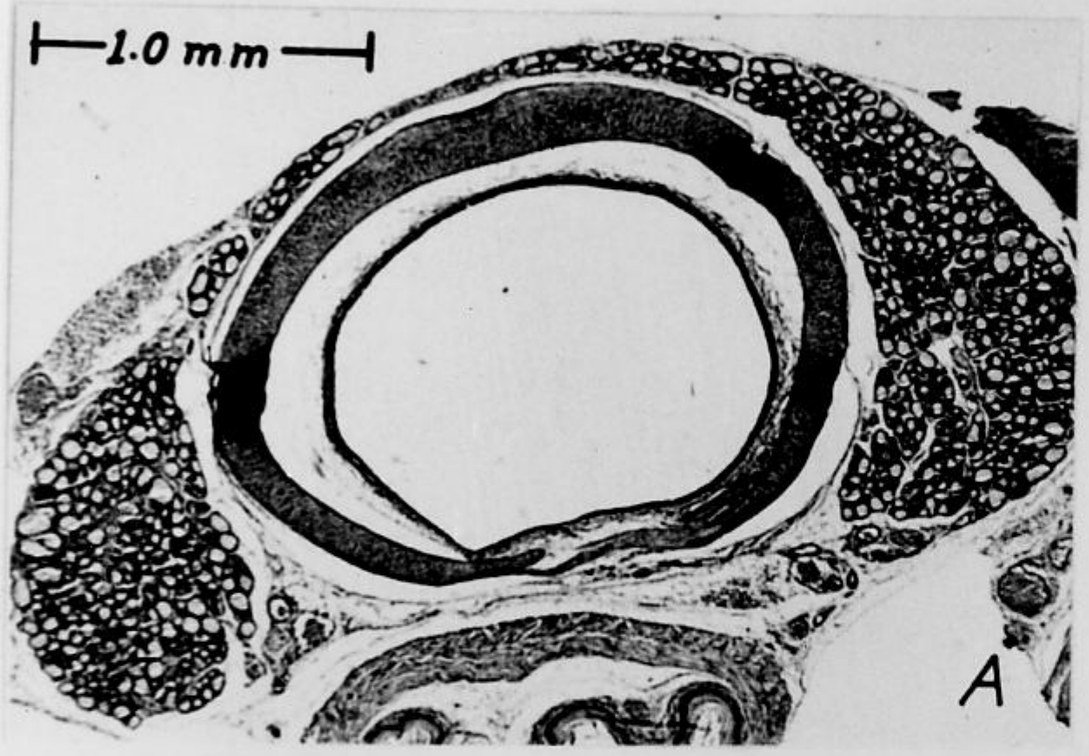
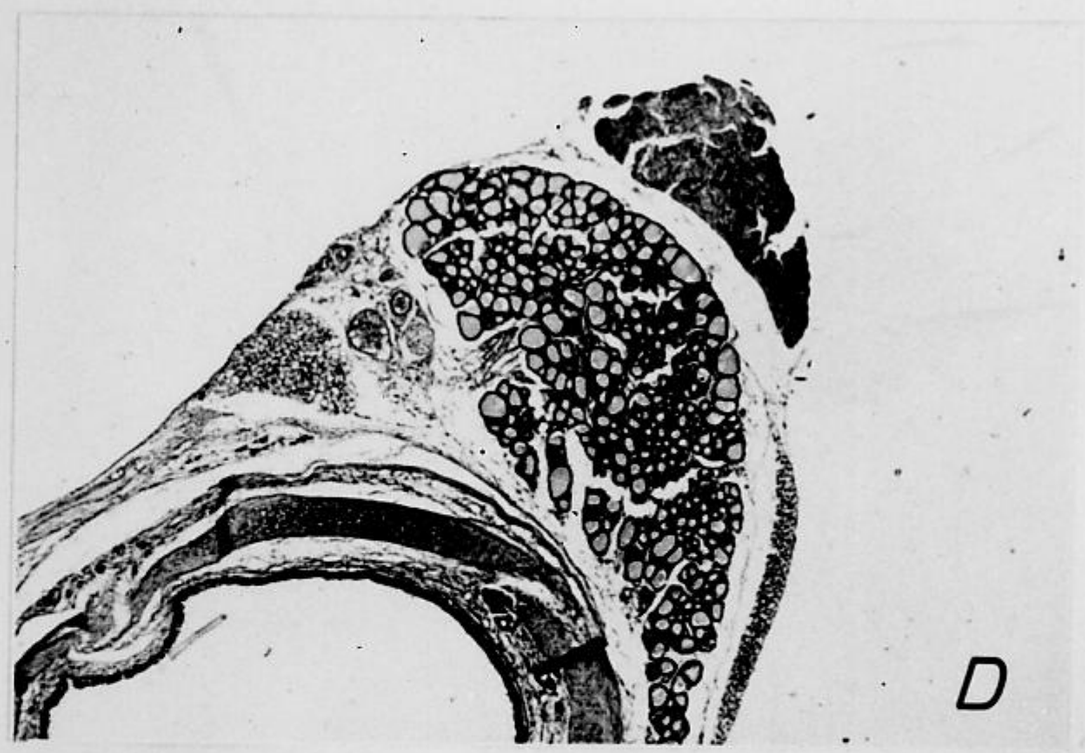


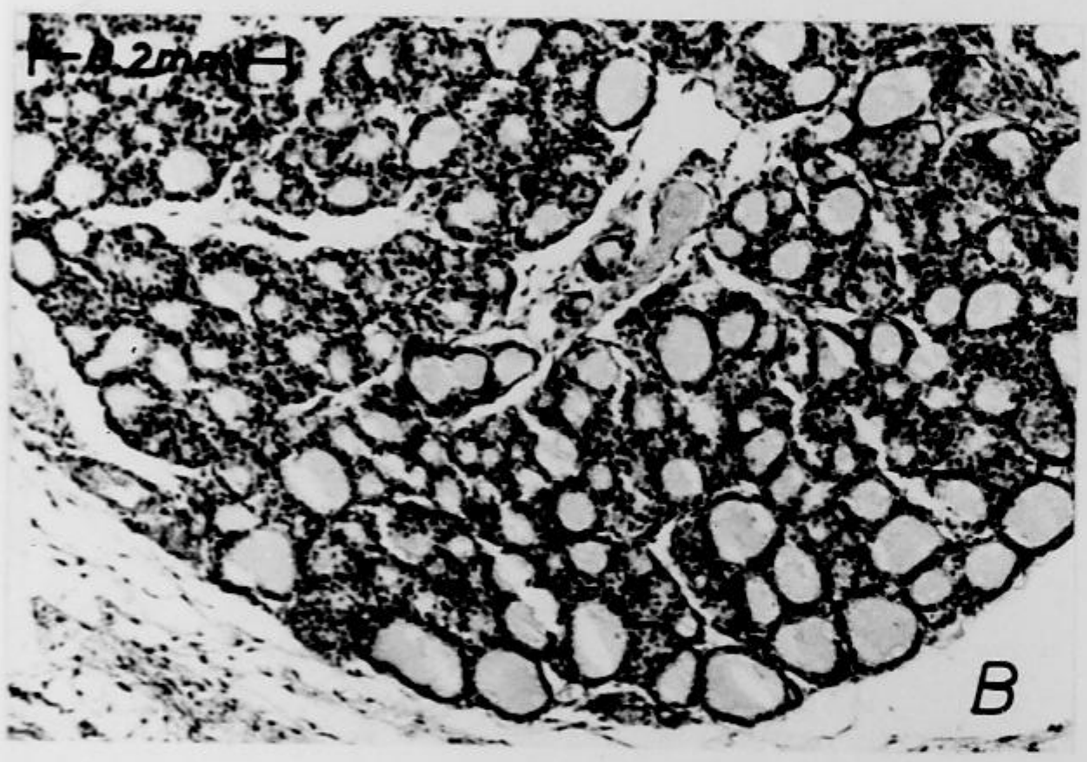
PLATE 2.

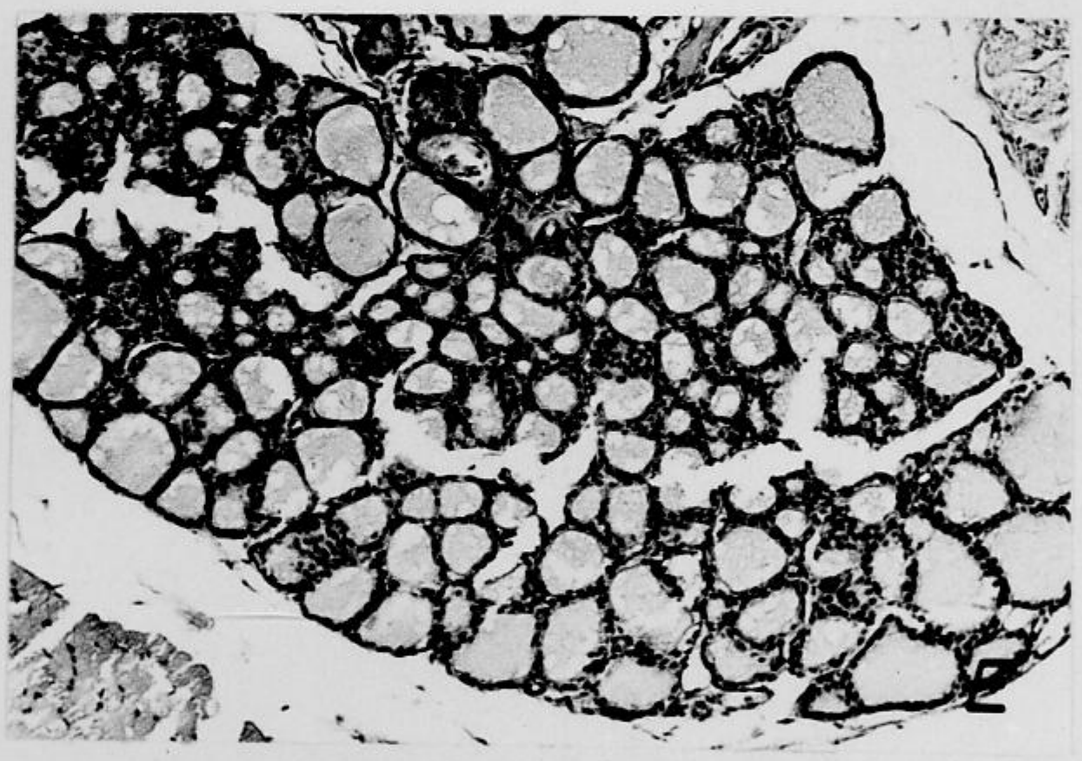
Fig. A. Thyroid gland (with trachae and esophagus) of 20 day old rat in C.S. (25X)
Fig. B. Left thyroid lobe of 20 day old rat (100X)
Fig. C. Left thyroid lobe of 20 day old rat (250X)
Fig. D. Left thyroid lobe (with trachae) of 30 day old rat (25X)
Fig. E. Left thyroid lobe of 30 day old rat (100X)
Fig. F. Left thyroid lobe of 30 day old rat (250X)

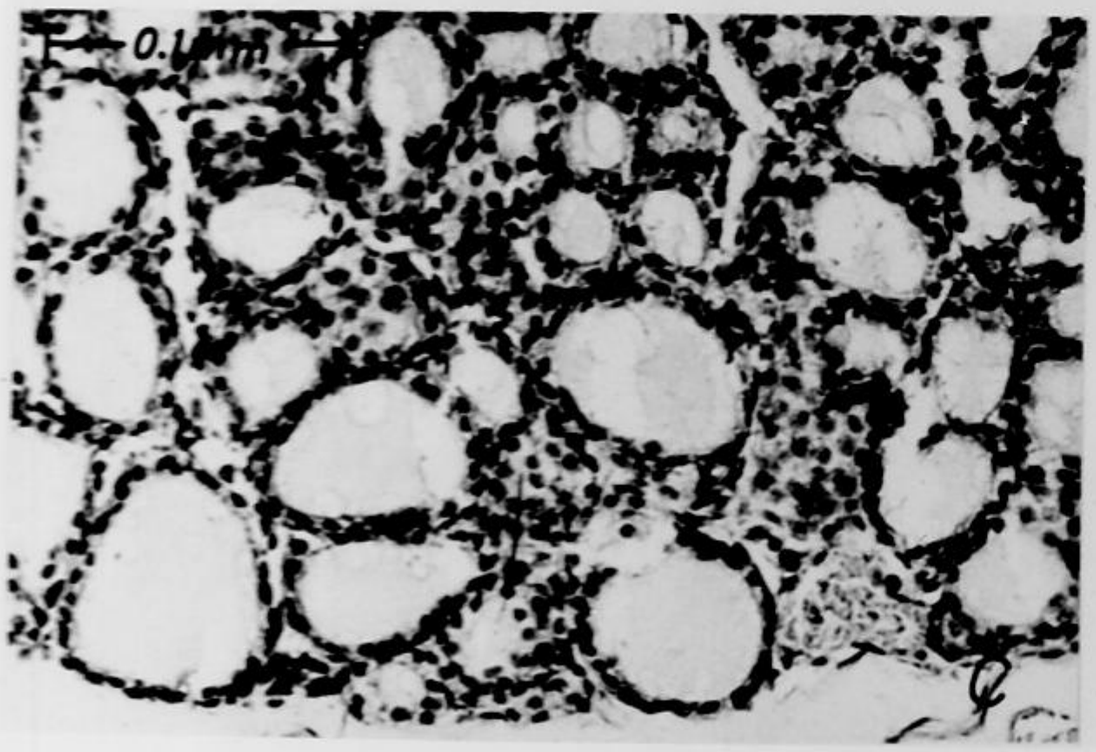
PLATE 2.











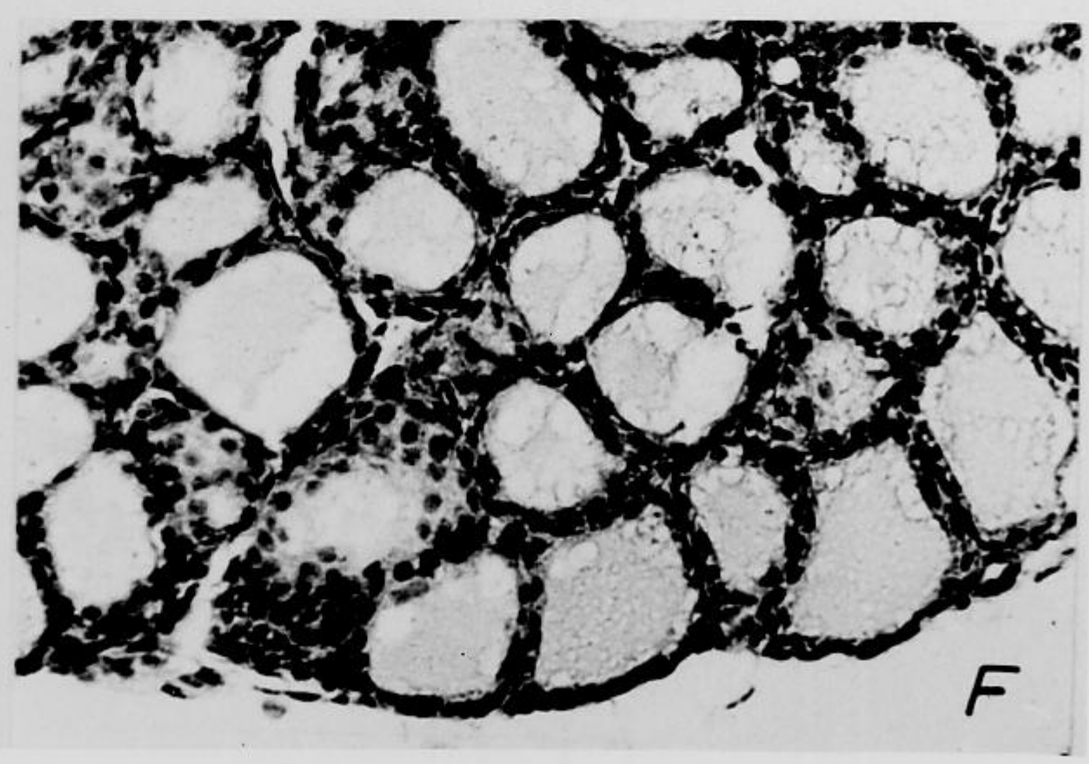
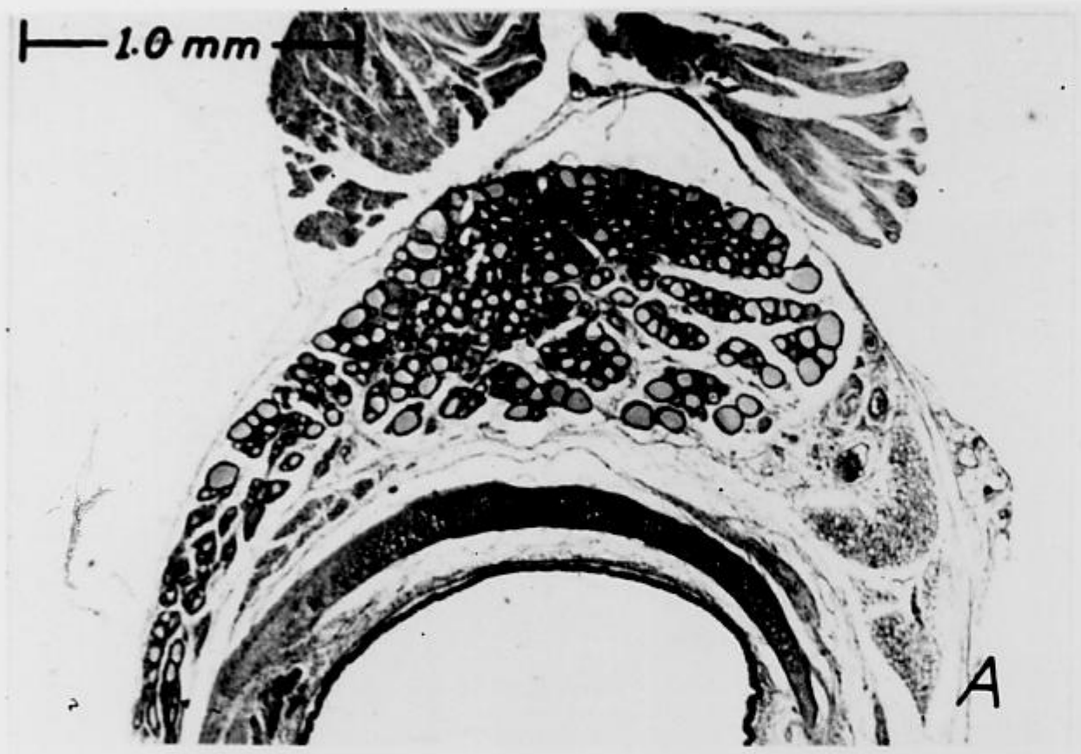
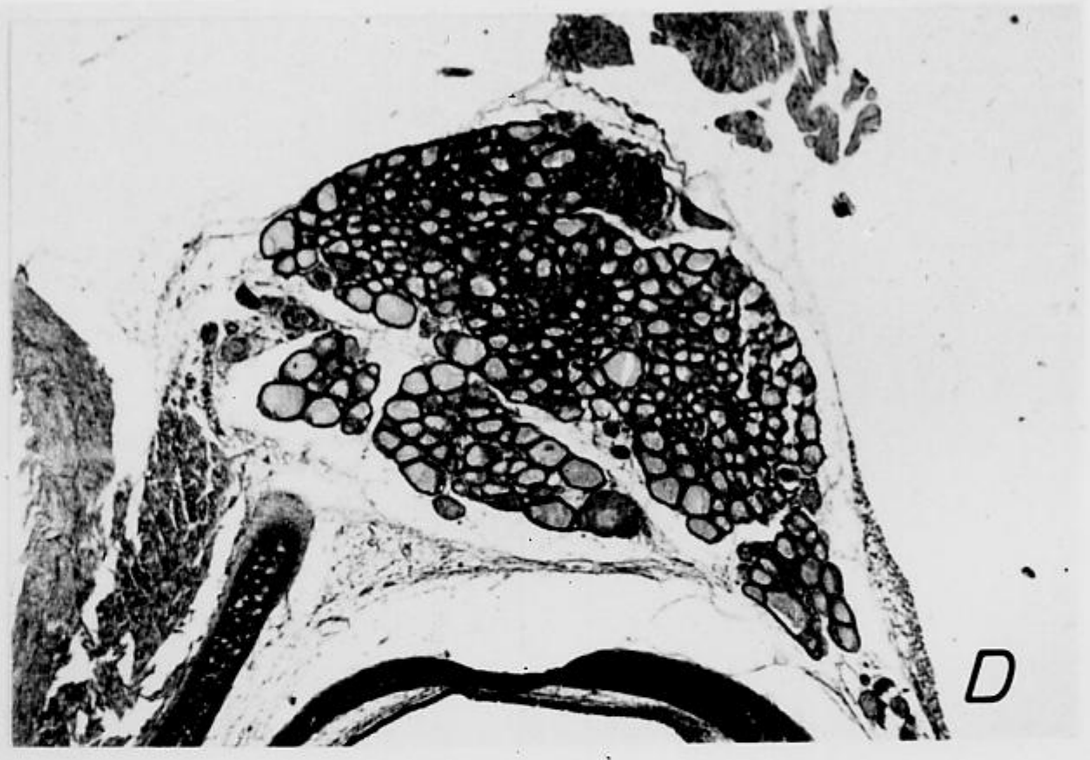


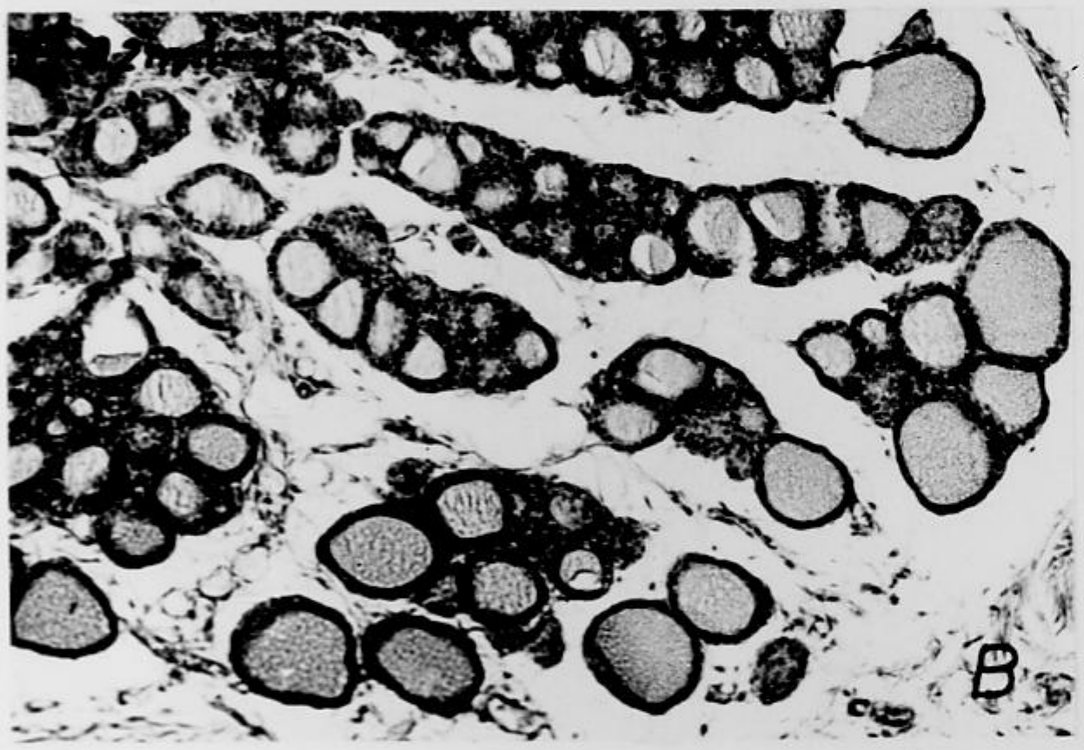
PLATE 3.

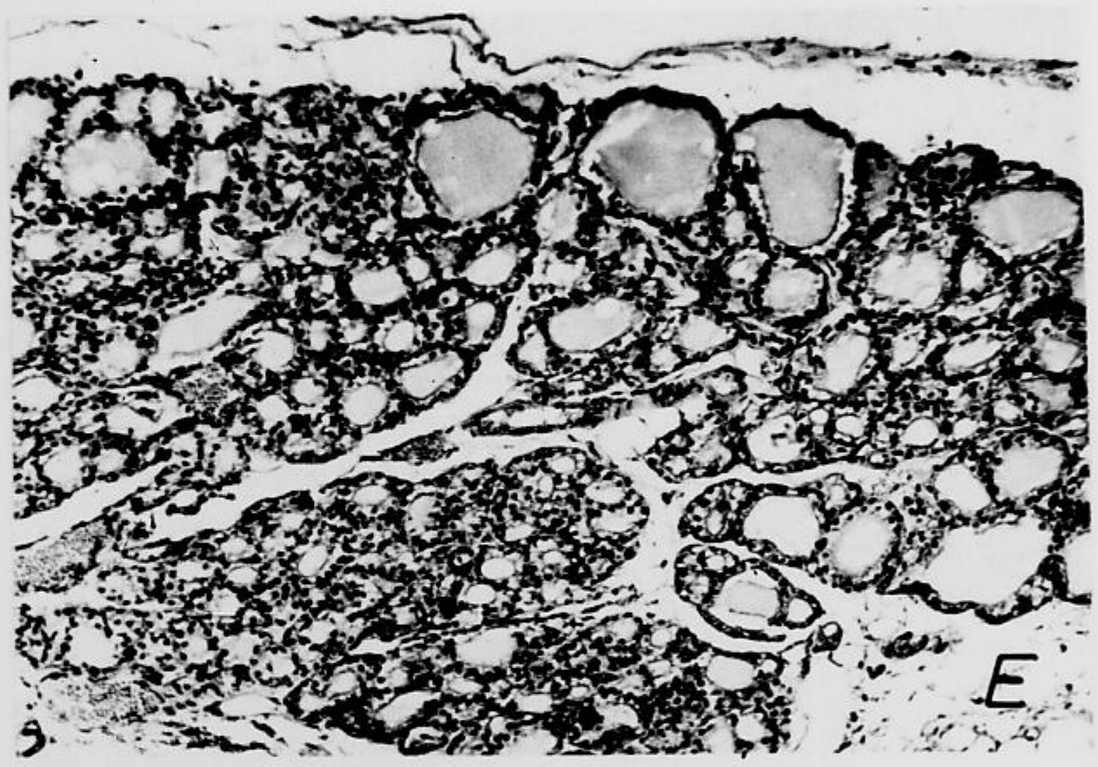
Fig. A. Left thyroid lobe (with trachae) of 40 day old rat (25X).
Fig. B. Left thyroid lobe of 40 day old rat (100X).
Fig. C. Left thyroid lobe of 40 day old rat (250X).
Fig. D. Left thyroid lobe (with trachae) of 60 day old rat (25X).
Fig. E. Left thyroid lobe of 60 day old rat (100X).
Fig. F. Left thyroid lobe of 60 day old rat (250X).

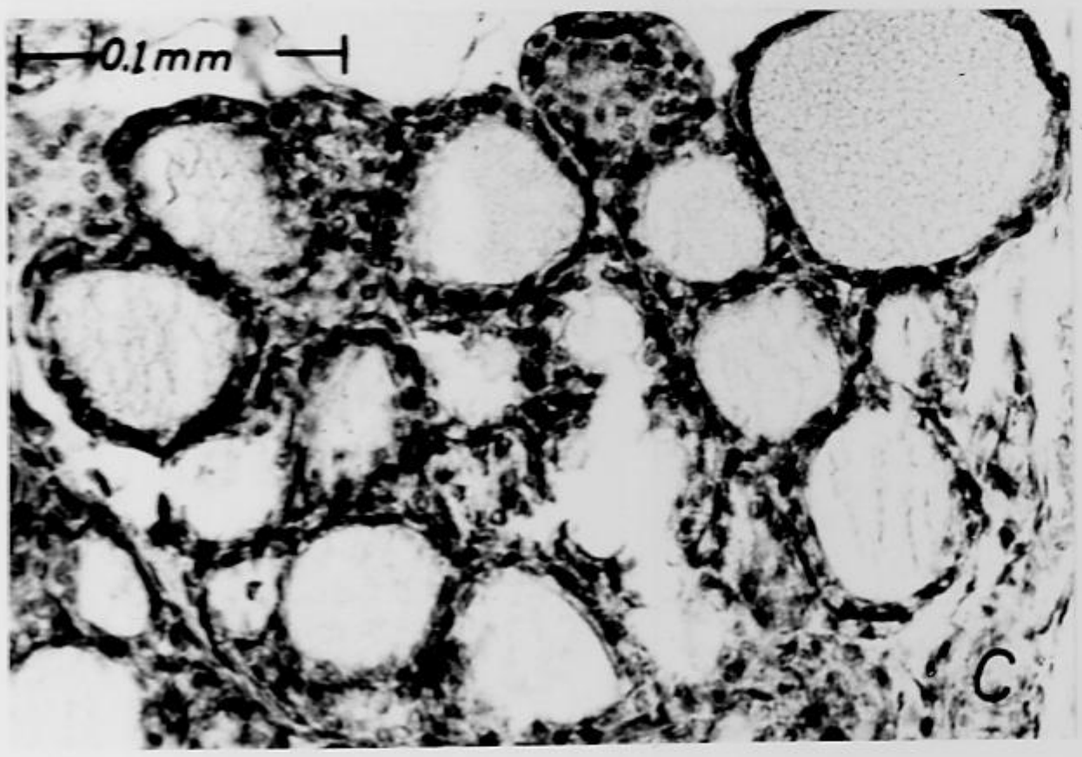
PLATE 3.











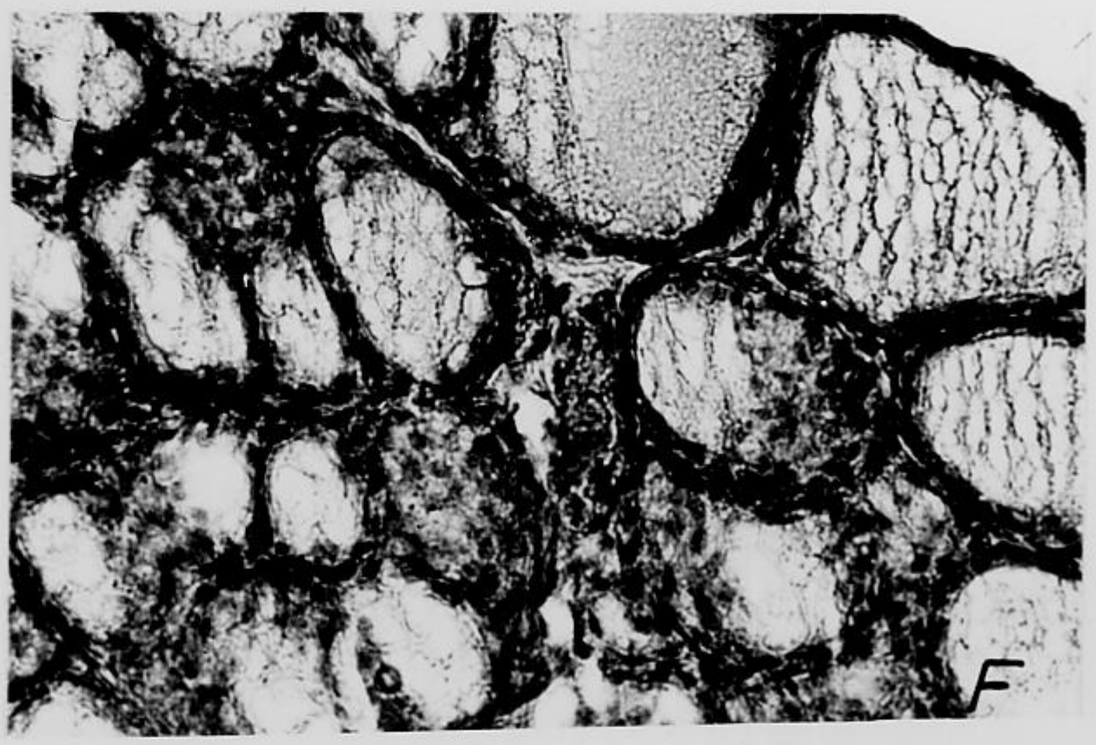
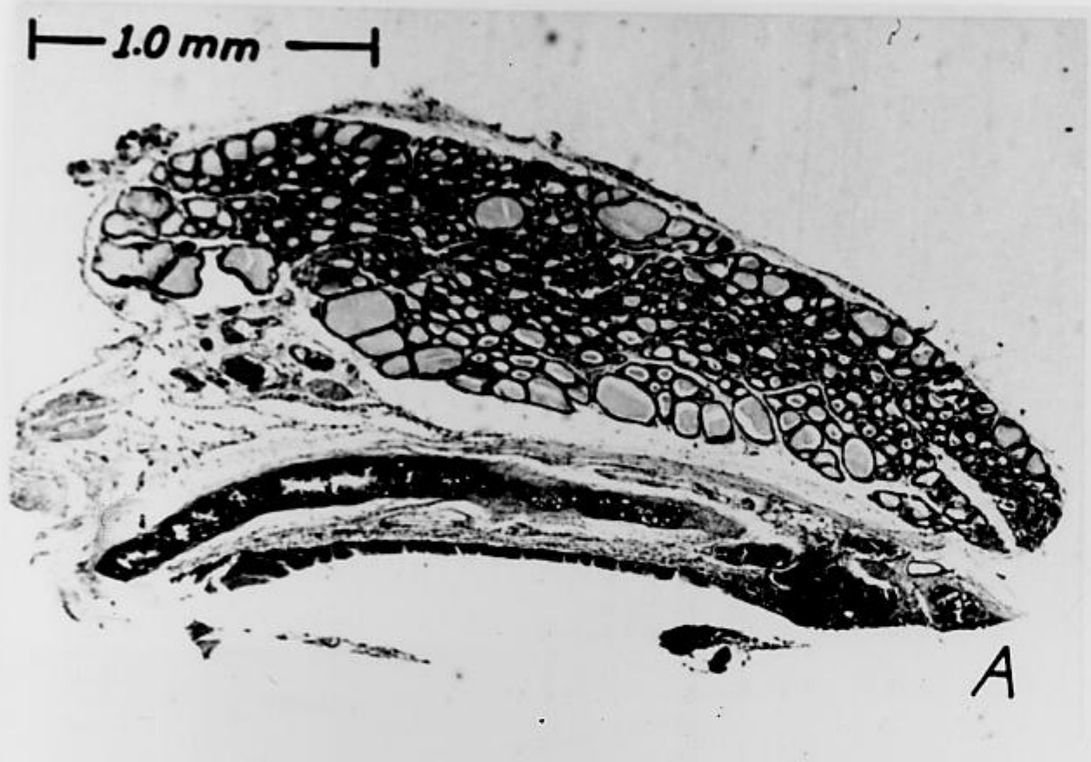
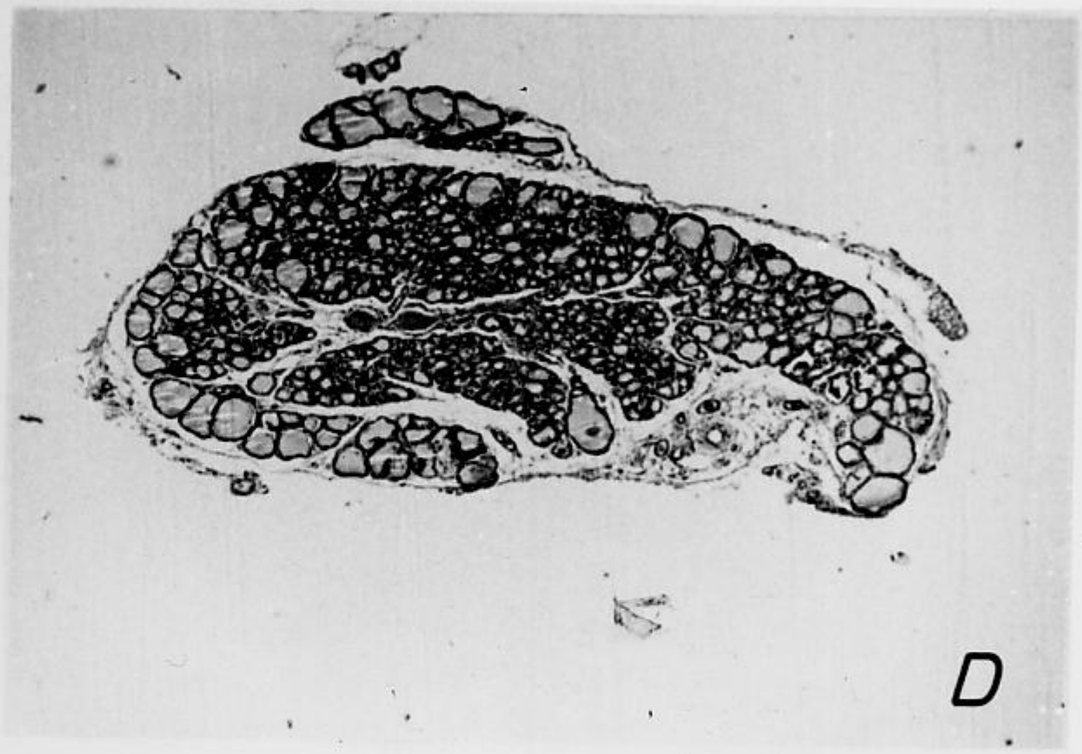


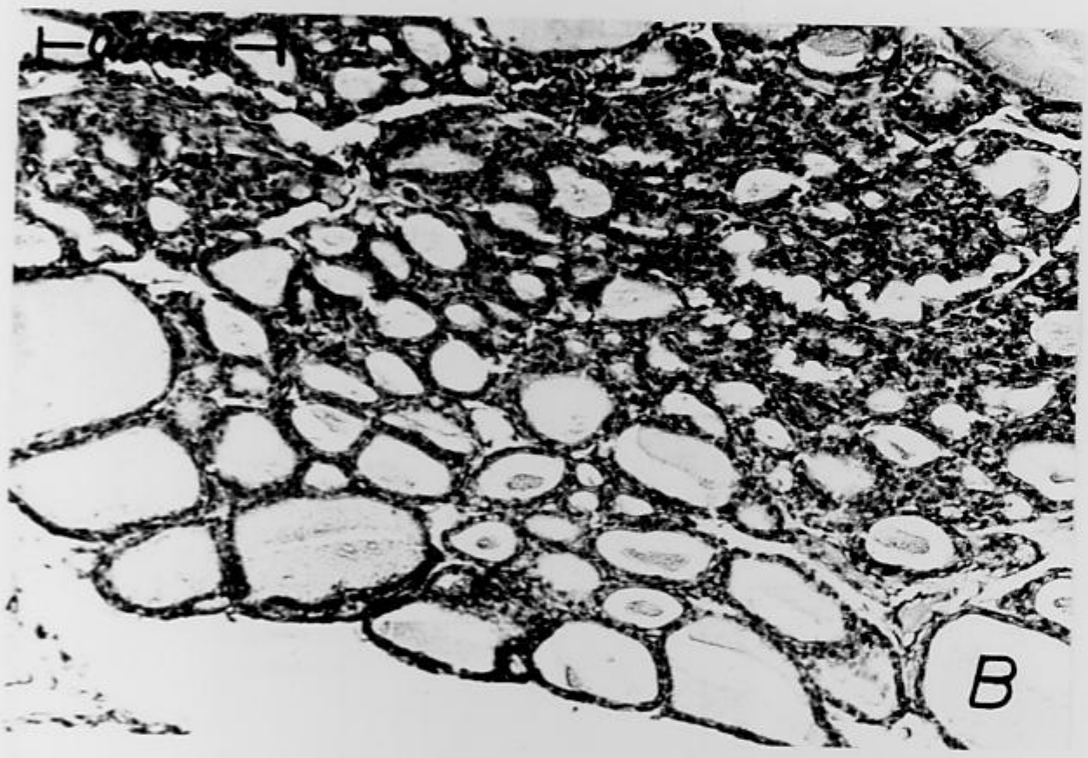
PLATE 4.

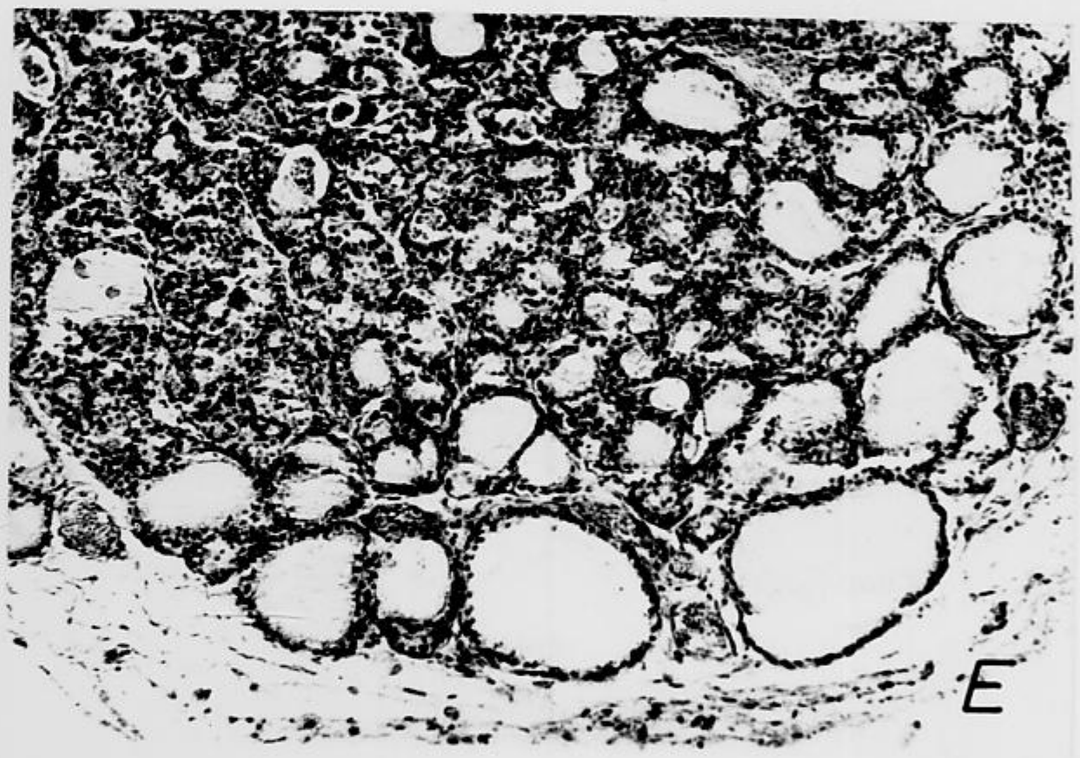
Fig. A. Thyroid lobe (with trachae) of 90 day old rat (25X).
Fig. B. Thyroid lobe of 90 day old rat (100X).
Fig. C. Thyroid lobe of 90 day old rat (250X).
Fig. D. Left thyroid lobe of 140 day old rat (25X).
Fig. E. Left thyroid lobe of 140 day old rat (100X).
Fig. F. Left thyroid lobe of 140 day old rat (250X).

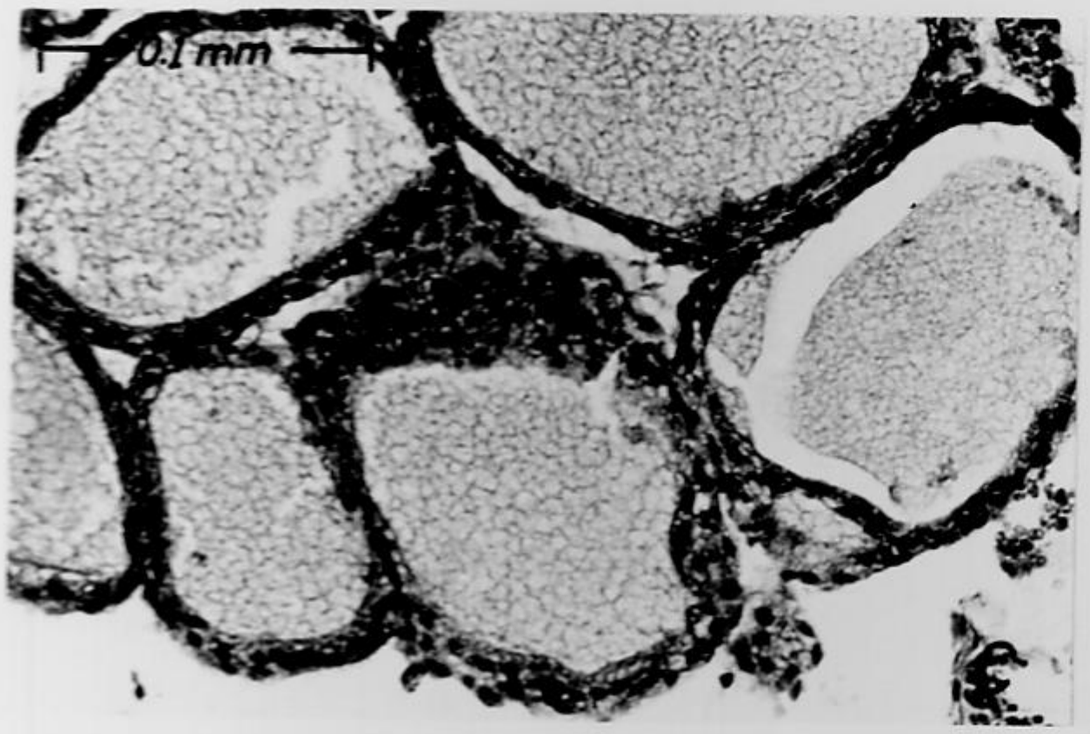
PLATE 4.











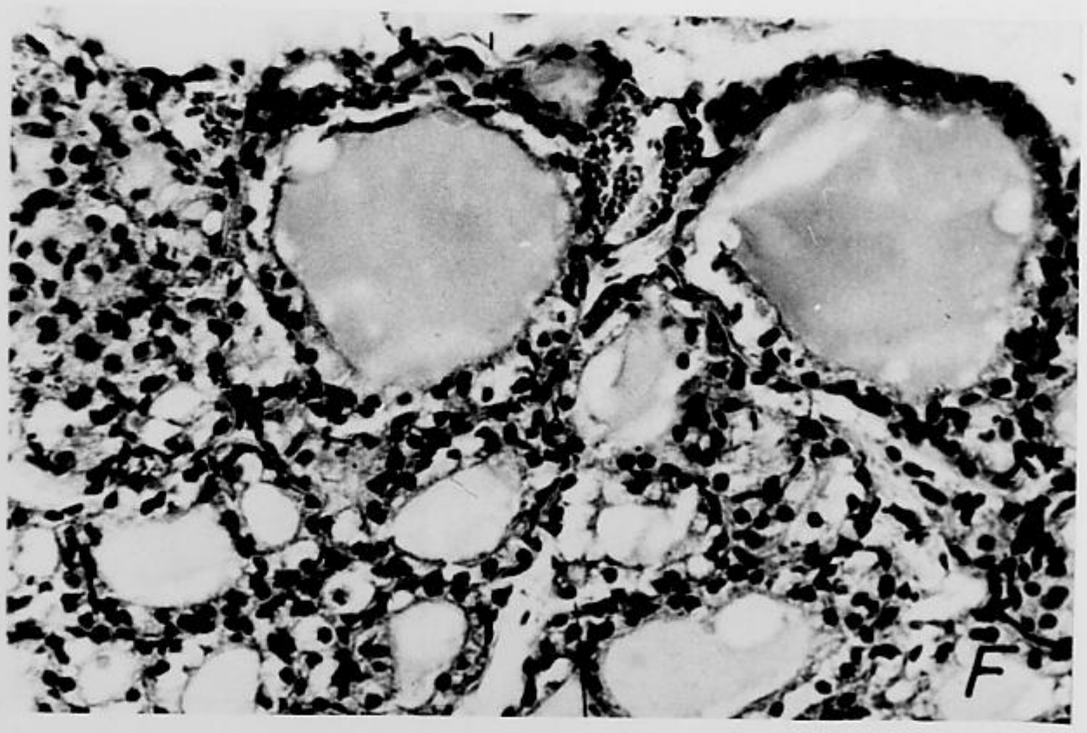


PLATE 5.

Fig. A. Left thyroid lobe of 180 day old rat (25X).
Fig. B. Left thyroid lobe of 180 day old rat (100X).
Fig. C. Left thyroid lobe of 180 day old rat (250X).
Fig. D. Left thyroid lobe of 900 day old rat (25X).
Fig. E. Left thyroid lobe of 900 day old rat (100X).
Fig. F. Left thyroid lobe of 900 day old rat (250X).

PLATE 5.