A COMPARATIVE HISTOLOGICAL STUDY OF THYROID STRUCTURE IN CERTAIN DOMESTIC AND LABORATORY MAMMALS, WITH EMPHASIS ON FOLLICULAR SIZE AND EPITHELIUM PERCENTAGE

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THYROID FOLLICULAR SIZE AND EPITHELIUM PERCENTAGE IN CERTAIN MAMMALS

ABSTRACT

For a comparative study of follicular size and epithelium percentage of the thyroid glands of various laboratory and domestic mammals, paraffin sections were prepared and stained with hematoxylin and eosin. From the gland of every animal, fifty follicles were drawn on graph paper at a known magnification with the aid of the camera lucida. For each of the three magnifications used, a stage micrometer was used for projecting and drawing on the graph paper, thus affecting calibration of the latter. The fifty follicles were then measured and their average taken. For each group of five animals representing one species, the average and range of follicular size was calculated. Epithelium percentage was obtained by drawing few lines at random through a drawn field, measuring the segments of the line covered by epithelium, dividing the total length of these segments with the whole length occupied by the follicles, and then expressing this as a percentage ratio. Again the average and range for each species was calculated.

The results show a tendency for follicular size, to increase with increasing body size of the mammal. However, this cannot be well generalized. Epithelium percentage decreases roughly, again with some exceptions, as follicular

size increases. Further studies using a larger number of animals per species are suggested. This might give the data obtained a more solid base.

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INTRODUCTION

The thyroid gland has been the subject of intensive study ever since Thomas Wharton ascribed to it an esthetic function in 1656 (Barrington, 1963). Through the years, a multitude of information has gathered about the different aspects of this endocrine organ with more still to be revealed.

The thyroid gland lies in man and other mammals on both sides of the trachea, hence we speak of a right and a left lobe which may or may not be joined by an isthmus. The basic histological unit of the thyroid gland in all vertebrates is the thyroid follicle. This is essentially a rounded or oval structure in all of the vertebrate group. The follicles are in every case discrete, individual structures never connected except by a small amount of connective tissue, as observed in turtles, frogs, chicken and mammals by Johnson and Gettlefinger (1932). In man, the follicle has been described by various authors as being rounded or oval (Wilson, 1927), or spherical (Rienhoff, 1929). Jackson (1930) studying ten normal glands of adult men and women, ascribed three shapes to the follicles he observed, namely angular, round or oval. The follicle is lined by epithelium which rests on a basement membrane as seen with the electron microscope (Waller, 1960). A viscous fluid of varying

consistency, the colloid, fills the follicular lumen. The thyroid is the only endocrine organ which stores its product in an extracellular site, while its hormone is the only one the specific molecular feature of which includes the possession of a specific chemical element, namely iodine. Histochemically, the colloid is argyrophilic, gives a positive Schiff reaction indicating the presence of a substance containing vicinal glycol groups (Dempsey and Singer, 1946). The third histological component of the thyroid besides colloid and epithelium, is the stroma which consists of interfollicular connective tissue along with the blood and lymph vessels and nerve fibers.

The thyroid follicles that are centrally located in the gland are the most reactive, reflecting functional ebb and flow (Dempsey and Singer, 1946; Johnson, 1950; Sugiyama, 1954; Sugiyama and Sato, 1954). In the ground squirrel (Zalesky, 1935), this central area is the first to exhibit heightened seasonal activity in spring. Radioautographic studies in the rat by Nadler, Leblond and Bogoroch (1954) indicate that the small follicles (generally located in the center of the gland) accumulate radioiodine in their colloid more actively, achieve a greater concentration of this element, and discharge it at a faster rate than large follicles. As previously mentioned, the centrally located follicles are the smallest in size, the largest ones occupying the periphery of the gland. This has been reported in cows, pigs,

horses, cats, dogs, rabbits, guinea pigs and rats (Tomonari, 1959), in the hamster (Knigge, 1957) and in two subspecies of the deer mouse, Peromyscus maniculatus (Yocome and Huestis, 1928). Histochemical differences have also been ascribed to these follicles of varying sizes. The large follicles are said to contain more protein in relation to RNA, DNA and phospholipid. This suggests a less rapid turnover of protein than in small follicles (Begg, McGirr and Munro, 1965). The small follicles exhibit acid glycerophosphatase activity while the large peripheral ones exhibit alkaline glycerophosphatase activity (Dempsey and Singer, 1946).

Variations in Follicular Size

The bulk of pertinent literature on normal thyroid histology seems to indicate the presence of considerable variations in follicular size from mammal to mammal, the trend being for larger mammals to have larger follicles (Begg, McGirr and Munro, 1965). These authors cite Yagizawa who computed the mean dimensions of the 50 largest follicles in several mammals. Where in the rat the longest diameter was 117μ and the shortest diameter 77μ, in the dog it was 277μ x 139μ and in the cow 337μ x 237μ . Klein (1935) found the average follicular diameter in the rat to be 251μ . In the sperm whale, Physeter megalocephalus, Grafflin and Geiling (1942) found the largest follicle to be 1.3 mm. in

diameter with many follicles of lesser size. Harrison et al. (1962) found the mean follicular diameter of 16 adult common seals, Phoca vitulina, to be 0.129 ± 0.005 mm. Wilson (1927) studying 400 human thyroid glands found the normal follicle to average 300μ in both horizontal and vertical diameters with a range from 150μ to 500μ, while Jackson (1930) found the average length of the normal follicle to be 163.24 ± 1.17μ. The largest follicle he measured in the normal gland was 1293.6μ. Follicles under 122 μ accounted for 41% of the normal gland. While studying variations in the size, weight and histologic structure of 725 human thyroids removed at autopsy, Nolan (1938) set arbitrarily 1 mm. as the upper limit of the follicular diameter.

Variations in Thyroid Weight

Variations in follicular size from mammal to mammal are accompanied by variations in total thyroid weight. Latimer (1951) studying 100 young adult male guinea pigs found the average thyroid weight to be 0.1040 ± 0.0021 gm. Also working on guinea pigs, Mixner, Bergman and Turner (1945) observed that although the thyroids in the male and female started at about the same level of weight, in the female the thyroid increased in weight at a greater rate than in the male, making the gland of the mature female heavier than that of the mature male. Hatai

(1914) on the other hand, noted that there was neither in the Norway nor in the Albino rat any sex difference in the weight of the thyroid. Such sex differences in weight, however, were found to be considerable in the Virginia deer, Odocoileus virginianus borealis. Grafflin (1942) found the weight in males to range from 3.17 to 16.94 gm. and in females from 2.75 to 6.70 gm. Not only this but there was also a difference in the weight between the two lobes of the same gland. In the gorilla, Gorilla gorilla beringei, the combined weight of the thyroid and parathyroid glands was 11 gm. (Grafflin, 1940b). The weight of the thyroid of one sperm whale, Physeter megalocephalus, was approximately 1400 gm., that of two other specimens 1000 gm. each (Grafflin and Geiling, 1942). Eleven adult common seals, Phoca vitulina, had a mean thyroidal weight of 5.56 ± 0.30 gm. (Harrison et al., 1962). Studying 35 normal male dogs, Mulligan and Francis (1951) obtained a mean weight of 0.95 gm. with a range from 0.35 to 2.03 gm. In humans, the normal thyroid gland has an average weight of 19.3 gm. in males and 17.7 gm. in females (Hull, 1955). These figures were based on 221 glands obtained at autopsy at Colorado General Hospital. According to Turner (1961) the normal thyroid gland of the adult weighs from 25 to 40 gm. Mortensen, Woolner and Bennett (1955) using a scattergram, observed that for adults after

20 years of age, the greatest frequency of weights was at 15 to 25 gm. followed by 25 to 35 gm. There was no essential difference in average weight of the thyroid glands of males compared to those of females.

Variations in Thyroid Morphology

The thyroid gland has been studied morphologically in many members of the Class Mammalia. In the opposum (Bensley, 1914), the gland consists of 2 ovoidal lobes on either side of the trachea. No connecting isthmus is usually present, while in swine the 2 lateral lobes fuse in the midline (Caylor and Schlotthauer, 1926). In the mountain gorilla, Gorilla gorilla beringei, the gla nd consists of 2 symmetrical lateral lobes connected by a thin isthmus (Grafflin, 1940b). The same author studying the thyroid of the baboon (1940a), found the gland to consist of 2 lateral lobes with no connecting isthmus. He also observed (1942) similar findings in the Virginia deer, Odocoileus virginianus borealis, where an isthmus was absent. Grafflin and Geiling (1942) studied the thyroid gland in 3 genera of whales viz. Physeter megalocephalus, Balaenoptera sibbaldii and Delphinapterus leucas. In these 3 whales the gland typically exhibited 2 lateral lobes connected superiorly by an isthmus, though the authors did not mention any except the first whale specifically. Frank (1960) in his report on the thyroid gland of the giraffe,

Giraffa camelopardalis L., did not comment on the morphology of the gland but claimed to have found 3 accessory glands lying also against the trachea. Gray and Loeb (1928) found an isthmus in only some of the guinea pigs they studied while in Latimer's (1951) study only 35 out of 100 male guinea pigs had an isthmus. Karski (1964) observing 120 Macacus rhesus and M. cynomolgus thyroids, failed to note any species differences in the gland. In 80% of the cases, the 2 lobes were united by a definite isthmus, in 5% the connection was nothing but a band of connective tissue, and in the remaining, the lobes were not connected. In 4.4%, accessory glands were found in the vicinity. The thyroid glands of the barasingha deer, Rucerus duvaucelli, is morphologically similar to that of the Virginia deer (Grafflin, 1939). In man, the two lateral lobes are connected by an isthmus (Bloom and Fawcett, 1962). Occasionally there is an irregular pyramidal lobe extending towards the thyroid cartilage. The literature reviewed does not reveal the absence of an isthmus in the humans.

Variations with Age

The thyroid gland also exhibits variability with age. In the Albino mouse (Smith, 1938), this is seen in the number and size of the follicles, differential amounts of colloid in them, vacuolation in the colloid and type of follicular epithelium. In the male rat, the weight of the

gland was found to increase throughout life (Leblond and Peets, 1951). New and old follicles continued to grow in size as a result of mitosis. In male dogs, Mulligan and Francis (1951) observed a direct correlation between the weight of the thyroid gland and age of the animal. In the common seal, Phoco vitulina, there was an increase of mean thyroidal follicular diameter from birth to adulthood (Harrison et al., 1962). The mean thyroidal weight at birth was 1.83 ± 0.13 gm. and in adult life 5.56 ± 0.30 gm. In man also such variations with age do occur. Hence Mortensen, Woolner and Bennett (1955) studying 1000 thyroids post mortem, observed that the weight of the thyroid glands from patients with clinically normal glands varied consistently with age. There was no significant variation related to residence of the donor-whether coming from goitrous or nongoitrous belt. Examination of 555 normal thyroid glands by Koch (1939) showed a mean weight of 2.5 gm. up to 5 years of age. At puberty (11 to 17 years) the mean weight was 12.5 gm., and at adulthood 18 gm. for males and 19 gm. for females. In this study, however, there was significant difference between glands from different thyroid areas. Jackson (1930) reported that young men did not have as many rounded follicles as the old men but rather more ovals; among the old the opposite being true. This author quoted Cooper as saying that thyroid follicles tended to be small in childhood, larger and to a degree irregular in adult life

and small again in old age. The weight of the thyroid gland at birth is subject to considerable variation depending principally on locality (Nolan, 1938). The average weight of the normal thyroid in the male stillborn was 2.1 gm. and in the female, 1.6 gm,, at from 1 to 30 days of age, the normal thyroid in the males weighed 1.9 gm. and in the females, 2.1 gm. According to this author, the weight varies with the geographic position but usually does not exceed 0.35 gm. per kg. body weight.

Histological Uniformity of Male and Female Thyroid.

We have so far seen how the mammalian thyroid gland is subject to variation specially in its weight and morphology. We have also noted the fact that the weight of the gland is also determined to a certain extent by the sex of the animal in question. However, one aspect of the gland seems to be constant in both sexes, namely the histology. The literature reviewed has failed to reveal any histological sexual dimorphism. While most of the experimental studies have dealt with only one sex to the exclusion of the other, it has been noted that most of the descriptive papers on thyroid histology have united both sexes into one study group. Some authors stated that histologically the male thyroid was similar or equivalent to the females. Ross (1938) found that in guinea pigs and rats there was no distinct sex difference in weight or histological structure of

the thyroids of normal males and females. Again in the guinea pig, Sugiyama and Sato (1954) observed that there was no noteworthy difference in thyroid patterns between males and females. One hundred human thyroid glands were studied by McFarland and Robson (1929). Four variations in these glands were taken into consideration, namely, the color of the glands, the texture, the lobulation of connective tissue partitions and the appearance of the colloid as to consistency, density and stainability. It was shown that these 4 variations had nothing to do with sex or race of the donor. The two authors concluded that the observed histological variations of the thyroid gland in man do not afford any information with respect to age, race or sex of the donor. Van Dyke (1945) could not find any essential differences in the thyroid glands of rams and ewes. The same thing can be said about the glands of males and females in the ground squirrel (Zalesky, 1935). Textbooks of Histology or Endocrinology do not brïng up any differences in the structure of male versus female thyroid glands in humans.

Quantitative Studies

Several methods have been devised for measurement of thyroid activity. The bulk of the histological methods is concerned with measurement of the height of the secretory cells - usually cuboidal or low columnar -, it being assumed that increased height is indicative of more abundant endo-

plasmic reticulum and subsequently greater secretory activity (Barrington, 1963). Other methods measure the protein-bound iodine content of the gland and still others measure the radioiodine turnover. Many histological methods have been devised and the trend towards objective, quantitative approaches is evident and encouraging. The elaboration of these methods took decades to achieve as shall be seen in the paragraphs that follow.

The histological changes which the thyroid gland manifests under the effect of thyrotropin released by the adenohypophysis were classified as to intensity by Junkman and Schoeller as early as 1932. Hence the first stage would consist of an increase of epithelial height; the second of uniformly increased cell height throughout the whole gland with beginning colloid vacuolation; and the third of complete disappearance of colloid with resultant empty lumina. Adams and Allen (1942) found that under the influence of the mouse adenohypophyseal thyrotropin, the thyroid gland would react in 4 ways: (1) by an increase in absolute and relative weight, (2) by a rise in the height of follicular epithelium, (3) by a change in consistency, vacuolation and staining reaction of the colloid, (4) by the presence of more follicles and mitoses. To assess mitosis, Gray and Loeb (1928) cut serial sections from the whole lobe. In every 10th or 11th section, the mitoses were counted, and on this basis the approximate number of

mitoses in the whole lobe was estimated. A similar procedure was followed by Rabinovitch (1928). The first epithelial height measurement is credited to Starr and Rawson (1936). These authors stained paraffin sections from formalin-fixed glands of female guinea pigs. Under oil immersion and by means of an ocular micrometer, the gland was systematically covered from end to end until 200 follicles had been studied. From each follicle the cell most representative for the average height was chosen and its height measured. From these measurements the mean cell height was calculated. Zalesky (1935) selected 50 follicles from central sections from the gland of the ground squirrel. An outline drawing was made on paper of the epithelial wall of each selected follicle by means of camera lucida, and the number of cells contained in this epithelial wall was noted. The total net area of magnified epithelium appearing in the drawing was measured by means of a Keuffel-Esser planimeter. The number of planimeter units contained in this area was divided by the total number of cells present in the corresponding follicles, and the area per cell thus obtained. The mean area per cell for each gland was computed and taken as basis of comparison between the thyroids chosen, and since only relative determinations were sought, it remained unnecessary to convert the planimeter units into metric units of area or to calculate the actual measurements from the magnification

employed in the process. Hypertrophy of the cell could thus be easily detected. In the Virginia deer, Grafflin (1942) used a method reminiscent of Starr and Rawson (1936). He counted, however, only 100 consecutive follicles which came into the field. Again he chose a cell of representative height from each follicle. Griesbach and Purves (1943) prepared stained sections passing through the center of the thyroid lobe of the guinea pig, placed them in the projector, and took measurements along a path parallel to the long diameter of the section. Well defined follicles were chosen. The measurements required from one animal were obtained from a single section of one of the thyroid lobes. The authors measured 50 follicles per section although, they stated 20 follicles per section for the tedious eye-piece micrometer method would have been enough. Cheymol, Delsol and Perrin (1955) studying 300 Wistar rats and 120 guinea pigs utilized the follicle diameter and the epithelial height methods. They studied 50 follicles traced by the micrometer. A frequency polygon was constructed by recording all heights on the absoissa and number of cells of each height on the ordinate. They concluded that epithelial height was a reliable criterion of gland variations. Follicular growth was assessed in the common seal by measuring the diameter of not less than 20 follicles (Harrison et al., 1962). Epithelial height was measured according to the Starr and Rawson method. While utilizing 200 follicles

per animal at the beginning, these authors eventually took only 50 to 100 follicles into consideration, a number later found to be adequate. Measurements were subsequently checked by microprojection at a known magnification against a calibrated scale. By measuring 100 follicles per rat and then averaging the diameters, Klein (1935) arrived at what he called a follicular index, which was an indication of the amount of colloid and which could be used as basis for comparing colloid content from gland to gland. Sugiyama (1954) studying the histogenesis of the guinea pig thyroid and the movement of cells, employed 20 fields at random in serial sections through the center of the lobe under high magnification to estimate the frequencies averaged per field of the occurrence of various thyroid cells. Sugiyama and Ohida (1954) devised a folliculogram to study thyroid follicular pattern in a puppy. Drawings were made by the use of an Abbe apparatus of each follicle previously marked by certain signs in the drawings. It was carefully traced serially in section with special emphasis on its follicular connections. Transverse sections of a follicle with a cavity or without were indicated as rectangles with a circle or without. On paper the rectangles were aligned serially and continuously in a craniocaudal axis. Accordingly, numerous horizontal paralleled bars of various lengths showed follicles of various sizes and from these the craniocaudal diameter of each follicle

and its cavity were approximately estimated. The foregoing method seems to be an elaboration on the method of Wilson (1927) where certain follicles would be followed through 60 serial sections and their spatial relationships preserved accurately by means of numerous wax and wire bridges. Lever (1948a; 1948b) noted that in the thyroid, when it was in one phase of activity, there existed in every follicle the same relation between outer and inner diameter, the number of cell and the epithelial height. Cordier and Herlent (1956) used the ratio of epithelium to colloid as an index of the effect of thyrotrophic hormone in the female guinea pig. This ratio increased in proportion to the logarithm of the dose.

More versatile methods of measuring different components of the thyroid gland have evolved in the last thirteen years. Tala (1952) used paper strips in performing the measurements. The limits of different histological components were drawn on them under the microprojector and the measuring proper was done afterwards on these paper strips. The procedure is said to be very accurate by that author. However, the method which has met with the most enthusiastic response is that developed by Uotila and Kannas (1952). With this method it has become possible to determine with speed and adequate precision, the percental proportions of colloid, epithelium and stroma in the thyroid gland. By this method a section is projected on

paper, a straight line is drawn in an arbitrary direction, and the segments of the line covered by colloid, epithelium and stroma are measured. The total length of each, expressed as a percental ratio of the whole length of the line, gives the relative amount of the component in question.

Lever and Vlijm (1955) used the method of Uotila and Kannas along with the colloid percentage method previously described, the d/n method whereby the ratio between the inner follicular diameter and the number of cells per follicle is deduced, and thyroid weight. All four methods gave essentially the same results for that particular experiment, the first method however, being the most informative.

Isotalo, Lofgren and Uotila (1955) eventually devised a scale-less ruler of acrylic resin on which the parts of the component to be measured were summed geometrically by drawing and the final result read directly from the picture thrown by the microprojector. By this alteration on the original Uotila and Kannas method, arithmetical calculations are said to be reduced.

Purpose of the Study

The aim of this study is to arrive at a better understanding of the mammalian thyroid histology by assessing the variations in follicular size from species to species along with variations in epithelium percentage. By employing one uniform method of evaluation for twelve

different mammals, it is hoped that the results thus obtained would contribute towards a more scientific approach to the ever growing interest in thyroid structure.

MATERIALS AND METHODS

The thyroid glands of twelve laboratory and domestic mammals were used in this study. As far as possible, only animals in apparent health were considered, taking care to include only sexually mature specimens and whenever possible, to have both sexes represented. An arbitrary minimum of five glands per species were studied. The sheep, goat, swine and cattle specimens were obtained from the local abattoir. Donkey specimens were obtained from the Government Veterinary Laboratory at Fanar, while the different laboratory mammals were secured through the facilities of the Medical School, A.U.B. The camel thyroids were obtained from the abattoir in Aleppo, Syria. As used in this study, the mice were 9 months old, the rats and guinea pigs, 12 months. For purposes of comparison, three human specimens were included in the study. These came from autopsies performed at the Department of Pathology on young adult patients who had died in the American University Hospital.

The preparation of specimens for microscopic study and evaluation was as follows:

Preliminary Handling of Tissues

The thyroid glands were removed as soon as possible after death to minimize the post-mortem changes. The glands were labeled, freed by means of scalpel and scissors from the adherent connective tissue and fixed *in toto* in 10% formalin which according to Holmgren and Nilsonne (1948) does not cause tissue shrinkage or swelling. After a period of at least one day in the fixative, the thyroids of the mice, rats, guinea pigs, cats and rabbits were removed from the solution and one lobe from each gland taken for subsequent processing *in toto*. For the relatively larger glands of the other mammals the following was done: one lobe was removed from each gland, bisected along its longest axis thus obtaining a representative section as advocated by Griesbach and Purves (1943). Such a section is called a 50% section by Uotila and Kannas (1952). One of the two longitudinal sections was subsequently taken for processing. Since the circular metal tissue receptacles have a diameter of only 36 mm., it was necessary to cut in half and sometimes in triple those 50% sections just mentioned so that they could be accomodated on the receptacles. The relation of these subsections to each other however, could be easily reconstructed.

Histological Tissue Processing

The tissue, in their receptacles, were processed by an automatic tissue processor[1]. The procedure was as follows:

1. Washing in distilled water, 30 min.
2. Dehydration
   a. 70% ethyl alcohol, 60 min.
   b. 95% ethyl alcohol, 60 min.
   c. 95% ethyl alcohol, 60 min.
   d. 100% ethyl alcohol, 60 min.
   e. 100% ethyl alcohol, 60 min.
   f. 100% ethyl alcohol, 60 min.
3. Clearing
   a. Xylol I, 60 min.
   b. Xylol II, 60 min.
4. Infiltration[2]
   a. Paraffin I[3], 60 min.
   b. Paraffin II, 60 min.

After infiltration was completed, the tissues were taken out of the automatic tissue processor and embedded in melted paraffin of the same grade as used in infiltration.

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1. Manufactured by the Technicon Co., Chauncey, New York.
2. Temperature of the paraffin bath was 65° C.
3. Hard paraffin, M.p. 56 to 58 deg. C.

Tissue Sectioning

The paraffin blocks were now transfered onto the block holder of a Reichert sliding-type microtome. Sections 6 micra thick were cut and affixed in the usual manner to 3 x 1 inch slides.

Tissue Staining

The staining was done with hematoxylin and eosin, a procedure found useful for thyroid studies by Uotila and Kannas (1952). The procedure was as follows:

1. Deparaffinization in 2 changes of Xylol.
2. Hydration in 6 descending grades of ethyl alcohol.
3. Washing in running tap water, 5 min.
4. Nuclear staining in Harris' hematoxylin, 15 min.
5. Differentiation in 2% acid alcohol (2% HCl in 70% ethyl alcohol) in the form of 3 dips.
6. Washing in running tap water where the alkalinity of the water would turn the nuclei blue. The sections were kept in running water for at least 10 min.
7. Counterstaining in a 0.25% aqueous solution of eosin for half a minute.
8. A brief washing in running water to remove excess eosin.

9. Dehydration in 2 changes of 95% and 3 changes of 100% ethyl alcohol.
10. Clearing in 3 changes of Xylol.
11. Mounting in a synthetic resin[4] and covering with micro cover slips.

The Optical Arrangement

In order to assess the size of the follicles of the thyroid glands in the different mammalian species and to evaluate the epithelio-follicular ratio, the Bausch & Lomb class microprojector was tried. This was the only available instrument which corresponded in construction to that used by Uotila and Kannas (1952) where the source of light was a high-pressure mercury lamp and an impulse of 900 volts. The Bausch & Lomb microprojector however, did not produce an image of sufficient clarity and resolution. Another attempt was made to use the Zeiss-Winkel micro-projector which utilized a carbon arc to project a slide on a screen. In here, the picture was fairly bright but the instrument needed continuous adjustments and the carbon rods constant replacing so that the efforts expended in project-ing one slide were not compatible with the aim of this study where a multitude of slides had to be assessed and reassessed. Hence it was decided to use the camera lucida.

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4. Manufactured by the Hartman-Leddon Co., Philadelphia.

The camera lucida approach for studying thyroid follicular patterns has been used before. Sugiyama and Ohida (1954) used an Abbe apparatus to draw outlines of follicles at a magnification of 588 fold of the thyroids of guinea pigs. Zalesky (1935) utilized the camera lucida to draw thyroid follicular outlines of the 13-lined ground squirrel from which he could later compute the relative area occupied by a particular cell in a particular follicle.

In this study, the camera lucida used was Zeiss manufactured. It was attached to an American Optical monocular microscope with a 10x eyepiece and 3 objectives 10x, 12x, and 43x respectively. The source of light in the microscope was the ordinary microscope illuminating bulb of 100 watts. The field of drawing was illuminated by the ordinary table lamp of 60 watts.

Experimental Procedure

Before starting to outline the follicle along its outer periphery and along the inner epithelial boundary which encroaches on the colloid, a suitable magnification was selected. Hence for thyroids with relatively small follicular size such as in mice, rats, rabbits and guinea pigs high power was used. In the larger mammals with large thyroid follicular size, medium and sometimes low power were used. In every case however, the magnitude of magnification was recorded on the drawing paper itself.

The fields to be drawn on paper with the aid of the camera lucida were selected so as to include the central part as well as the periphery of the thyroid lobe under study. By so doing, the drawings would include the follicles of different sizes which occur in the same gland as mentioned previously in the Introduction. Fields with artifacts as well as those with tangentially cut follicles were avoided.

The total number of follicles drawn from each thyroid lobe was uniformly in the vicinity of 50. Such a number was found to be adequate in the guinea pig by Griesbach and Purves (1943). These authors stated that studying even only 20 follicles per animal would have been enough. In a survey of 300 rats and 120 guinea pigs, Cheymol, Delsol and Perrin (1955) also considered 50 follicles per animal. In the ground squirrel, 50 follicles per animal were selected by Zalesky (1935). In the common seal, Harrison et al.(1962) assessed follicular diameter from 20 follicles per animal and epithelial height from 50 per animal.

The fields containing the follicles were drawn directly on graph paper, fool-scap size divided into 1 mm. sections. After the necessary drawings had been made from the thyroid lobe under study, a stage micrometer calibrated in units of 10 and 100 micra was projected and drawn on the same graph paper and in a fashion comparable to the

follicular drawings. Now it was possible to equate units of the graph paper to the units of the stage micrometer. Such an equation will hold true in any case where the same magnification is used. If, however, a different magnification is subsequently used, a new equation will have to be computed.

Measuring from the Graph Paper

Measurements of follicular size were done by counting the maximum number of horizontal graph units occupied by each follicle. In order to transform graph units into actual units of measurements, the number of graph units was multiplied by the factor derived from equating stage micrometer units to graph paper units as discussed previously. This gave the horizontal measurement of the follicle in micron units. For every animal the average follicular dimension of 50 follicles was computed.

In order to derive the epithelio-follicular ratio for a particular animal, a graph line was followed through a drawn field with its follicles and epithelium. Recordings were made of the number of segments of that line occupied by epithelium alone and by the follicles as a whole. The number of segments occupied by the epithelium divided by the number of segments occupied by the follicles represented the epithelio-follicular ratio for the animal under study. Such a ratio when multiplied by hundred would give the

epithelium percentage. Several such measurements were made for every animal in order to improve the accuracy of the ratio.

RESULTS

Measurements of Follicular Size

The results of the measurements of the greatest horizontal follicular diameter of the thyroid gland in the twelve mammalian species studied as well as in man are presented in Table 1. From every species the thyroids of 5 animals representing both sexes were assessed. The Table shows the difference range among the 5 animals belonging to one species and also the average of the follicular dimensions in those animals. In the case of the human thyroids, the data represents the evaluation of only three specimens, those being the only ones available and meeting the criteria of this study.

From a close scrutiny of the results, the following are noted:

1. There exists among the different species studied in Class Mammalia an appreciable difference in thyroid follicular size. Between man, with an average follicular dimension of 198.1μ and the mouse with an average follicular dimension of 045.7μ , there were gradations in follicular size among the different mammals. As for the results of Yagizawa in a Japanese article cited by Begg, McGirr and Munro (1965), they cannot be compared with those obtained in this study because

TABLE 1

THE RANGE AND AVERAGE IN MICRON UNITS OF THE FOLLICULAR SIZE IN THE VARIOUS MAMMALIAN SPECIES[5]

Animal	Range	Average
Mouse	041.8 - 052.6	045.7
Rat	055.9 - 066.8	059.7
Cat	056.0 - 066.4	060.8
Guinea pig	062.6 - 090.5	073.2
Rabbit	071.0 - 103.5	081.6
Sheep	082.2 - 097.3	087.9
Donkey	084.8 - 095.6	089.6
Goat	089.7 - 102.8	094.6
Dog	082.6 - 107.3	096.5
Cattle	169.1 - 182.0	172.9
Pig	159.3 - 211.5	179.6
Camel	155.3 - 240.7	186.7
Man	178.4 - 221.5	198.1

5. Photomicrographs of the thryoid glands of the various mammalian species appear in PLATES I-IV.

Yagizawa took only the 50 largest follicles of the glands he studied in the rat, rabbit, cat, dog, pig and cow specimens. On the other hand, the findings of Klein (1935) on the rat give a value much higher than that obtained in this study. This author did not mention the region of the thyroid from which he measured the 100 follicles, a fact of important bearing on the subject. The present findings on the human follicular size, however, fall within the range calculated by Wilson (1927). It is important to note here, that this author chose a "typical" area from each gland for study. Jackson (1930) on the other hand, obtained values for man smaller than those in this study. This can be explained by the fact that he included in his materail specimens from men over 70 years. Jackson cited Cooper as saying that thyroid follicles tended to become small in old age. Hence his low values can be explained on this basis.

2. The range of variation in thyroid follicular size varies in different species. Thus while there was a difference of only 10.4μ between the two extremes in the cat, this difference

amounted to 24.7μ in the dog and to 85.4μ in the camel.

3. Within reasonable limits, the claims of Begg, Mcgirr and Munro (1965), based on a table by Yagizawa that mammals with large body size tend to have a greater average follicular size compared with smaller mammals are confirmed in this investigation.

The findings in this study fall within the framework of the above mentioned generalization. Thus the mouse has the smallest body and also smallest follicular size, and the camel is the animal with the largest body and greatest follicular size, with the exception of man. However, deviations from the above are noted. The cat was found to have a smaller average follicle than the guinea pig, the dog a larger follicle than the goat, sheep and donkey; and as mentioned, man's average follicular size is larger than that of the camel.

Measurements of Epithelium Percentage

The results of measurements of the epithelium percentage in the twelve mammalian species studied are presented in Table 2, together with the measurements for man. The same animals as utilized for follicular measurements were used here for calculation of epithelium percentage. While the different species in Table 1 are arranged in order of

TABLE 2

THE RANGE AND AVERAGE OF THE EPITHELIUM PERCENTAGE IN THE VARIOUS MAMMALIAN SPECIES

Animal	Range	Average
Mouse	24.1% - 31.5%	28.6%
Rat	18.9% - 30.5%	24.9%
Sheep	12.0% - 19.4%	17.5%
Cattle	12.5% - 23.3%	17.3%
Cat	14.5% - 24.4%	15.6%
Guinea Pig	13.6% - 18.4%	15.5%
Rabbit	12.5% - 17.9%	15.3%
Pig	09.8% - 17.6%	14.3%
Donkey	13.2% - 14.3%	13.6%
Goat	12.1% - 15.2%	13.2%
Dog	10.7% - 15.7%	12.8%
Camel	09.0% - 10.9%	10.0%
Man	06.4% - 09.7%	08.4%

increasing follicular size, in Table 2 the arrangement is in order of decreasing epithelium percentage. By so doing, any correspondence between increasing follicular size and decreasing epithelium percentage would be easier to note.

Upon inspection of Table 2 the following are noted:

1. There exists among the different species studied in Class Mammalia a difference in epithelium percentage in the same way as a difference in follicular size.

2. Between man, with an average epithelium percentage of 08.4% and the mouse, with an average of 28.6%, there are gradations in epithelium percentage.

3. The epithelium percentage is seen to decrease roughly with increasing follicular, size as can be noted from Table 2. Variations from this are, however, observed. For example, the dog has a lower epithelium percentage than all the mammals studied except the camel and man. Similarly, the rabbit has a lower epithelium percentage than both sheep and cattle.

4. Excluding the mouse and the rat at the top of the Table, and the camel and man at the bottom, it is seen that all the remaining 9 species have an epithelium percentage that lies between 12.8% and 17.5%, a rather close range.

Follicular Size in the Male VS. Female

Although not within the main scope of this study, an attempt was nevertheless made to note any differences in thyroid follicular size between the males and females of the various mammalian species. Table 3 shows the individual measurements for rat, cat, donkey and camel specimens. These species were selected at random to represent the orders Rodentia, Carnivora, Perissodactyla and Artiodactyla, respectively. It is seen that within any species, no consistent difference in the follicular size between the thyroids of males and females is found. Similar observations are noted in all the species studied. These findings are in line with those of Zalesky (1935) on the squirrel, Ross (1938) on guinea pigs and rats, Van Dyke (1945) on sheep and Sugiyama and Sato (1954) again on the guinea pig, about the similarity in structure of the thyroid glands of both sexes within one species.

Variations in Follicular Size from the Periphery to the Center of the Gland

From the material available in this study it is possible to extend the observations of a decrease in follicular size from the periphery towards the center of the thyroid lobe made by Yocom and Huestis (1928) on the deer mouse, Knigge (1957) on the hamster and Tomonari (1959) on the cow, pig, horse, cat, dog, rabbit, guinea pig and rat,

TABLE 3

AVERAGE FOLLICULAR SIZE IN MICRON UNITS OF THE MALE AND FEMALE THYROID IN FOUR MAMMALIAN SPECIES

Rat No.	Sex	Average Size
1	M	057.5
2	M	059.0
3	F	066.8
4	F	055.9
5	F	059.4
Overall average		059.7
Cat No.		
1	F	059.2
2	F	056.0
3	M	061.2
4	F	066.4
5	F	061.2
Overall average		060.8
Donkey No.		
1	M	095.6
2	F	091.8
3	M	084.8
4	M	086.3
5	F	098.8
Overall average		089.6
Cattle No.		
1	M	182.0
2	M	169.1
3	M	169.1
4	F	168.4
5	F	176.1
Overall average		172.9

to species not covered by their reports. By observing the slides prepared from all the mammalian species with the low power of the microscope, it was noted that all the species without exception showed this change in follicular size. This phenomenon in the rat is depicted in PLATE IV, Fig. B.

DISCUSSION

The results obtained in this study lend further evidence to the generally accepted indications that the thyroid gland, although serving one main function in all the vertebrate groups and consequently in Class Mammalia, namely the regulation of the metabolic rate, is nevertheless subject to variations from species to species both in gross anatomy and histology.

The main contribution of this investigation is that it brought into perspective two aspects of the comparative microstructure of the thyroid gland, namely, follicular size and epithelium percentage of mammals which had not been the subject of investigation from the approach mentioned.

This investigation was primarily a general survey of some of the common mammals around us, in the field and in the laboratory. Further studies, using a larger number of animals per species might perhaps give the data obtained in this study a more solid base. Another approach would be to take one species or two and follow the change in epithelium percentage (or height) and follicular size from birth to senility. This may explain to a certain extent, the variations reported by different investigators. Such a study has been conducted on the seal, Phoca vitulina, by Harrison et al. (1962).

It is however, useful to remember that the thyroid is avvery labile gland. It is not uncommon to find in one follicle both columnar and cuboidal cells. The arrangement and the height of the secretory epithelium of the follicle are continuously in a state of flux (Bloom and Fawcett, 1962). The age, the sex, the diet and certain environmental and pathological conditions can all influence the follicular epithelium. This should prompt us to accept all histometric evaluations with an open mind to their limitations (Barrington, 1963). In this respect it is interesting to note the findings of Nadler, Leblond and Bogoroch (1954), who reported that in the rat the small follicles were more active in accumulating iodine per unit volume than the large ones. They attributed this to the fact that in relation to their size, the small follicles had a greater surface and cell number.

It is also pertinent to recall that the thyroid gland functions with the hypophysis in a state of mutual excitation and inhibition, governed by the well known "feed-back mechanism". The height and the activity of the follicular epithelium are also affected by the physiological demands for the thyroid hormone, an adequate supply of dietary iodine being indispensable for the maintenance of normal thyroid activity. Hence any investigations, specially those utilizing grazing animals such as cattle, sheep, goat, swine and camel should be conducted with

caution before any substantial generalizations are made. The type of food the animal consumes is also important. Leaves and seeds of Brassica plants are reported to contain goitrogenic substances which have been proved experimentally to cause hyperplasia and hypertrophy in the thyroid gland (Barrington, 1963). According to Latimer (1951), the amount of the endocrine secretion is so minute in reference to the weight of the gland, that total gland weight might not be an indication of the amount of the secretion produced. He cites the example of the enlarged thyroid which may be found in either hyper- or hypothyroidism.

It should not be surprising therefore, to see eventually the emergence of more correlative studies combining histological, nutritional and biochemical approaches.

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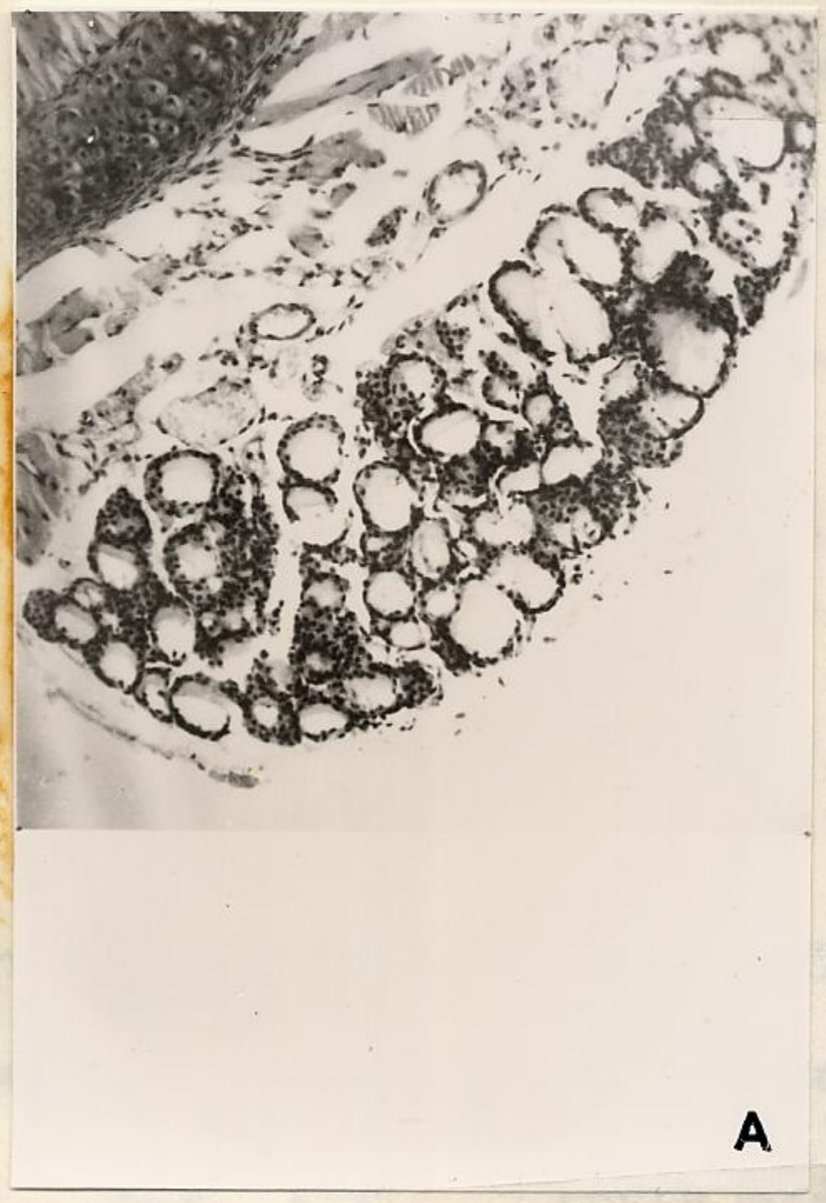
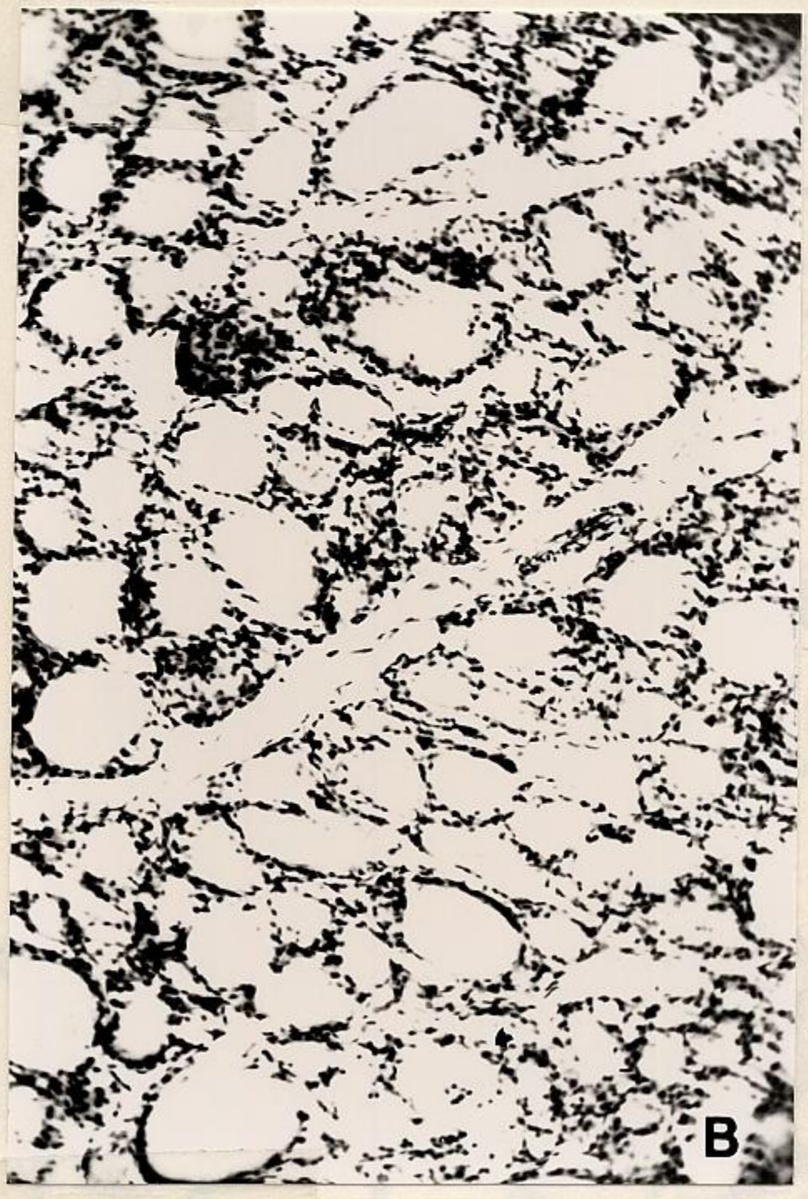
PLATE I

Figs. A, B, C and D: The thyroid glands of the mouse, rat, cat and guinea pig respectively. All 240X.

PLATE I





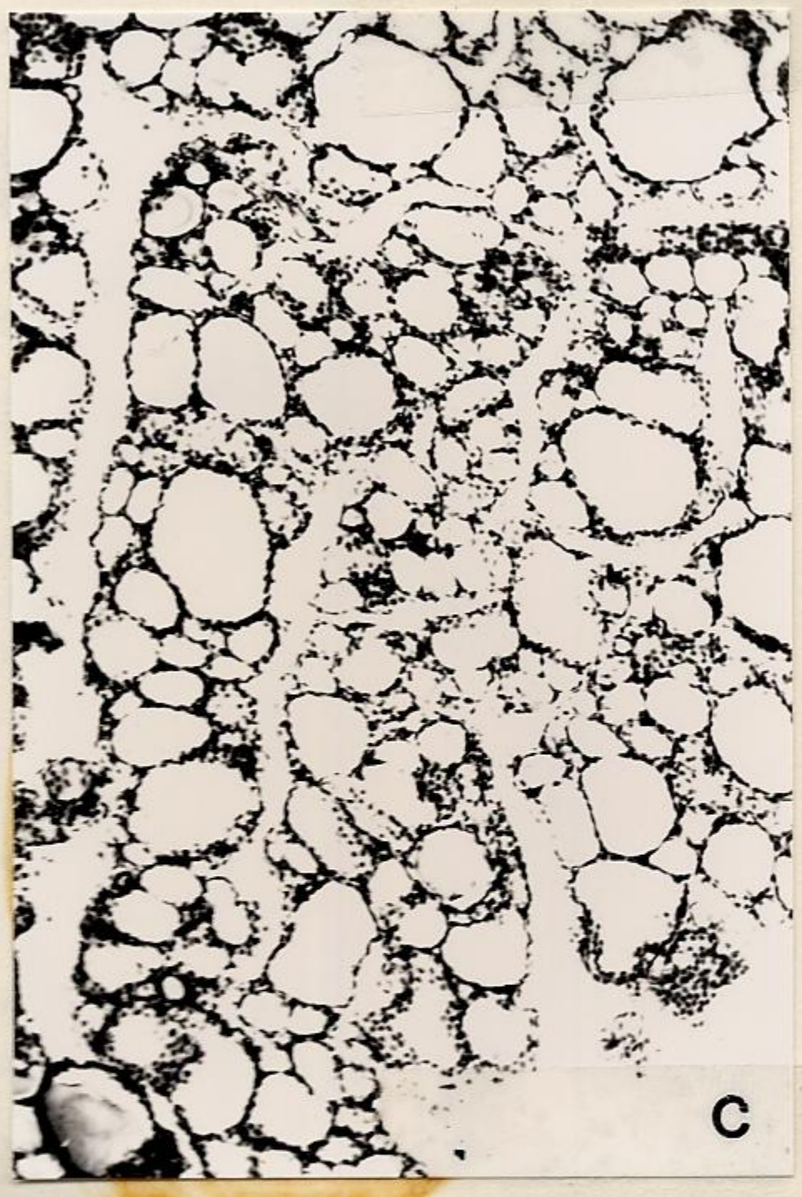


PLATE II

Figs. A, B, C and D: The thyroid gland of the rabbit, sheep, donkey and goat respectively. All 240X.

PLATE II

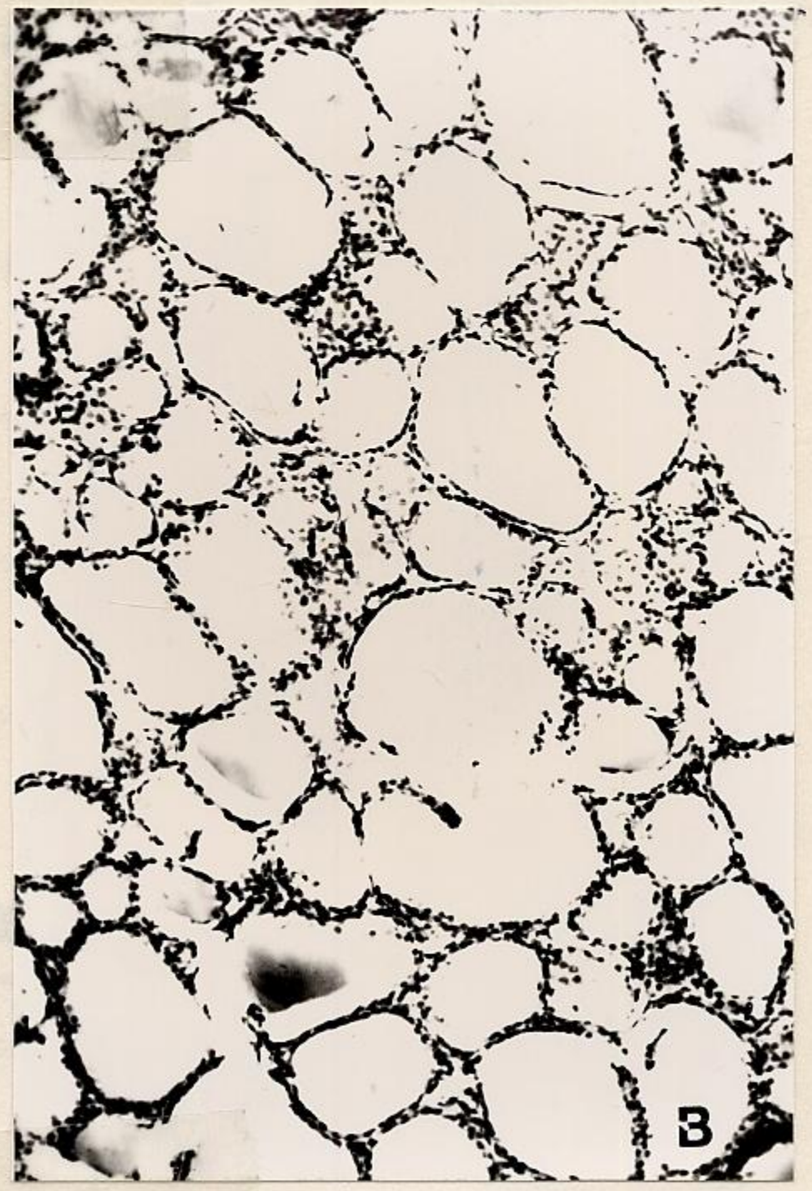


PLATE III

Figs. A, B, C and D: The thyroid glands of the dog, cattle, pig and camel respectively. All 240X.

PLATE III

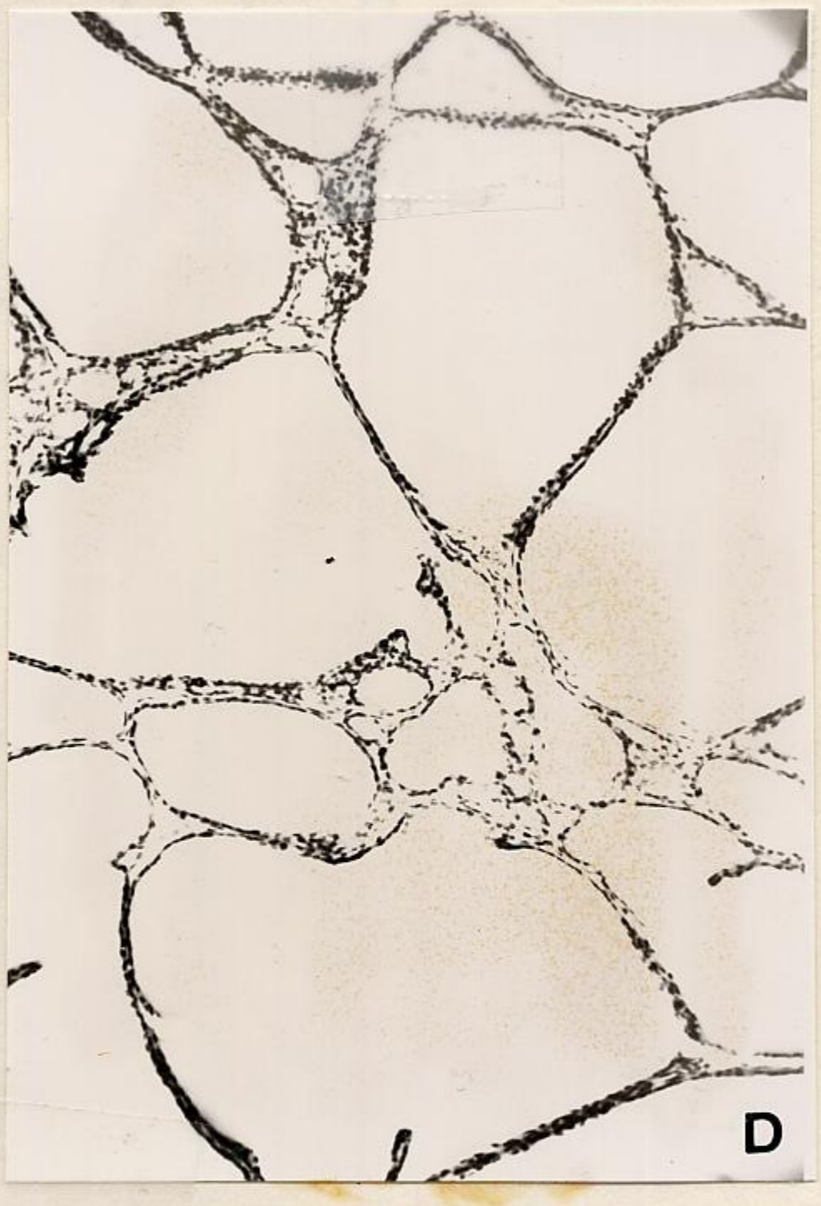
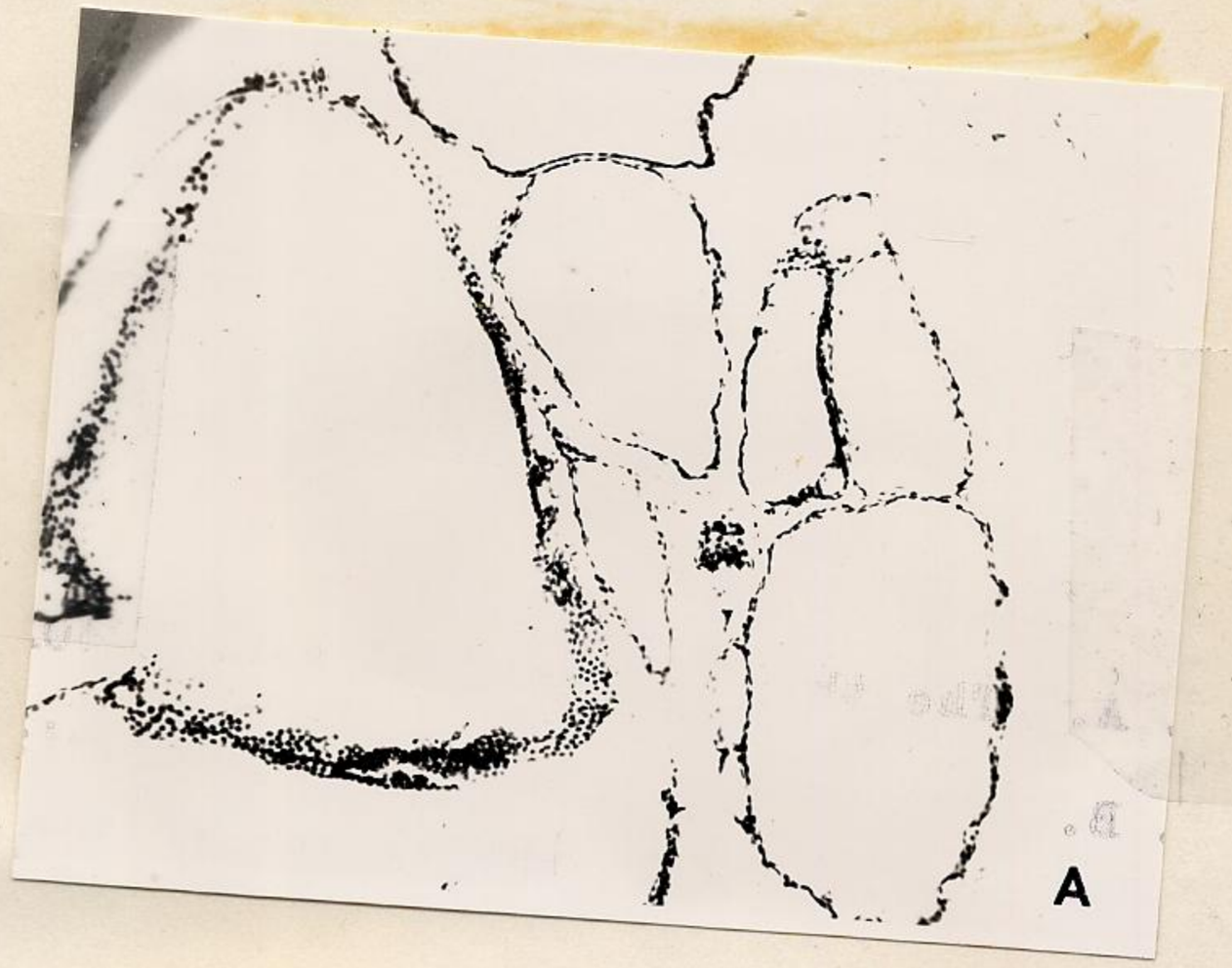


PLATE IV

Fig. A. The thyroid gland of man. 240X.

Fig. B. The variation in follicular size from the periphery to the center of the thyroid gland of the rat. 240X.

PLATE IV



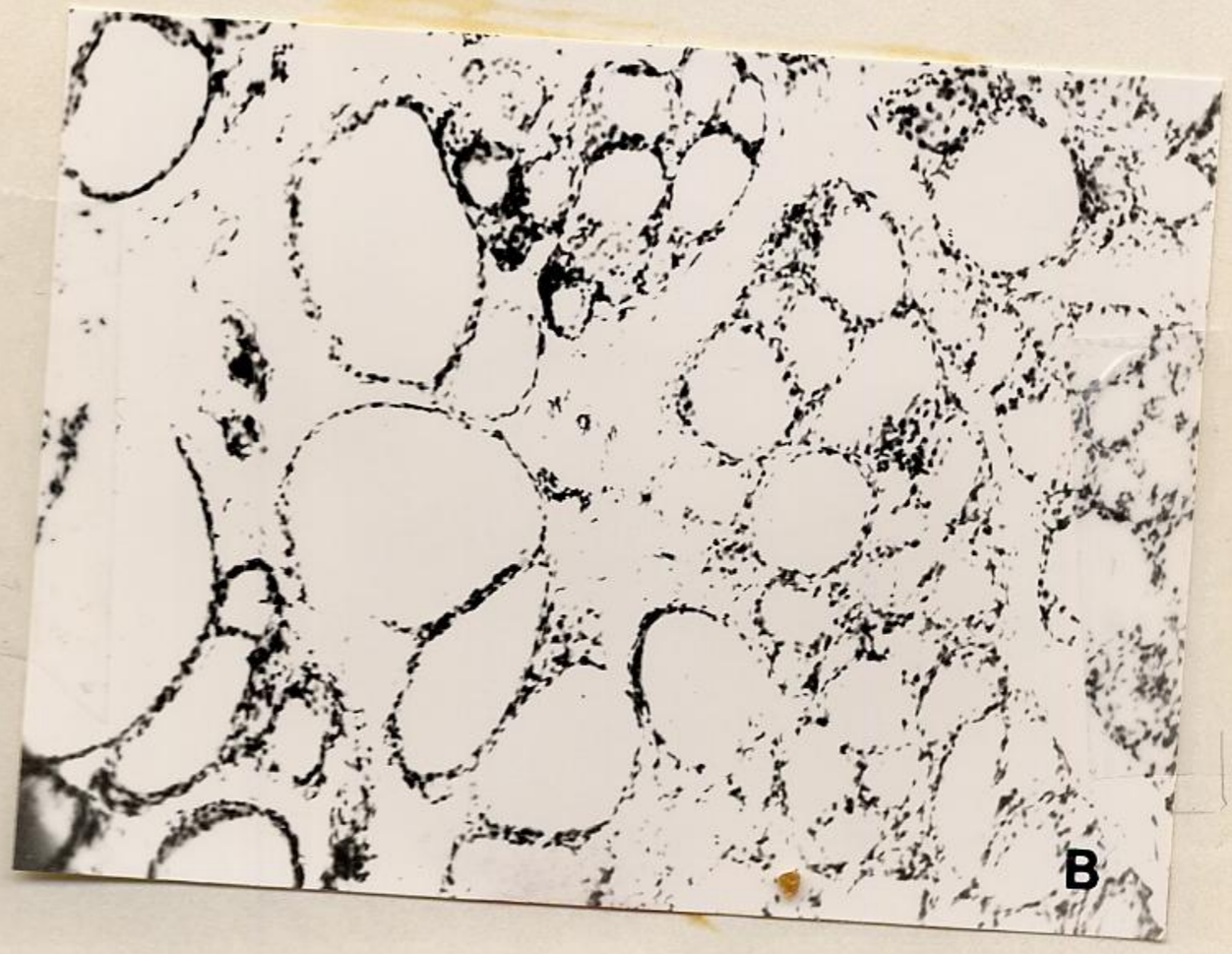


PLATE IV