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DEVELOPMENT
of the
THYROID GLAND
in the
CHICK EMBRYO

by

Marie Lockett Hopkins

Presented in partial fulfillment of the
requirement for the degree of
Master of Arts.

State University of Montana

1933

Approved:

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TABLE OF CONTENTS

	Page
I. Introduction	1
II. Aims of Present Study	2
III. Summary of Previous Investigations of the Thyroid Gland in Chickens	2
IV. Summary of Previous Investigations of the Thyroid Gland in Animals Other Than the Chicken	6
V. Materials and Methods	10
VI. Observations and Experimental Results . .	14
VII. Summary and Conclusions	29
VIII. Bibliography	31
IX. List of Footnotes	33
X. Plates and Explanations of Figures	38

I INTRODUCTION

Due to certain abnormal conditions of health resulting from aberrations in thyroid function, the effect of this gland upon the organism as a whole is rather well understood. In this branch of endocrinology, however, pure science has not kept pace with applied science. For example, although the active physiological principle, thyroxin, was isolated by Kendall in 1919, the exact relation, either chemically or functionally of this substance to the colloid which fills the thyroid follicles, is still an unsettled problem. Moreover, many facts regarding the histological development of the gland, as well as the details of colloid elaboration and release, remain controversial or obscure.

This unsettled state of information regarding the relation of histology to physiology in the thyroid, has not been due to lack of research. In fact since about 1850 the scientific literature contains records of countless extensive investigations in this field. These studies, dealing with various aspects of the condition of the thyroid in the vertebrate classes, are not in accord with respect to the following points: (1) number and development of embryological anlagen; (2) relation of colloid accumulation to gross morphology; (3) exact method of elaboration and storage of secretory products; (4) normal method of

rendering the secretory products available to the organism.

There is a general consensus of opinion among investigators that among the higher vertebrates, the structural plan of the gland is fundamentally similar. Phylogenetically speaking, the gland is said by Vincent (1925) to be homologous to the endostyle organs of the primitive chordates.

II AIMS OF THE PRESENT STUDY

The present study¹ was undertaken with the following aims in view: (1) to bring together and summarize the results of the few studies which have been made by previous investigators of the thyroid gland in chickens; (2) to make supplementary contributions to the morphogenesis of the gland, especially from the standpoint of the cell types and cell inclusions which are present; (3) to test Minot's law of cell differentiation by investigating the C/W ratios² of several developmental stages in the thyroid.

III SUMMARY OF PREVIOUS INVESTIGATIONS OF THE THYROID GLAND IN CHICKENS

According to Marine (1932), "Thomas Wharton (1659) first accurately described the thyroid and gave it its present name".

Work on the aspects of its embryological development in chickens did not begin until Remak (1835) described its

development from an unpaired, median, ventral invagination of the pharyngeal epithelium near the first gill cleft. He also described its division into a T-shaped mass and its migration to the thoracic location it occupies in the adult.

Steida (1881) claimed that the gland had a lateral as well as a median origin.

Hall (1881), in his work with chick embryos, sustained the original conclusion of Hensen, as did also Muller (1871). Marine (1932) says that subsequent investigations have established the correctness of their concept regarding the development of the chick thyroid from a median ventral anlage only.

One of the classic investigations of the nineteenth century was carried on by Vordun (1898). A translation of his summary given in Paris in 1898 is as follows:

"Derived like that of mammals from an invagination in the anterior part of the pharynx near the second branchial arch, the outline of the median thyroid of chickens divides at the 172nd hour into two large lobes which migrate laterally to either side of the trachea toward the derivatives of the third gill slit with which they become connected. They assume from then on their definite position, and in the adult one finds them a little below the bifurcation of the brachio-cephalic trunks, between the internal carotid arteries and the jugular veins, which are behind and to the side."

In the same article Vordun advances the view that the "lateral thyroid" of other investigators represents a complex ensemble most of which is derived from the fifth branchial

pouch. That concept makes the so-called "lateral thyroid" of the early investigators homologous with the "ultimobranchial bodies" of current scientific literature.

Hertwig (1912), seems to still cling to the idea of two lateral as well as a median anlage for the thyroid. In Hertwig's compendium of 1906, however, Maurer (1906) voices the idea of a single median anlage and shares Verdun's views of the lateral anlage representing the ultimobranchial bodies from the fifth visceral pouch.

Lillie (1919) contributes several interesting observations of the early development of the chick thyroid.

The next contributions to the literature on the subject are three recent papers from the laboratories of the Universities of Oregon, Tokyo, and Iowa respectively. In the first of these Bradway (1930) gives a comprehensive account of the morphogenesis of the gland in embryonic chicks. Her results are summarized in Table I, page 5.

In the second recent contribution Yoshikawa (1930) working along lines similar to those of Bradway supplemented the knowledge of the more detailed morphology. His results are also listed in Table I, page 5.

In the third recent contribution Sun (1932) gives a brief summary of some of the morphogenetic stages. However the chief aim of Sun's investigation was to establish the percentage of iodine present in various stages of development

1990

Comparative Results of Previous Investigations of Morphogenesis of Celiac Thyroid

	Age	: in hours	: Ago	: in days	: Ago	: in days	: when plates	: when	: Age	: Nature	: Method of
	:	: when thy-	: when gland	: when	: when plates	: when	: of	: of	: of	: of	: of
	:	: thyroid	: rold an-	: becomes	: gland	: of epitho-	: first	: first	: first	: first	: first
	:	: calage	: tage loses	: completely	: acquires	: lial colls	: colloid	: colloid	: colloid	: colloid	: follicles
	:	: first ap-	: pharyngeal	: paired	: non-fol-	: separated	: appears	:	:	:	:
	:	: tears	: connection:	:	: lissular	: by cirou-	:	:	:	:	:
	:	:	:	:	: cavities	: labory	:	:	:	:	:
	:	:	:	:	:	: spaces de-	:	:	:	:	:
	:	:	:	:	:	: cone dis-	:	:	:	:	:
	:	:	:	:	:	: tipotly	:	:	:	:	:
	:	:	:	:	:	: visible	:	:	:	:	:
Verdan	:	:	:	172	:	:	:	:	:	:	:
Lillie	33	96	168	:	:	:	:	:	:	:	:
Bradway	54	120	120	6	8	11	Chromo- phobe	prolifer- tion of follicle cells. Very rarely by budding or fission	Interfol- licular epithelial cells form new folli- cles	:	:
Yoshikawa	42	72	120	3.25	3.5 - 11	11.5	Chromo- phobe	Interfol- licular epithelial cells form new folli- cles	:	:	:
Sum	--	--	--	--	6	9	:	:	:	:	:

of the embryonic chick thyroid. He found that the first recognizable percentage appeared at about 10 days. After that there was a general trend of increase up to a maximum of 1.31% of iodine in the fresh gland at six hours after hatching. Developmental stages described by Sun are listed in Table I, page 5.

IV SUMMARY OF INVESTIGATIONS CONCERNING THE ANATOMY AND PHYSIOLOGY OF THE THYROID GLAND IN ANIMALS OTHER THAN CHICKENS.

It is beyond the scope of this study to cover thoroughly the comparative embryology, anatomy, and physiology of the thyroid gland in the various other vertebrates. It does seem necessary, however, before going further, to emphasize some facts and ideas about the relation of thyroid structure to function.

Beneley (1916) studying the thyroid glands of opossums in an effort to settle the question regarding the normal mode of secretion, concludes that the thyroid,

"prepares and secretes into the vascular channels of the gland a secretion, and that this secretion is formed in the outer poles of the cell, and excreted from it directly under normal conditions of functioning without passing by the indirect route through the follicular cavity."

He also claims,

"that in addition to the direct mode of secretion there is an indirect mode, which consists in the condensation of the secretion into the form of droplets having a high content of solids, and the

extrusion of these droplets into the follicular cavity. These droplets are formed in the same zone as that in which the primary or direct secretion is formed and it is probable that they are formed at the expense of the latter."

Cowdry (1922) on the basis of certain reversals in polarity of the Golgi apparatus in the thyroid, comes to the same conclusion as Bensley, namely that the gland discharges sometimes into the lumen, sometimes into the blood.

Bowen (1929) calls attention to the fact that the position of the Golgi apparatus is not an infallible sign of secretory polarity, but admits it is in some way related to the secretory process.

Uhlenhuth, (1925, 1928) has published a series of articles dealing with the morphology and physiology of the thyroid gland in various salamanders. Since metamorphosis in these animals is seemingly dependent upon the proper functioning of the thyroid gland, he reasons that the condition of the gland in animals beginning metamorphosis should represent the height of functional activity. He claims that at this stage there are in the follicular cells two definite morphological evidences of heightened functional activity. The first of these evidences is the presence of large distinctly visible secretion vacuoles³ of chromophobe colloid in the cells and edges of the follicles. The second of these evidences is the transformation of the follicular cells into high columnar-type cells having conspicuous

gatherings of secretion granules at their apical poles. Uhlenhuth (1925) further claims that in *Amblystoma opacum* as well as in man and other mammals, the two secretory products of the gland (the chromophobe and chromophile colloid) are always elaborated in and excreted from the same cells at the same time and into the same place, although in varying amounts.

Two English investigators, Williamson and Pearce, after an extensive survey of the human thyroid have advanced a new conception of its structural plan. They claim that the lobules of the gland are subdivided by strands of fibro-elastic tissue into well defined "gland units" which are, rather than the follicles, the functional units of the gland. To describe a gland unit briefly, one may say that it is really a lymph sinusoid enclosed by the fibroelastic tissue mentioned above. Inside this capsule one or more cylindrical columns of epithelial cells float. Usually the columns are greatly coiled. Up and down these columns, colloid accumulates at discrete intervals to form at first "vesicles of colloid", and later "lacunae". The latter, it is said, represent the active secretory stage of the gland. Sometimes the process of lacunation goes on so profusely that the successive lacunae along the center of the columns fuse, thus giving the effect of a fluid-filled tube floating in the lymph of the gland unit. Blood and lymph vessels of

course enter and leave the capsule of the latter and form extensive anastomoses over the surface of the epithelial columns.

One other rather revolutionary idea advanced by Williamson and Pearce is in connection with the follicular structure. They claim that a syncytial condition exists within each follicle, and that, between the accumulated colloid and the nuclei in the epithelial wall there is a system of intracellular tubules. These tubules, judging from the illustrative photomicrographs accompanying the article, are thought to lead into one somewhat larger tubule which drains the follicle. Just where this drainage tubule is supposed to conduct the follicular contents, and what relation the colloid of the follicular stage bears to the lacune is not made clear by these authors.

Uhlenhuth, (1928) although he disagrees with Williamson and Pearce on many points, admits that in *amblystoma opacum* there is a similarity in the morphology of the entire thyroid gland to one gland unit as described by the latter.

Zeschel, (1931) who has published several articles about the morphology of the normal and regenerating gland in dogs, agrees that there is confluence and coalescence of follicles when the gland is in a high state of activity. Also, on the basis of one hundred cells measured for each stage, he concludes that there are quite distinct morphological differences

between the interfollicular epithelial cells and the follicle cells. The former, it is said, are larger and have relatively smaller nuclei which take a deeper stain. He thinks the functions of these interfollicular cells are to help form new follicles, produce colloid, and start follicular destruction.

V MATERIALS AND METHODS

The first step in the present investigation was the making of slides of chick embryos from three days to hatching. Up to and including five days the entire embryo was imbedded in paraffin. From six through eight days only the lower neck region, and, from nine days on, only the excised glands were imbedded.

The fixatives and staining combinations employed are listed in Table II, page 11.

Most of the sections used in the making of my slides were cut at 4-6 μ , but some of the slides made by Miss Kimball¹ were cut at 10 μ . The total number of finished slides used in this investigation was 226, and represented serial sections from 64 chick embryos.

In making the comparative C/N ratio study of three different developmental stages, 1,000 cells from each stage, 3, 12, and 19 days respectively, were drawn to scale and measured. The drawings were made with the aid of a camera

-11-

Fixative	Stain Used	Counter Stain Used	Special Purpose
Serum without Acid	Iron (Alum) Hematoxylin	Eosin	
"	"	"	
"	"	Ethyl Green	
"	Gallory's Connective Tissue Stain		Stain Connective Tissue
Zenker	Iron (Alum) Hematoxylin	Eosin	
"	Berlin Blue	Orange G.	Differentiate Connective tissue
"	Gallory's Connective Tissue Stain		Differentiate Connective tissue
Fluoroc with Reduced CH ₂ COOH	Iron (Alum) Hematoxylin	Eosin	
"	Aniline Acid Fuchsin	Ethyl Green	
Fluoroc with Strong Solution	McLafferty's Hematoxylin	Eosin	
"	Hematoxylin and Fuchsin	Ethyl Green	
Alcoholic Acetic Sublimate	Iron (Alum) Hematoxylin	Eosin	
"	Aniline Acid Fuchsin	Ethyl Green	
Acid	Iron (Alum) Hematoxylin	Eosin	
"	Carfrain	Light Green	
"	Hematoxylin	Ethyl Green	
"	Gallory's Connective Tissue Stain		Differentiate Connective tissue
"	Aniline Acid Fuchsin	Ethyl Green	Differentiate Mitochondria
Reagent	"	"	"
"	Iron (Alum) Hematoxylin	Eosin	

lucida of slides under the oil immersion lens. Measurements were made with a polar planimeter the accuracy of which was first checked on areas of known dimensions. Since the cell boundaries in the younger stages were either indistinct or absent, it was necessary in making all the drawings, to simply draw all the cytoplasm and nuclei in a given microscopic field at a given focus, and consider that the number of nuclei represented the number of cells present. Since the nuclear areas to be traced were small, all nuclear boundaries were traversed four consecutive times and an average figure obtained to reduce the error of manipulation. The cytoplasmic areas were obtained by subtracting the nuclear areas, and such extraneous areas as stored colloid etc., from the total areas of the sections drawn. The necessary mathematical calculations were made with a comptometer. The ratios obtained for comparison represented the sum total of the area of the cytoplasm divided by the sum total of the area of the nucleoplasm; or in other words the number of times greater the area of the cytoplasm was than that of the nucleoplasm.

Previous investigators, Dolley (1909-1917-1925) and Young (1931) have reduced their results to volumetric ratios. There is no doubt but that this gives a better picture of the actual situation. In some of the younger stages of development in the thyroid it is impossible to determine the exact shape of the cells. Moreover where it was

possible to distinguish cell boundaries there was great variation in their shapes. For these reasons I did not feel it was justifiable to attempt to reduce my C/N ratios to volumetric terms.

There are in the literature pertaining to the thyroid repeated hints that cells of the reticulo-endothelial system are present in the thyroid. Williamson and Pearce (1926) think that they have a part in the normal functioning of the gland. To determine whether or not such phagocytic cells are present in the embryonic chick thyroid, some vital staining was employed. The procedure used was that suggested in a report by E. D. Hannan (1925) of the University of Buffalo. Briefly summarized, it consisted of injections of from $\frac{1}{2}$ to 1 c.c. of a .25 distilled water solution of various dyes into the air chambers of incubating eggs. Aseptic technique was used. I made duplicate injections with each of three dyes beginning when the embryos were eight days old. By that time the gland is quite vascular. After the dye had remained in the incubating eggs from 12-22 hours, I dissected out the thyroid glands and examined teased preparations of them in physiological salt solution. Inspection was made by means of oil immersion. These experiments were carried on at one or two day intervals from 8 days of age until hatching. To check observations two series were run. Substances used for vital injection included toluidin

blue, Berlin blue, aniline blue-black, neutral red, janus green, carmine, thionine and India ink.

VI OBSERVATIONS AND EXPERIMENTAL RESULTS

In chick embryos just before hatching the thyroid glands are located along the jugular veins just above the branching of the sub-clavian and carotid arteries. (Plate IV, Fig. 25) At this age there is an intimate association of position with two other glands. The thymus, a lobulated organ of soft texture, is partially dorsal to the thyroid and extends some distance farther headward. The parathyroid gland, a rounded organ of lesser bulk, lies immediately caudad of the thyroid.

Besides being ensheathed in a casing of connective tissue which anchors it in position, the thyroid is found upon dissection to be an oval pinkish body which has its own true capsule of connective tissue. To and from the gland on each side lead no less than four relatively large vessels. Study of a gland vitally injected with India ink via the blood stream reveals that its substance is traversed by many branches from the main blood vessels, and that the smaller vessels formed a mesh-work between the follicles. The latter are sac-like vesicles filled with a thick fluid substance called "colloid". (Plate IV, Fig. 26)

Study of serial sections of the 64 chick embryos revealed the morphogenesis of the gland to be as follows.

At three days the thyroid anlage is a somewhat spherical diverticulum from the floor of the pharynx opposite the second branchial cleft. In cross sections through the visceral arches of this region, the thyroid anlage, posterior to its connection with the pharynx, appears as a doughnut-shaped structure. (Plate I, Fig. 1) When viewed with the oil immersion lens, it is seen to be made up of narrow cells of high columnar type. These cells, having elongated, oval, reticular nuclei, radiate out from the central lumen. The nuclei are practically all located in the peripheral half of the cell. Occasional cells at this stage show mitotic figures. The outer edges of the gland are quite distinct. (Plate I, Fig. 1)

At four days the gland has increased its bulk many times. It is now almost completely separated from the pharynx, only a narrow strand of tissue, two cells in thickness, remaining as the so-called thyroglossal duct. The large peripheral nuclei of the preceding day have apparently undergone rapid division, as countless smaller nuclei are evenly distributed everywhere except in the region immediately surrounding a tiny remnant of the original lumen. Cell boundaries are invisible or lacking. While more mitotic figures are in evidence than in the three day stage the total number seems insufficient to explain the extremely rapid growth which is obviously in progress. The rather indistinct

ventral border of the gland shows a tendency to become bilobed. (Plate I, Fig. 2)

By five days division has been completed and the two glands are seen just ventral to the trachea. Horse shoe shaped strands of tissue, a sort of vestigial isthmus, may be seen connecting them. It is still almost impossible to distinguish any individual cells. The borders of the gland are more distinctly visible than in the preceding stage. This is due to the fact that sections of about a dozen small vessels form an interrupted border about each gland. In some specimens mesenchyme containing vessels divides the glands into lobes. (Plate I, Fig. 3)

The most noticeable change in the glands at the sixth day is their position relative to the trachea. They have become entirely lateral to that organ and lie between it and the carotid arteries. The mesenchyme invasion is much more pronounced and has caused the gland to assume the appearance of plates separated by circulatory spaces. Although this appearance becomes much more pronounced during the next five days, this stage probably represents the beginning of the "plate stage" described by Norris (1910) for human thyroid and the "vascular stage" mentioned by Yoshikawa for chicks. (Plate I, Figs. 5 and 6; and Plate II, Fig. 10)

Little change besides growth occurs in glands on the seventh, eighth, and ninth days except that vascularity

increases and the epithelial plates, which are now two or three cells in thickness, have become more convoluted.

The tenth day is interesting inasmuch as the first colloid, which is chromophile rather than chromophobe in nature, becomes visible. This colloid, in the form of droplets of smaller size than the nuclei, accumulates between three or four adjoining cells, the boundaries of which are still indistinct.

No changes worthy of note are visible in eleven and twelve day stages except that chromophobe colloid appears.

At thirteen days the gross anatomy, except for the shape of the epithelial plates, is very suggestive of a gland unit in the follicular stage described by Williamson and Pearce (1923). Inside the gland capsule is a large sinus continuous with the circulatory spaces between the convoluted epithelial plates. Whether this sinus is a blood or lymph sinus I am unable to say definitely. Although they contain erythrocytes, it seems, in general appearance and morphological relationship, that the spaces between the columns more nearly approximate the lymph spaces which Gale Wilson (1927) demonstrates in his wax reconstruction models of foetal human thyroids. (Plate I, Fig. 6)

In Fig. 7 of Plate I is shown a typical follicle from a 14 day embryonic chick thyroid. The aggregation of secretion granules toward the central lumen in this follicle and

the lines of seepage of colloid from adjacent cells in the follicle below is of interest.

From 15-22 days there is decided growth both in size and number of follicles, the former being achieved apparently by fusion of follicles which is a very prevalent occurrence. Growth in the number of follicles seems to be accomplished as Seckel describes it in dogs by the formation of follicles out of interfollicular epithelial cells. Even as early as fifteen days there are colloid droplets in some of these cells. In size they seem a little larger than the cuboidal type of follicular cell. However they are no larger than the columnar type of follicular cell which Uhlenbuth (1924) described as being characteristic of the state of heightened activity. Plate III, Fig. 18 shows similar large follicular cells in the embryonic chick thyroid.

In the late embryonic stages of chicks one noticeable morphological feature is the increased secretion of droplets of chromophobe colloid which seems to have the effect of diluting the more dense chromophile colloid. Not only are these two kinds of colloid present in different cells of the same follicle at the same time as Bradway (1929) observed, but are occasionally observed in the same cell at the same time. (Plate III, Fig. 17)

The question of the exact method by which the follicular

contents reach the circulatory medium has been, and still is controversial. In contrast to such views as those of Bensley (1916), Bowen (1929) and Uhlenhuth (1928), already described in connection with the history of the thyroid investigations, there are numerous advocates of the view that the colloid escapes into the lymph spaces between the follicles. Sechel (Feb. 1931) claims that follicular disintegration permits the escape of colloid. There are frequent reports of colloid being present in the lymph spaces. Wilson (1927) observed extrafollicular colloid in four percent of 400 human thyroid glands studied. He refers to experiments performed by Cannon and Smith (1926) in which they found it possible to engorge the lymphatics of cats by simply massaging their thyroid glands for a few minutes. That the lymphatics rather than the blood stream were involved was indicated by the length of time (30-60 minutes) it took for the denervated heart of the animals to show acceleration, and the corresponding slowness with which the effect wore off.

Yoshikawa (1930) mentions that on some occasions the chromophile colloid is found to be discharged into the blood sinus attached to the follicle wall.

Definite, single, tube-like openings have, during the present investigation, been repeatedly observed, leading from the lumina of follicles to the surrounding circulatory spaces. They show even more conspicuously in fresh gland

tissue under oil immersion than they do in the slide material. However several camera lucida drawings from the latter source may be seen on Plate II, Figs. 11-13 inclusive. It is my belief that such an opening is functional during each functional cycle of a follicle, and that it furnishes one route by which the chromophile colloid (diluted by the chromophobe) reaches the circulation.

There was some evidence that the disintegration of a cell in the follicle wall formed the exit for colloid. On the other hand the apparent tube may be merely an intercellular crack through which seepage occurs as Uhlenhuth (1923) believes is sometimes the case in salamanders. A third possibility is that a circulatory capillary taps the follicle.

As compared with the size of the follicles these single tubular exits are small, their diameter being usually no greater than that of an erythrocyte. In Fig. 14 of Plate II an erythrocyte may be seen blocking such an opening. It seems probable that the short length and diameter of these tubes so reduces their chances of being sectioned radially that they have either been overlooked by previous workers or thought unimportant. The probability of their being observed in the fresh material is also slight, being determined by two possibilities: first, that they be in the horizontal plane; and second, that the follicles be distended

with colloid.

After completing the study of thyroid morphogenesis, a survey of the C/N ratio was made of three of the developmental stages--3, 13, and 19 days respectively. Contrary to the general rule given by Linot (1908) there was a definite gradual decline in the relative amount of cytoplasm to nucleoplasm. Thus at three days the C/N ratio for a thousand cells was $\frac{252.95}{76.09}$. In other words the total cytoplasmic area for that stage was 3.324 times greater than the nuclear area.

In the 13 day stage the corresponding C/N ratio was $\frac{88.02}{44.17}$, showing that at this stage the total cytoplasmic area was only 1.95 times greater than the nuclear area.

By the 19 day stage the C/N ratio was still further reduced to $\frac{76.00}{51.14}$, showing that the area of the cytoplasm was only 1.46 times greater than the nuclear area.

For reasons already stated it was not feasible to convert the figures for areas to their volumetric equivalents. If volumetric terms could have been secured, the disproportion in the ratios would obviously have been decidedly greater.

Before attempting to interpret the possible meaning of these results, I should point out one probable explanation for the ratio of cytoplasm to nucleus being so high in the three day stage. This is the only stage where the cells of

the entire gland show any very definite "grain". Due to this "grain", resulting from their radial arrangement around the lumen, it seems probable that a majority of the cells were sectioned more along their longitudinal than their cross axes. This would naturally tend to favor the cytoplasm more than the nucleoplasm as they are high columnar type cells. (Plate IV, Fig. 9)

Such selection would ordinarily tend to make questionable the unexpected results of this study. However the same definite trend toward a reduction of the C/N ratio is noticed in comparing the 13 and 19 day stages. In these older glands there is no definite plane of orientation of cells and the sections were all cut in a plane transverse to the long axis of the gland so that no selection was possible here.

The possibility suggests itself that this progressively reduced C/N ratio may be due not to a decrease in cytoplasm but to an increase in nucleoplasm. However, in comparing the total areas of nuclei of the 9 day stage to those of the 13 day stage, there is found to be an actual decrease of 31.92. Therefore the cytoplasm must have actually decreased in amount during the time between these two stages. A further comparison shows an increase in nuclear area of 7.97 from the 13 to the 19 day stage. At the same time there was a decrease of 10.03 in the cytoplasmic area, thus taking

a further reduction in the C/N ratio as above noted.

This study indicates that up to 13 days there is an actual decrease in both cytoplasm and nucleoplasm. This may be naturally accounted for by the rapid cell division and differentiation in the growth of the gland during this period. Nevertheless there is a steady decrease in the proportion of cytoplasm from the 3 day to the 19 day stage, which is only partially explained by the increase in nucleoplasm after 13 days. Whether the reduction in cytoplasm indicates contributions to the formation of colloid or to the nuclear growth it is impossible to say without further investigation. It seems logical to suppose that cytoplasm elaborating a secretory product for use by the entire organism would reduce its bulk during a state of high activity. However, exactly the opposite opinion is held by Dolley (1925) of the C/N relationship in the functioning pancreatic cells. Why there should be so much variance between glands as similar in general function as the thyroid and pancreas it is difficult to understand. The difference in results obtained from the studies of these glands may be due to the fact that Dolley (1925) studied different states of activity in the adult gland whereas the present investigation deals only with developmental stages. However, with the results of Sun's (1932) investigation of iodine content in the embryonic chick thyroid paralleling the morphological

evidence of activity as closely as they did, there seems no doubt but that the gland in these animals becomes increasingly active from an age of ten days to hatching.

The results of the C/N ratio study in the thyroid gland of chickens fails to substantiate Minot's law of cell differentiation, which states that the relative amount of cytoplasm to nucleus increases as cells differentiate.

The mitochondria in the thyroid have been studied in guinea pigs by Nicholson (1934), and in albino rats by La (1935). The former study dealt with changes from the normal condition brought about by such experimental technique as reducing the blood supply, and feeding drugs. In the latter study La (1935) noted mitochondrial changes occurring during the functioning of normal glands. According to him the mitochondria originate as cytoplasmic granules which change into rods and filaments. He claims that they dissolve in and contribute to the colloid.

Marino (1932), summarizing what is known about mitochondria in the thyroid, says they occur in the form of thin filaments which are parallel to the long axis of the cells, and are most numerous between the nucleus and the follicular lumen.

I do not feel that the present study included a comprehensive enough investigation of changes in the mitochondria to contribute any conclusion as to their relation to functional

stages in chick thyroid. However, in slides of the late follicular stages their position was found to vary. They were found most frequently in the perinuclear position shown in Plate I, Fig. 23. In many follicles they were grouped more toward the central lumina as in Fig. 24, of Plate III. Still less frequently they were scattered indiscriminately through the cytoplasm. In form they were straight thin filaments of varying length. None were observed in the colloid either within the cells or the lumina of the follicles.

In connection with the study of the morphogenesis of the gland, endothelial cells with somewhat fibrillar ends were found lining the circulatory spaces and adhering tightly to the periphery of the follicles. (Plate II, Figs. 10-14) Occasional apparently unattached cells of a similar type were also seen. The position and appearance of some of these cells suggested a phagocytic function. That is, they were located at strategic angles in the sinuses between follicles, and seemed able to change shape to form more rounded structures with pseudopodia-like projections. Probably they represent cells of a similar type, in which, to those referred to by Williamson and Courne (1923) as similar in appearance to the Kupfer⁴ cells of the liver. These investigators found such cells occurring normally in the human thyroid and believed they had special

significance.

Downey (1916) criticized the assumption that phagocytosis of particles of vital dyes is a specific reaction for cells of the reticulo-endothelial system. However in the latest edition of Cowdry's "Special Cytology", repeated mention is made of this method of identification being considered diagnostic for that system. McClung (1929) in his recent book on microscopical technique claims a high degree of specificity for the vital dyes. According to him epithelium, ordinary endothelium, and other tissues of the body do not take them up; whereas phagocytic cells of the reticulo-endothelial system show a decided affinity for them all.

McJunkin (1919) used the specific staining reaction of vital dyes in rabbits and dogs as a basis for concluding that the phagocytic nonnuclear blood cells originate from the endothelial cells of vessels.

Plate IV, Figs. 27-31 inclusive, show the types of phagocytic cells containing dye granules and vacuoles which were found in thyroids of vitally stained chicks. Two types of phagocytic cells occur and most frequently in the vitally stained thyroid glands. The first type were narrow cells: Fig. 29, A and B; Fig. 30, B, C, E, F; Fig. 30, C; and Fig. 31, A. These cells resemble strongly the endothelial cells of the prepared slides, not only as to shape but as to arrangement of granules and lack of any conspicuous nuclei.

The second type usually appeared rounded as in Fig. 22 A. On rare occasions these living cells were seen to assume their spherical shape after a change from more elongated cells of irregular shape. This change of form was observed in cells being viewed with the oil immersion lens, and was not due to pressure from any surrounding cells. Of these two types of cells the rounded ones were usually the larger, although the cells of elongated type varied in size ^{from} extremely small cells like C, in Fig. 22 to cells approaching the size of an erythrocyte.

Since these cells were observed in teased preparations of the gland, it was impossible to say what their normal position had been with respect to the follicles and circulatory spaces. Time did not permit any attempt to get permanent cross sections of fixed vitally stained glands. I doubt whether the coloration would have withstood the necessary manipulation involved in making permanent slides. Even in the fresh material it was evident that the dyes taken up were being changed either physically or chemically. For instance, if too long a time interval elapsed between injection of stain and examination, the characteristic dye color could not be detected. Instead, scattered throughout the gland, cells, similar to the characteristic types usually stained, could be seen to be filled with abnormally large numbers of vacuoles having the greenish yellow color of a lipoidal

material. Repeated observations of this phenomenon leads me to agree with the claim of Berger (1929) that phagocytic elements of the reticulo-endothelial system pick out particles of dye and incorporate them as colloidal particles or store them as fat droplets. It is possible that the phenomenon of phagocytosis demonstrated in the figures of Plate IV might have merely been incidental in the blood which happened to be in the glands at the time they were excised. To determine whether this phenomenon was merely characteristic of the blood, I made smears of blood taken from the hearts of vitally stained embryos. Some of these smears were studied in the fresh state, others were stained with Wright's blood stain. No clear-cut cases of phagocytosis of dye particles were observed in the fresh smears although the stained slides showed leucocytes of some sort present.

While studying a gland supra-vitally stained with janus green numerous instances were noted of leucocytes phagocytosing erythrocytes. The material was fixed with weak osmic acid solution, and, after being permitted to dry, was mounted in balsam. Camera lucida drawings of this phenomenon are shown in Fig. 27 of Plate IV. Although repetition of this occurrence was sought in other glands, no clear cut cases of it were evident. Whether the instance cited represents a pathological condition or not, it is of interest as showing an unusual method of erythrocyte destruction inasmuch

as they were entire and of normal appearance when attacked.

The significance of the results shown by these vital staining experiments is problematical. The idea suggests itself that possibly in the walls of the thyroid sinusoids certain endothelial cells become specialized for phagocytic function, cease to be fixed to the walls by fibrillar ends, and, after passing through transition stages, become true macrophages. However, no such conclusion is justifiable merely on the results of the experiments outlined here. It does seem clear, however, that reticulo-endothelial elements of some sort are present in embryonic chick thyroids.

VIII SUMMARY AND CONCLUSIONS

1. The morphogenesis of the gland in chicken embryos was found to be quite similar to the stages described by Bradway (1929) and Yoshikawa (1930) except in some minor details. Considerable variance in results as compared with earlier investigations exists.
2. Contributions to the knowledge of mitochondria in chicks are to the effect that they exist as filaments of varying lengths which vary in position being most frequently perinuclear.
3. Not only is the chromophobe and chromophile colloid present in different cells of the same follicle at the same time, but are occasionally present in the same cell at the same time.

4. Evidence of heightened activity in chick thyroid cells are as Uhlenhuth (1928) describes them in salamanders: namely, high columnar cells, apically located secretion granules, and vacuoles of chromophobe colloid.
5. As the thyroid gland develops in chick embryos there is a progressive decrease in the C/N ratio. This result is contrary to the generally accepted opinion regarding cell differentiation.
6. Follicles in the late developmental stages in chick thyroids have, at some time during their functional cycles, tubular exits which are thought to represent one path through which colloid reaches the circulatory spaces.
7. Some experiments of vital staining have established the view that elements of the reticulo-endothelial system are present in the developing chick thyroid.
8. Phagocytic cells in the thyroid gland of one chick embryo were observed phagocytosing entire erythrocytes.

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IX LIST OF FOOTNOTES

1. This investigation was carried on in the Biology laboratory of the University of Montana, under the supervision of Dr. R. T. Young, for whose helpful guidance I wish to express my appreciation. I am also indebted to Miss. A.C. Millball, former student of the University of Montana, for some of the slides used in making this study.
2. In the notation C/N as used in this paper, C refers to the mass area of cytoplasm, and N refers to the mass area of nucleoplasm.
3. The "Andersons Vacuoles" of the literature.
4. The Kupffer cells are phagocytic cells in the lining of the liver sinusoids. Their functions include the phagocytosing^{of} the red blood cell puscles and salvaging of their pigment.

PLATE I

Explanation of Figures

All drawings are of the chick embryo, and made with the camera lucida.

1. Section through viscerol arches and pharynx--3 day stage. P, pharynx; T, thyroid. X23.
2. Section through pharynx--4 day stage. P, pharynx; D, thyrogllossal duct; T, thyroid; L, lumen of original invagination. X133.
3. Section through neck--5 day stage. A, artery; G, ganglion of vagus nerve; V, internal jugular vein; Th, thymus anlage; E, esophagus; Tr, trachea; T, thyroid. X130.
4. Section through neck--7 day stage. N.T., spinal cord; N, notochord; other abbreviations same as Fig. 3. X130.
5. Section through thyroid gland--8 day stage. S, circulatory sinus; Pl, plate of epithelial cells; N, nucleus; V, vein; J.V., jugular vein; C.S., circulatory space. X50.
6. Section through thyroid gland--13 day stage. C, colloid; other abbreviations same as Fig. 3. X550.
7. Section through thyroid follicle--14 day stage. C, colloid; M, lines of intercellular seepage of colloid; N, nucleus. X1300.
8. Section through thyroid gland--19 day stage. F, empty follicle; C, colloid filled follicle; A, artery; V, vein. X550.

PLATE I

PLATE II

Explanation of Figures

All drawings are of chick embryos, and made with camera lucida.

9. 3 day stage. Portion of anlage showing high columnar type of cells with peripherally placed reticular nuclei. L, central lumen of anlage. Oil immersion X1300.
10. Portion of thyroid gland in 17 day stage. Pl., plates of epithelial cells; C.S., circulatory spaces; R, erythrocyte; E, endothelial cell. Oil immersion X1300.
11. A thyroid follicle, 17 day stage, showing endothelial cell partially blocking drainage tubule of follicle. E, endothelial cell; C, colloid; E.C., colloid in circulatory spaces; C.S., circulatory space. Oil immersion X1300.
12. thyroid follicle, 17 day stage, showing endothelial cell of circulatory space in contact with colloid. Abbreviations same as Fig. 11. Oil immersion X1300.
13. Follicle of 17 day stage showing branch from circulatory space in almost direct connection with colloid. X1300.
14. Colloid of 19 day stage showing erythrocyte, R, blocking drainage tubule to circulatory space. Other abbreviations same as in Fig. 11. Oil immersion X1300.
15. Colloid of 19 day stage showing fusion of follicles, and direct contact of erythrocyte with colloid. Note extra cellular colloid, E.C., in circulatory space. X1300.

PLATE II

PLATE III

Explanation of Figures

All drawings are of chick embryos, and made with camera lucida.

16. Thyroid follicle, 17 day stage, in process of excreting chromophobe colloid droplets into lumen. Note high columnar type of cell associated with heightened activity. The rounded apical ends of cells indicate lack of tension within follicle. C₁, chromophobe colloid; C₂, chromophile colloid. Oil immersion X1300.
17. Cell from follicle in 15 day stage. The chromophile, C₂, and chromophobe, C₁, droplets are present in same cell at same time, and almost replace cytoplasm. N, nucleus. Oil immersion X1300.
18. Isolated follicular cell from teased fresh gland--15 day stage. G, secretion granules; N, nucleus; V, vacuole of chromophobe colloid.
19. An isolated cell, apparently interfollicular, from teased thyroid at 15 days. G, secreting granules; N, nucleus; E, endothelial cell. Oil immersion X1300.
20. Isolated follicle cell from teased thyroid at 15 days. C, colloid droplets; G, secretion granules; N, nucleus. Oil immersion X1300.
21. Young follicle from teased thyroid at 15 days. Note approach toward columnar type of cell, and the aggregation of secretion granules in apical ends. N, nucleus. Oil immersion X1300.
22. Follicle from teased thyroid at 15 days. G, secretion granules. Oil immersion X1300.
23. Follicle from 17 day thyroid stained with aniline acid fuchsin and methyl green to differentiate mitochondria, oil immersion X1300.
24. Follicle stained by special mitochondrial technique showing mitochondria in apical ends of cells. Oil immersion X1300.

PLATE III

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PLATE IV

Explanation of Figures

All cells are from embryonic chick thyroid.

23. Free hand sketch to show relative positions of thyroid, thymus, and parathyroid in 20 day chick embryo. Tr, trachea; Th, thymus; P, parathyroid; T, thyroid.
25. Section of fresh thyroid, 13 day, supravitaly stained with janus green and fixed with osmic acid to blacken colloid. Note circulatory network between follicles. Camera lucida, oil immersion X1300.
27. A, B, C. Phagocytosis of red blood corpuscles. From 11 day fresh thyroid, stained similarly to Fig. 25. L, leucocytes. Camera lucida--oil immersion X1300.

The following drawings are of cells of thyroids from vitally stained embryos. The coloring substance was sterily injected into air chamber 24 hours before the specimen was examined.

28. 2 cells from 3 day thyroid--neutral red injections. Both cells contain red granules and small vacuoles.
29. From 10 day thyroids. A, berlin blue injection, cell is stained a blue-yellow with many vacuoles. B, neutral red injection, the small vacuole in the end of cell is bordered by a red fringe. C, berlin blue injection, the whole cell is bright blue. D, carmine injection, the 2 large vacuoles are fringed with carmine, and each have 4 radiating lines of carmine granules.
30. From 13 day thyroids. A, neutral red injection, 1 large red granule in the cell. B, carmine injection, profuse carmine granules are throughout most of the cell, and in 3 prominent, dense clusters. C, neutral red injection, red granules scattered through cell.
31. From 13 day thyroid--carmine injection. A, small circles represent yellowish fat-like globules; small dots are carmine particles. B, the black portion of cell, represents a reddish black color; the small circle stained red, and the remainder of cell is yellow.

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