REPORT
of
Committee on Thesis

The undersigned, acting as a Committee of
the Graduate School, have read the accompanying
thesis submitted by Edgar H. Norris.
for the degree of Master of Arts.
They approve it as a thesis meeting the require-
ments of the Graduate School of the University of
Minnesota, and recommend that it be accepted in
partial fulfillment of the requirements for the
degree of Master of Arts

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May 30, 1916

REPORT
of
Committee on Examination

This is to certify that we the undersigned,
as a Committee of the Graduate School, have given
Edgar H. Norris final oral examination
for the degree of Master of Arts. We recommend
that the degree be
conferred upon the candidate.
Minneapolis, Minnesota.

May 30, 1916.

C. M. Jackson
Chairman

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The Morphogenesis of the Follicles in the Human Thyroid Gland.

A Thesis

Submitted to the Faculty

of the

Graduate School

of

The University of Minnesota

by

Edgar H. Norris

In partial fulfillment of the requirements for

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The Morphogenesis of the Follicles in the Human Thyroid Gland.

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I. Introduction.

In spite of numerous investigations, many questions concerning the development of the thyroid gland are still unsettled. This applies particularly to the morphogenesis of the thyroid follicles. The various and contradictory views in the literature on the development of the follicles are doubtless due in part to lack of adequate series of successive embryonic stages (especially of human embryos) available for study. The greatest difficulty, however, has arisen from the use of inadequate methods of investigation. The method hitherto almost exclusively used, that of direct observation of the microscopic sections, is insufficient. By the use of reconstruction methods, however, it has been found possible in the present study to reach a satisfactory solution of this difficult and important problem, at least regarding some of the more fundamental features.

This study was undertaken in the Anatomical Laboratory of the University of Minnesota at the suggestion of Prof. C. M. Jackson, under whose supervision the work was conducted. I wish to thank Dr. Jackson for his valuable aid and criticisms.

II. Literature.

The literature concerning the thyroid follicle will be considered in chronological order. First, it is desirable to mention briefly the various views which have been held concerning the morphology of the adult (human) thyroid follicle. Then follows a brief statement of the conclusions concerning follicle development (including also prefollicular stages), which have been arrived at by the observers who have worked upon human material. A few observations, made on lower forms, which have seemed especially pertinent

to the present problem, are also included. Unless otherwise indicated, however, all statements refer to human material.

The follicular structure of the adult human thyroid gland has long been known. According to Boéchat ('73), Lalouette (1750), who was the first to describe the minute structure of the thyroid gland, found vesicles which seemed to communicate with each other. Bardeleben ('41) is said by Zeiss ('77) to have been the first to describe the adult thyroid follicles as isolated structures. Five years earlier, however, Jones ('36) described the thyroid follicles in considerable detail as completely closed vesicles. Although there has been considerable disagreement concerning the structure of the adult thyroid follicle, the majority of the later observers have, like Jones, described the vesicles of the adult gland as closed, spheroidal bodies. Cruveilhier ('43), Virchow ('63), and more recently Boéchat ('73), Zeiss ('77), and Hitzig ('94), however, have followed Lalouette in describing the follicles as forming a system of branched and communicating cavities within the gland. Still others, like Streiff ('97), have maintained that both branching forms and isolated vesicles occur in the adult gland.

Jones ('36), who was perhaps the first to describe the microscopic structure of the human fetal thyroid, found that in a fetus of 4-1/2 months the cells of the gland had become partially arranged into solid, globular masses; but no vesicles were observed at this stage.

Remak ('55) describes^d in chick embryos in the wall of the primitive saccular, epithelial thyroid anlage the formation of thickenings which become separated and later give rise to the thyroid follicles. He also thought that the original saccular

anlage might persist for some time and form new secondary vesicles by a process of constriction. In summarizing his observations upon the thyroid of chick embryos, he says:

"Die Epithelialbläschen, welche im erwachsenen Zustande die bindegewebigen Alveolen der Schilddrüse auskleiden, entstehen aus der Wand der primitiven Schilddrüsenblase. Was aus der Höhle der letzteren wird, vermag ich nicht mit Bestimmtheit anzugeben. Sicher ist nur, das sie in dem Maasse sich verengt und unkenntlich wird, als ihre Wand sich verdickt. Es ist daher möglich, dass sie nicht ganz schwindet, sondern die sie zunächst begrenzende Zellschicht der Wand nicht zur Bildung von sekundären Epithelialblasen verwendet wird, sondern eine centrale Epithelialblase bildet, welche durch feinere Abschnürung in kleinere Blasen zerfällt."

Remak also described similarly the origin of secondary follicles, both by constrictions and by solid budding, in the thyroid of pig fetuses four inches and above in length.

Peremeschko ('67) stated that the structure of the thyroid varies with age. He noted the presence of vesicles and colloid in mammalian fetuses, and described the division of primary into secondary follicles. Colloid is described as arising partly by secretion and partly by colloid metamorphosis of epithelial cells.

W. Müller ('71), who considered the fully developed thyroid vesicles as closed globular bodies, described an earlier developmental stage in which, before the formation of vesicles, the gland consists of a network of cylindrical tubes. ^{Such tubes were found} in ~~the~~ 24 mm. fetus and in decreasing numbers in later fetuses and even in a three year old child. These tubes arise from solid epithelial cords by the development of a central lumen. The segmentation of the tubes with the formation of the gland-vesicles is produced by ingrowth from the mesoblast. All of the early follicles have lumina which are distinctly outlined by clear cut margins. Müller found that there is great variability in the shape of the thyroid follicles between the fifth fetal month and the third year of postnatal life.

Horcicka ('80) found the thyroid gland of a four months' fetus to be made up for the most part of solid cell masses; but there is some change toward lumen formation in the central cells of these masses. Typical gland structure is found after the fifth fetal month.

Wölfler ('80) described the formation of follicles from solid masses of epithelial cells. Toward the end of the fetal period and after birth the peripheral cells of the groups dispose themselves in a circle. The central cells become at first granular, then

degenerate and disappear in the pale, granular mass which fills the lumen of the vesicle thus formed.

L. Stieda ('81) noted that the anastomosing epithelial cords ("Epithelstränge") of the embryonic, mammalian thyroid are at first always solid, but that:

"Im Laufe der Entwicklung tritt erst^s hie und da am Ende eines Strangs ein Lumen und darauf eine ganz allmähliche Abschnürung des betreffenden Abschnitts des Drüsenstrangs auf, es bilden sich sehr langsam, wie es scheint, die Blasen der Thyreoidea".

Baker ('81) described the fully formed follicle as spheroidal in form, but observed also branching follicles which are probably giving rise to secondary follicles by a process of division.

Wölfler ('83) described the process of later development in the human thyroid gland as centrifugal. He distinguished a cortical and a medullary portion, which are respectively youngest and oldest, least developed and most developed portions.

His ('85) in the thyroid gland of an embryo between 16.5 and 22 mm. in length, found as follows:

"Bei Z^W sind zwar auch noch Gruppierungen der Zellen um längliche Spalten herum vorhanden, daneben aber zahlreiche Complexe, die den Habitus eigentlicher Acini tragen. An jeder Zelle ist nunmehr ein leicht tingirbarer körniger Abschnitt von einem hellen (colloiden) geschieden, dabei lagern sich die Elemente so, dass die die Kerne tragende körnige Zone derselben die Peripherie des Acinus bez. des Zellenrohres bildet, dagegen der helle Antheil nach einwärts gekehrt ist".

Biondi ('89) found that after filling, the (postnatal) thyroid vesicle discharges its contents, collapses and finally rearranges itself in the form of a number of small acini which repeat the process. He held that the colloid arises by cell secretion, and not by cell degeneration.

Ribbert ('89) described a centrifugal growth of the organ, and distinguished cortical and medullary portions in embryos and newborn children. Follicles are formed by the outgrowth of solid buds or sprouts from the old follicles. These buds are cut off by the connective tissue, and a lumen appears into which colloid is secreted.

Lustig ('91), who studied the thyroid gland in the pig and other animals, affirmed that colloid and follicles appear synchronously as the result of the same process,--the degeneration of the central cells of the preexisting solid masses.

Podack ('92) found well formed follicles in a fetus of five months. In some parts of the gland the follicular structure is only suggested and many cell-masses and cell-cords are present.

Marshall ('93) found that the thyroid in chick and frog embryos presents a stage in which the gland is made up of communicating, epithelial tubes. In the rabbit he described the presence of outgrowths, some solid and some hollow, from the primitive epithelial anlage. Concerning the early development of the thyroid in the human embryo he stated: "At an early stage the lobes are excavated by a number of detached cavities, which become the vesicles of the adult thyroid".

Zielinska ('94) found the structure of the thyroid in newborn children variable both in size and number of the follicles, and also in the amount of solid cell masses. She remarked that:

"Solche Lobuli, in denen mehr oder weniger central ein oder zwei mit cubischem oder cylindrischem Epithel versehene Drüsenlumina und im Uebrigen solide Zellhaufen sich finden, erinnern an acinöse Drüsen und erwecken den Gedanken, dass hier ein sich verästelnder Drüsenkanal vorliegt, als dessen Endbläschen die solide Zellhaufen gelten können."

Hürthle ('94) described in the thyroid of young dogs scattered masses of interfollicular epithelium, whose cells were supposed to migrate into the walls of the follicles and thus contribute to their growth. New (primary) follicles were also described as arising in the interstitial epithelial masses. Colloid is secreted into the angles between adjacent cells, which become separated and bound the lumen.

Anderson ('94) described secondary follicles (postnatal) arising from the collapsed epithelium of emptied follicles in various mammals. The new lumina are formed by cell-secretion of chromophile spherules, and not by degeneration of the central cells. No constriction or division of follicles was observed.

According to L.R.Müller ('96), the origin of small secondary follicles from the larger follicles, as described by Ribbert ('89) is clearly evident, even in the human adult.

Tourneux and Verdun ('97) in a careful study of the branchial derivatives in the human embryo described the transformation of the (median) thyroid plate into a richly anastomosing network of solid epithelial cords by ingrowth of vascular connective tissue in a 14 mm. embryo. Similar changes take place later in the portion derived from the lateral thyroid anlage. The network of solid cords was likewise observed in embryos of 19 mm., 24 mm., 26 mm., and in one of 37 mm. in length. In another of 37 mm.: "Les cordons anastomosés de la thyroïde montrent quelques vésicules". The early follicles are described more fully in a fetus of "32/40 mm." as follows:

"La thyroïde se compose de cordons cellulaires ramifiés et nettement anastomosés qu'englobe une trame conjonctive lâche dans laquelle rampent de larges capillaires. Ces cordons n'affectent pas une forme régulièrement cylindrique, mais ils présentent sur leur parcours des renflements sphériques ou ovoïdes, au niveau desquels ils sont creusés d'une cavité centrale; leur épaisseur, de 30 à 40 μ en moyenne, peut s'élever à 80 μ au niveau des dilata-tions. Ils sont constitués par de petites cellules polyédriques tassées les unes contre les autres, et se disposant en un revete-ment cubique ou cylindrique peu élevé, au pourtour des excavations glandulaires. En beaucoup de points, la paroi épithéliale d'une vésicule paraît épaissie, et se soulève sous forme de bourgeons pleins faisant saillie à l'extérieur, et qui donnent à l'ensemble un aspect variqueux."

Soulié and Verdun ('97) describe in a rabbit embryo of 15 mm. the morphogenesis of the thyroid follicles similar to that found by Tourneux and Verdun ('97) in the human fetus.

Streiff ('97) made wax reconstructions of normal, adult human thyroid tissue, and found it to be made up of closed follicles, ovoidal or spindle-shaped. Branched forms due to budding or to secondary fusion were also described; some of these more complex forms he thought may represent persistent branching,--a continuation of the embryonic process. He concluded (from a study of the adult structure) that:

"Die Glandula thyreoidea des Menschen entsteht nach Art einer verästelten tubulösen Drüse. Am Schluss der Entwicklung aber bilden sich an den Zellschläuchen vielfach Erweiterungen aus. Diese Erweiterungen werden als Vesiculae, -noch nicht erweiterte Stücke der Schläuche als geschlossene Tubuli durch die wuchernden Gefässe und Bindegewebeszüge von einander getrennt."

Schreiber ('98), in a fetus of three months, found the thyroid gland for the most part arranged into follicles which contained much colloid.

Kürsteiner ('99) in fetuses from 8 to 30 cm. in length found the thyroid lobules made up of round or elongated, solid or hollow follicles. The lumina are few in number up to about 20 cm., but in the older fetuses they are numerous and evenly distributed throughout the gland. In the four older fetuses (17.5 cm.; 21 cm.; 22 cm.; 30 cm.) a few vesicles measuring as much as $1/2$ mm. in diameter were found particularly in the lower and posterior part of the gland. Some branching vesicles were also noted at 17.5 cm.

(p.13)
Prenant ('01) stated that in the embryonic thyroid the solid epithelial cords are transformed into a network of tubes from which the follicles arise by a process of constriction.

Von Ebner ('02) found numerous well developed follicles in older fetuses and newborn. In the adult he described the follicles as of variable size, usually spherical in shape, but frequently elongated. Diverticula may occur, and two neighboring follicles may fuse. Between the follicles are found, even in the adult, frequent solid strings and nests of epithelial cells, which are in the majority during development.

Elkes ('03), who studied the thyroid in fetuses from $4-1/2$ to $6-1/2$ months in age, found that it presents both solid cords and well developed follicles in variable number. In the newborn the earlier follicles have largely disappeared, leaving only a few at the periphery of the gland.

Hertwig ('10) (pp.444-446) described in the embryonic thyroid anastomosing epithelial cylinders. These become tubular; varicose dilations are by ingrowth of the adjacent connective tissue cut off

to form the permanent follicles.

Isenschmid ('10) found that in the thyroid of children the gland grows not only by the increase in the size of the follicles, but by the formation of new follicles by two methods: budding and division. The lumen of the bud may be continuous with that of the mother-follicle, or may arise independently. He found no evidence that follicles are formed from solid cell-masses (interfollicular epithelium) retained from the embryonal period.

Division of a follicle is produced by ingrowth of epithelial spurs from the side wall. Fusion of follicles may also occur. Follicles show the greatest conceivable variation in form, even in the same gland. A relatively large amount of desquamated epithelium was observed.

According to Hesselberg ('10): "Die Ausbildung der Thyreoidea in der fötalen Periode erfolgt durch Zerfall der ursprünglich soliden Zellplatte in solide Zellstränge. Diese schnüren sich zu Bläschen ab, die zuerst am kaudalen Pol auftreten". The normal structure of the thyroid is established from the fourth fetal month on. Desquamation of epithelial cells was found in about half of the cases from the seventh to the ninth fetal month, and the follicles are almost entirely obliterated in the newborn. During the first week of postnatal life the follicles are reformed and increase in number by a process of budding.

Prenant and Bouin ('11) give an account of the development of the median thyroid anlage similar to that given by Prenant ('01).

Broman ('11) described in the differentiation of the thyroid anlage a tubular stage, transformed by constrictions into beaded chains and finally into separate follicles.

According to Grosser ('12), the thyroid anlage begins to separate into solid cords in the human embryo of 8 mm. In the 50 mm. fetus the cords, especially in the periphery, appear beaded. The beaded cords become divided into separate cell-masses, the anlagen of the follicles. The lumina may appear as independent cavities (no tubular stage), before the follicles are detached, or they may arise later, even in early postnatal life.

Simpson ('12) referred to the tubular structure of the thyroid and describes the gland of a seven months old child as tubular in character.

Aschoff ('13) stated that in the developing thyroid^{gland} connective tissue separates round epithelial balls from the anastomosing cords, and it is in these "balls" that the follicular lumina develop.

Sobotta ('15) described the first lumina as appearing in the peripheral parts of the lateral thyroid lobes in a fetus 50 mm. in length. The final breaking up of the cell cords into single groups, which will later form follicles, progresses very gradually, so that the final structure of the gland is arrived at only after birth. Interfollicular epithelium persists, which may later give rise to follicles.

Kingsbury ('15) in describing the early development of the thyroid states that:

"Lumina (follicular cavities) within the cell cords appear at about this period (32 mm.) and at first contain no demonstrable colloid. In the 40 mm. embryo some of the follicles contain colloid, although not much is present in the oldest embryo (48 mm.). At 10 cm. the colloid-containing follicles are numerous."

III. Material and Methods.

This study is based upon the collection of human embryos in the Anatomical Laboratory of the University of Minnesota. Several of the series used are in excellent condition for histological study. The embryos of the collection have been variously fixed and stained. The additional glands specially prepared have, for the most part, been fixed in Formal-Zenker; embedded in paraffin, sectioned at 10 μ , mounted serially and stained with alum-haematoxylin and eosin, or iron-haematoxylin and eosin.

Besides the specimens mounted in the collection, other glands from newborn children and from children in the early years of life were obtained at autopsy. These were used merely for purposes of comparison, and have neither been listed below, nor discussed in this paper.

The following table shows the materials used in this work. The embryos and fetuses used are arranged in the order of their crown-rump lengths. "Minn.E.C." refers to the Minnesota Embryological Collection. An asterisk (*) following the number signifies that the thyroid gland alone was sectioned. Otherwise the entire embryo was available in serial sections.

Serial No.	Minn. E.C.No.	C.R. Length in mm.	Fixation	Section Thickness in Microns.
1	H 6	6.0	Zenker	10
2	H 13	7.5	Alcohol	15
3	H 60	11.0	Alcohol	20
4	H 68	11.0	Bouin	15
5	H 134	12.0	Alcohol	20
6	H 1	15.0	Alcohol	12
7	H 23	15.0	Alcohol	10
8	H 18	15.5	Formalin	10
9	H 28	16.0	Alcohol	10
10	H 62	16.0	Formalin	20
11	H 58	17.0	Formalin	20
12	H 260	18.0	Formalin	15
13	H 24	19.0	Zenker	12
14	H 2	20.0	Picro-sulphuric	15
15	H 7	20.0		10
16	H 265	21.0	Formalin	20
17	H 3	22.0	Zenker	12
18	H 15	22.0	Alcohol	12
19	H 64	23.0	Alcohol-formalin	20
20	H 304	24.0	Zenker	20
21	H 56	24.0	Alcohol	20
22	H 5	25.0	Zenker	15
23	H 21	26.0	Alcohol	15
24	H 29	26.0	Alcohol	12
25	H 99	26.0	Alcohol-formalin	20
26	H 48	27.0	Bouin	12
27	H 10	29.0	Alcohol	15

Serial No.	Minn. E.C.No.	C.R. Length in mm.	Fixation	Section Thickness in Microns.
28	H 375	29.0	Formalin	10
29	H 259	30.0	Formalin	20
30	H 108	30.0	Zenker	10
31	H 57	31.0	Formalin	20
32	H 16	33.0	Zenker	15
33	H 313	35.0	Formalin	20
34	H 122	39.0	Formalin	20
35	H 8	41.0	Formalin	12
36	H 12	41.0	Zenker	15
37	H 121	46.0	Formalin	60
38	H 115	50.0	Formalin	40
39*	H 290	56.0	Formalin	10
40*	H 85	60.0	Formalin	10
41	H 26	65.0	Formalin	30
42*	H 285	66.0	Formalin	10
43*	H 81	83.0	Alcohol	10
44*	H 286	86.0	Zenker	10
45*	H 75	90.0	Formalin	10
46*	H 267	115.0	Formalin	10
47*	H 34	120.0	Formalin	10
48*	H 49	126.0	Bouin	10
49*	H 187	158.0	Formalin	10
50*	H 381	163.0	Formol -Zenker	10

The ordinary reconstruction methods, both plastic (Born's wax-plate method) and graphic, were utilized in the present study. In all cases where a determination of the follicular form or structure was attempted, special precautions were observed in making the reconstructions as accurate as possible.

The drawings for reconstruction were made with the camera lucida on transparent paper. After the drawings were completed, those of successive sections were superimposed upon a tracing-table, and each epithelial structure in the section given a letter or number. The drawings were controlled by careful microscopic observations, to determine the frequently complicated relations of neighboring follicles. By this method it was possible to determine with certainty what the limits of any particular mass or follicle might be.

IV. The Morphogenesis of the Thyroid Follicle.

(a) Prefollicular Period.

A brief consideration of the thyroid gland in the prefollicular period is essential to an understanding of the follicular development. For it is during this prefollicular period that the anlagen of the primitive follicles are derived from the original epithelial mass; and, to a certain extent at least, the size, form, and arrangement of the earlier follicles are thereby predetermined.

The earlier well-known stages in the development of the (median) thyroid anlage in the human embryo are not considered in the present paper. The original thyroid diverticulum becomes detached and transformed into a solid epithelial plate, well shown in the 6 mm. embryo (No.1 of the present series). This and several succeeding prefollicular stages of the thyroid were carefully reconstructed by Born's wax-plate method, but for the present purposes it is unnecessary to figure or describe these models.

As is well known, the (median) thyroid epithelial plate soon presents irregularities, as shown in the 7.5 mm. embryo (No.2), and rapidly becomes transformed into what appears in cross-sections to be (as heretofore almost universally described) a network of anastomosing epithelial cords.

With the details of this process of transformation the present study is not concerned. One feature of the end result, however, which comes out clearly in the reconstructed models, is that the cord-like appearance seen in the sections is largely an illusion. Fundamentally the plate-like structure of the thyroid anlage persists for a considerable time, although somewhat modified by a complicated process of fenestration, splitting, and budding during the

growth of the primitive epithelial plate. The resultant structure consists essentially in a mass of irregular, branching and fenestrated plates, for the most part longitudinally arranged (parallel to the long axis of the body), so that in cross-section they appear as "cords" of epithelial cells (fig.1). This type of structure, with varying degrees of complexity is found in the various prefollicular stages of embryos from about 10 mm. to 22 mm. in length. (Numbers 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 of the present series).

Such a description, however, does not apply to the so-called lateral thyroid anlage (ultimobranchial body). As described by Tourneux and Verdun ('97) and others, this body long remains as a compact, deeply staining mass of epithelium (the "inner condensation" of Kingsbury '14) on the dorso-medial aspect of each lateral lobe. Whether in the human thyroid the lateral anlage later atrophies or finally becomes transformed into permanent thyroid tissue is still uncertain. This region is therefore purposely excluded from the following descriptions, which apply primarily to the process of morphogenesis in the structures derived from the median thyroid anlage.

The thyroid gland in the final stage of the prefollicular period is shown in a fetus of 22 mm. (No.18). This stage will be described in some detail, in order to make clear the subsequent process of morphogenesis of the thyroid follicles. When studied in sections, the thyroid at this stage is seen to be made up apparently of epithelial "cords" which are interrelated in a complex manner (fig.1). A loose, anastomosing network is thus formed, the inter-spaces of which are filled with a vascular mesenchymal tissue (fig.1). The "cords" are, in general, only two cells in width, a

feature characteristic of the thyroid plate and its derivatives at various stages.

Upon reconstruction (figs. 24 and 25) it is found that the epithelial network seen in sections, and described by so many observers as consisting of "rods" or "cords", is merely a section of flat slab-like plates, or bands, representing portions of fenestrated plates. It is true that some few of the epithelial masses are actually cord-like in form, but by far the greater number are better described as bands or plates. A definition of terms is necessary at this point. The term "plate" will be used to signify a structure of relatively slight thickness, presenting expansive surfaces which are more or less smooth, and which may or may not be perforated (fenestrated). A band is a narrow plate. Therefore a fenestrated plate may be considered as made up of a number of anastomosing bands. There is great variability in the way in which these plates and bands are arranged. Some are mere slabs, which may or may not be perforated; others form irregular prisms or rounded cylinders, which open at both ends into the surrounding mesenchyme. The length and width of these plates is also quite variable. In three respects, however, they are in general agreement. They are two cells thick; present fairly smooth surfaces; and are longitudinally placed. Within these epithelial bands (fenestrated plates) the primitive follicles of the thyroid gland develop.

(b) Follicular Period.

A thyroid follicle will be defined as a completely closed sac whose wall is usually made up of only a single layer of epithelial cells. This definition includes all the features of the follicle which may be regarded as absolute and constant. The size and shape of the follicle may vary, and great differences are found in these respects in follicles of the same gland as well as in follicles of fetuses at different stages. Typically the thyroid follicle may be considered spherical or spheroidal in shape; but, as will appear later, this type is subject to considerable variation. The term primary follicle will be used to include those follicles developing independently of preexisting follicles. The follicles derived from preexisting follicles, by budding or otherwise, are termed^{ed} secondary follicles.

In the present series, the first primary thyroid follicles appear in a fetus 24 mm. in crown-rump length (No.20). In this fetus the thyroid gland has essentially the same structure as has that of the last (No.18) described in the prefollicular period, i.e. it is made up chiefly of longitudinally placed epithelial plates or bands, only two cells in thickness. But in this stage, the plates, which have in previous stages been characterized by comparatively smooth surfaces, now present surfaces which are more or less roughened by the appearance of scattered, short ridges (figs.26, 27 and 28). These ridges are never very extensive. They might therefore better be called hillocks or mounds than ridges. They are placed very irregularly with respect to one another, and may appear for the first time in any part of a plate, at its periphery or in a more central region.

When studied in cross sections (fig.2) it is found that these

hillocks are the immediate anlagen of the early thyroid follicles. It is further seen that the hillocks are apparently produced by the concurrence of four different processes in the epithelial^{ura} cells. The first process is that of cell rearrangement, the second that of cell proliferation, the third that of absolute cell growth, and the fourth that of lumen formation. These processes, although described separately, may occur simultaneously.

The first departure from the two-celled plate arrangement, in the process of follicle formation, is found in a rearrangement of the cells of the plate (figs. 2 and 5). The cell outlines can be made out only with difficulty in most cases. But in those places where they can be seen, they bound cells which are more or less columnar in form. The nuclei are ovoidal or elliptical in outline and are placed with their long axes perpendicular to the surface of the plate. Here and there along the course of the plate (figs. 2, 3 and 5), it will be noticed that certain of the nuclei have shifted their axes and have changed their relative positions. Certain of the nuclei have rotated thru an arc of 90° so that their long axes, in their final position, are at right angles to their original position in the plate. As a result of this shifting process, little circlets (really spheres) of nuclei are formed in the plate.

This shifting of nuclei is but the visible expression of the changed position of the cell. For while it is impossible to observe the cell boundaries in most cases, it is hardly probable that the nuclei shift their axes independent of the cytoplasm; moreover, the few faint cell-boundaries which may be made out show the same changes in position as do the nuclei. Further, it is found (figs. 2 and 5) that at the point from which a nucleus has shifted toward the center of the plate, a slight depression appears

on the surface of the plate, indicating that the cytoplasm has shared equally with the nucleus in the movement. From these three facts it may be concluded that the first process manifested in follicle formation is the shifting of the axes of certain cells of the epithelial plate through an arc of 90° . There is no evidence that the depressions on the surface of the epithelial plate are due to invasions or activity of the adjacent mesenchyme (figs. 2, 3, 4, and 5).

This process results in the transformation of the smooth surfaces of the bands (fenestrated plates) into surfaces which are somewhat roughened. The irregularities are apparently not, at first, due to swellings on the plates, but rather to the slight indentations produced by the shifting of certain cells toward the center of the plate as above described. When studied in cross section (fig. 2) such a plate appears as a sort of beaded chain, with alternate swellings and constrictions. But, as noted above, the initial swellings due to this process are only apparent and are actually not greater in thickness than is the plate in other parts of its extent where indentations have not yet occurred.

The extraordinary cellular activity of the epithelium at this stage is clearly manifested by the large number of mitotic figures to be seen. There is no section of the gland in the 24 mm. fetus (No. 20) which does not present several cells in process of mitosis. But the localization of these mitoses is even more significant than is their number. A typical area is shown in figure 6. It will be noticed that the nuclear figures in this area are found only in those places in the epithelial plates where actual thickenings on the plates are being formed. Therefore the little mounds which appear on the plates, as the immediate anlagen of the early follicles, may

be formed not only by the rearrangement of the already existing cells of the epithelial plates, but also by the formation of new cells as well. Consequently, it can easily be seen how the apparent swellings on the plates, produced by the rearrangement of the existing cells, may be transformed into actual swellings, by the absolute increase in number of the cells found in a localized area. These swellings become roughly spheroidal in form.

The third process referred to above is the absolute increase in size of the cells. While the cells are shifting their axes and proliferating, they are also growing in size. This fact results in the paradoxical appearance seen in figure 7, where the solid, two-celled plates are found in some cases to be no greater in cross section than the one-celled plate which surrounds the follicle lumen. It might be thought that the cells do not actually increase in size, but only increase in height by a closer crowding together. But a study of figures 2, 3, 4 and 5 will show that such is not the case. For the cells are not more closely packed together in the newly formed follicles than they are in the two-celled plate.

This progressive increase in the height of the cells corresponds to the progressive stages in the differentiation of the two-celled plate into newly formed follicles. So that the thyroid gland of a 30 mm. fetus (No.30) presents in different regions epithelial cells varying greatly in height. The lowest cells are found at the beginning of the process, in the two-celled plate; the highest being found at the other extreme, in the completely formed follicle.

Three of the four processes above mentioned as apparently involved in the evolution of the follicle from the epithelial plate have now been reviewed in detail. The formation of the lumen remains to be considered. Just preceding the appearance of the lumen,

the spherule (in which it is about to develop) appears in cross section as a circlet of very tall columnar cells, whose nuclei are peripherally placed. This arrangement results in the formation of a striking picture. The nuclei are regularly placed at the periphery of the circle and form a dark band, which surrounds an expansive, clear, central cytoplasmic portion. The magnitude of this cytoplasmic area and the sharp contrast between the two portions (in the stained preparations) are most striking features (figs. 2,4 and 5). It is in the center of this cytoplasmic area that the lumen makes its appearance as a tiny spherical space outlined by a definite and regular margin. It is as though the cells had but drawn a little apart, so that their central ends, instead of remaining in contact one with another, might be separated by an interval. The relation of the early lumina to one another is well shown in figure 8 which is a graphic reconstruction of a plate. It is important to note that no tubular stage is found in the process of lumen formation. The lumina appear as absolutely independent spaces.

As the lumina first appear they apparently have no content; but undoubtedly they contain some substance which is not stained with the ordinary methods, and which increases in amount with the size of the follicular cavity. Certain of the larger lumina (not all of them), which are perhaps older, are found to contain a hazy, granular substance. Typical colloid does not appear until later in the 60 mm. stage (No.40).

The various possibilities as to methods by which the follicular lumen may arise will be considered later in the discussion.

From the time of their first appearance, considerable variability in the size of the lumina found in any particular gland is to be noted. The first isolated follicles are found in the more

peripheral parts of the thyroid gland, and it is in these regions that they first attain large size and considerable complexity. For some time after the formation of follicles has begun, all stages previously described may be found in different parts of the same gland, a considerable portion of which retains the irregular plate-like type of structure characteristic of the prefollicular stages.

In the foregoing account, the cell masses in which the lumina develop have been described as spherules whose cross section is circular in outline. While this is true for typical follicles and in most cases, some variation within comparatively narrow limits is found. Ovoidal or somewhat irregular follicles occur, but these are not more numerous than would be expected in a rapidly growing tissue.

The foregoing descriptions of the early primary follicles have been taken in large part from observations made on two fetuses, one of 24 mm. (No.20) and one of 30 mm. (No.30). The excellent condition of these specimens has made possible studies of considerable detail. The members of the series (Nos. 21,22,23,24,25,26,27,28,29) intervening between these two, although not favorable for such intensive studies, show substantially similar structure. These stages may be summarized very briefly. The comparatively smooth epithelial plates of the prefollicular period have been transformed into plates with rough surfaces. The roughened plates are but the early indication of the follicles about to be formed. With the progressively increasing number of follicles the plates are transformed into bands, which in turn give rise to groups of solid or hollow masses of cells. In the 30 mm. stage, however, the thyroid gland is still largely made up of anastomosing bands (fenestrated plates), although some entirely isolated follicles are found.

While the fetus is increasing in length from 30 mm. to 50 mm. the thyroid gland presents merely a continuation of the process above described; so that by the time the 50 mm. stage is reached the band or plate formations are relatively insignificant, while the isolated epithelial masses make up the greater part of the organ. All stages in the breaking up of the bands or plates may still be found, however. At this stage (50 mm.) the gland is therefore characterized by the presence of few epithelial plates or bands, very many isolated, solid cell-masses (anlages of follicles) and relatively few well developed follicles.

At 56 mm. (No.39) the thyroid gland presents slightly more follicles than at 50 mm. Not only are the follicles increased in number, but they also show some changes in both size and form. In the previous stages the follicles have been relatively of about the same size (^{average diameter} 35 to 55 microns), and typically spherical in form. But in this stage many of the follicles at the periphery of the gland have enlarged considerably and have departed from the more usual spherical form of previous stages. This condition is more strikingly seen in the next fetus of the series (60 mm., No.40) where both increase in size and change in form of the follicles are very evident. The number of follicles at this stage appears about equal to the number of solid epithelial masses. Figure 16 is a graphic reconstruction of a number of epithelial masses from which follicles are being formed and of certain isolated follicles. A study of figure 9 will show follicles situated at the peripheral portions of the lobe which are three or even four or five times as large as those of greatest magnitude located more centrally. The average diameter of the centrally placed follicles is about 15 microns, while the average diameter of the follicles in the peripheral

regions is approximately 55 microns. There is much less absolute variation in the size of the central follicles than in those of the periphery. Centrally placed follicles range from 13 to 17 microns, while peripherally placed follicles range from 20 to 103 microns in diameter. It will further be seen that these same large peripheral follicles are quite irregular in form. Instead of being circular in outline, they have a more or less regularly elliptical or ovoidal form, and may present a number of solid or hollow buds. Figures 13 and 14 represent reconstructions of a typical one of these large budding follicles, and figure 15 shows the same follicle in cross section. Figure 16 is a graphic reconstruction of a number of epithelial masses found in the thyroid gland of the 60 mm. fetus (No.40) to demonstrate stages in the breaking up of the plates and the formation of isolated follicles, which are still present at this time.

The 65 mm. fetus (No.41) is the first member of the series in which practically no epithelial plates or bands are found. From this stage on through the remainder of the series the thyroid gland is made up almost entirely of independent solid or hollow, epithelial cell-masses. The stages (Nos. 42 and 43) included between this embryo and the one of 86 mm. (No.44) may be passed over briefly with the statement that the processes of budding and formation of new follicles just described are advancing rapidly parallel with the increasing length of the fetus.

The 86 mm. fetus (No.44) deserves special attention for it exhibits what appears to be the culmination of the budding processes referred to above. A study of figures 10 and 17 is sufficient to demonstrate the extreme complexity of the gland at this stage. The hollow and solid epithelial masses are still about equal in number.

The extreme variability in the form of the follicles is the most striking feature (figs. 10, 17 and 19), and the reconstructions (figs. 29 and 30) reveal more clearly the complexity of these structures than is apparent in sections. Elongated follicles (figs. 10, 17, 29 and 30) with numerous hollow or solid buds, as well as numerous varieties of other more or less complex arrangements are to be seen. The whole picture is one of active growth. There is great variability in the size of the follicles, the largest being for the most part located in the peripheral zone; but the extreme variation in the follicular form makes absolute measurements for comparison of follicles of little value.

This condition of the gland prevails in the remaining members of the series, up to and including the 158 mm. fetus (Nos. 45, 46, 47, 48, 49). There are but two differences to be noted. In the first place, as shown in figures 9, 10 and 11, the relative number of follicles is increasing from stage to stage, so that by the time the fetus is 158 mm. in length, the follicles are far in excess over the solid, interfollicular masses. Secondly, the number of irregular and complex forms, although still occurring, ^{is} ~~are~~ becoming fewer (fig. 31).

The thyroid of the 163 mm. fetus (No. 50) has quite a different structure from that described for the members of the series just preceding (Nos. 44, 45, 46, 47, 48, 49). The irregular and branching follicular complexes are comparatively few in number, while small, spherical follicles make up almost the whole of the tissue. There are, however, a few solid, interfollicular epithelial masses still present at this stage. A comparative study of figures 10 and 12 will show that the picture has changed from one in which great numbers of new follicles were being formed to one of almost complete

organization. In other words, the epithelial structures of the tissue instead of being complex in their form have become relatively simple in character and for the most part are organized into small follicles.

My material from this stage on up to and including newborn children shows to a greater or less extent the process of epithelial desquamation described in the thyroid follicles by Elkes ('03), Hesselberg ('10), Isenschmid ('10) and others. Whether this process is physiological or pathological is as yet undetermined. Because the material at hand is not sufficient to warrant the drawing of any conclusions in this matter, it is not listed with the materials nor discussed in the paper.

In four of the fetuses studied (Nos. 40, 42, 43, and 44) a number of cyst-like follicles located in all cases in the lower and posterior part of the lateral lobe, were observed. Some of these measure as much as 200 microns in diameter. The size of these structures is not much greater than that of some of the larger normally appearing follicles. But in structure they are quite different, having walls made up of very much flattened epithelial cells, whose nuclei cause the cells to bulge and are separated from one another by much greater distances than is the case with the nuclei in the more usual follicles. The lumina of these cysts are quite regularly circular in outline and in many cases contain a granular substance. It is as though a follicle had been greatly distended, the cells of the wall being stretched and flattened.

5. Discussion and Conclusions.

Remak's theory of the derivation of the thyroid follicles directly from the primitive saccular thyroid anlage has not been confirmed. In the prefollicular stages, the thyroid is by recent investigators quite generally described as assuming the form of irregular, anastomosing "cords" or masses of epithelium. This undoubtedly appears to be the case when sections of the gland are observed (figs. 1 and 7). But the reconstruction methods used in the present investigation reveal a surprisingly different ^{condition} result. It is found that, as a matter of fact, in the great majority of cases the cords are illusions and in reality are merely sections of fenestrated epithelial plates longitudinally arranged.

As to the further steps in the process of morphogenesis of the follicles from these anastomosing "cords", widely divergent views have been held as noted in the section on "Literature". While differing considerably in detail, these views may be classified according to their principal features. The morphogenesis of the primary follicles and of the secondary follicles will be considered in their order.

According to the apparently most widely accepted view, based perhaps largely upon preconceived ideas or theories concerning the evolution of the thyroid gland, there are two distinct stages in the transformation of the epithelial "cords" into follicles. First the anastomosing "cords" acquire lumina, so that the gland becomes a more or less definite network of hollow epithelial tubes. The tubes then become constricted (by ingrowth of vascular connective tissue) into spheroidal segments, each of which becomes a small sac or follicle whose cavity represents a portion of the originally continuous lumen of the tube. This view has been advocated by

W. Müller ('71), Marshall ('93) (in chick and frog), Streiff ('97), Prenant ('01), Hertwig ('10), Prenant and Bouin ('11), Broman ('11) and others. Some like Streiff ('97) and Simpson ('12) have described this branching tubular condition as persisting in part throughout fetal life and even in postnatal life.

Other investigators, however, have described the lumina of the primary thyroid follicles as appearing directly and independently, with no preceding tubular stage. The anastomosing solid cell-cords are usually described as becoming varicose, with successive enlargements and constrictions, so as to present an irregular beaded appearance. Sooner or later each of these spheroidal swollen masses acquires a lumen and becomes separated so as to form an independent follicle. This method of follicle formation (with no tubular stage) has been described by Tourneux and Verdun ('97), Soulie and Verdun ('97), Grosser ('12), Aschoff ('13), Sobotta ('15), and Kingsbury ('15).

It is impossible to decide from direct observation of sections which of the preceding theories is correct. By reconstruction methods, however, both graphic reconstruction and wax-plate models, evidence has been secured in the present investigation which definitely disproves the tubular theory and establishes in the human thyroid the independent origin of the lumina of the thyroid follicles. The follicles, however, appear not in epithelial "cords" as described by earlier observers, but in the fenestrated epithelial plates above mentioned. The view that the thyroid is a modified branching tubular gland (Streiff, Simpson, and others) therefore obtains no support from its morphogenesis, aside from the initial stage of the primitive diverticulum.

In the glands studied in the present series the first follicles appear in a fetus of 24 mm. in length (No.20). This is earlier than the time of appearance described by most observers. His ('85), however, described follicles in a fetus ($Z\overset{w}{W}$) whose absolute length is not recorded, but is placed in series between fetuses of 16.5 and 22.0 mm. in length. Kingsbury ('15) described follicles in a fetus of 32 mm.; Tourneux and Verdun ('97) in one of 32.4 mm.; and Grosser ('12) and Sobotta ('15) in fetuses of 50 mm. These are the only cases found in the literature, where the presence of early follicles has been noted in fetuses of definite length. Several observers refer to the age of fetuses in which the thyroid follicles appear, but in terms too indefinite to be of value for comparison.

Although by definition the prefollicular period ends abruptly with the appearance of the first follicles, it is not true that the structure of the gland undergoes any corresponding sudden change with the ushering in of the follicular period. The epithelial bands (fenestrated plates) are only gradually replaced by the primary follicles, and structures characteristic of the prefollicular period may be present through a considerable part of the follicular period, at least until the fetus has attained a length of 65 mm.

Concerning the first three processes (rearrangement of the cells, cell proliferation, and increase in the size of the cells) involved in the development of the follicle from the epithelial plate, no further discussion is necessary. The fourth process, however, that of lumen formation, calls for further consideration. The follicular lumen might arise in various ways, which have been suggested by earlier investigators. Most of the workers, however, do not mention the process by which the lumen of the follicle is

formed.

Wölfler ('80), and Lustig ('91) have described the formation of lumina in the solid cell masses by a degeneration of the more centrally placed cells. The present investigation does not support this view, however. In the first place none of the so-called central cells have been found; and secondly no evidences of degeneration have been observed in the primary follicles.

It might be supposed, as Hürthle ('94), and Anderson ('94) have suggested, that the lumen is formed by the accumulation of colloid between the angles of the cells which compose the solid mass. Such a process would leave a colloid-containing space surrounded by epithelial cells. But in no case in the present observations was "colloid" found within the very early follicles; although the accumulation of some other (precolloidal?) secretory product between the angles of the cells, might result in lumen formation. The fact is that the smaller lumina, which are probably the most recent in formation, have been universally found to be devoid of any demonstrable content, and that some of the larger and supposedly older lumina do contain a stainable substance. As to the nature of the precolloidal substance or substances, nothing definite is known.

One might suggest that the lumen could be formed by a degeneration or liquefaction of the central ends of the cells which later form its outline. In this case it would be expected that the early follicles would present a lumen outlined by an irregular or ragged margin. Without exception, however, the lumina of the early follicles are clearly outlined and marked off by a very sharp margin.

Having studied the way in which the follicle forms within the plate, it is of further interest to determine the method by which the follicle frees itself from the plate and comes to take up an isolated existence. Earlier observers, in describing the formation of follicles from anastomosing rods or tubes, have laid emphasis upon the activity of the adjacent mesenchymal tissue as the factor operating in the separation of the follicles. In the present study no definite morphological evidence appears in favor of this view. For as shown in the drawings (figs. 2,3,4, and 5) there is no special differentiation of the mesenchyme or increased vascularity in the regions in which follicles are being separated off from the plate. The evidence seems rather to indicate that the follicles themselves are the active agents in their separation. Thus as certain of the cells leave their original positions to assume a position more nearly in the center of the plate, the indentations previously described appear on the plate. These may be considered as weak points. And as the cells increase in number between these indentations it is not difficult to see how the increased pressure due to the increased mass might force certain follicles out from the row in which they formed and thus isolate them from the parent plate. The vascular mesenchyme doubtless takes some slight part in the process, however.

As previously pointed out, the primary follicles may not in every case be at once separated completely from the plate. Instead of being in all cases sharply outlined and spheroidal in form, small portions of the plate (epithelial tags) may be left hanging to the follicle wall. The significance of such epithelial tags is readily understood when it is considered how easily they might be mistaken

for epithelial buds arising from the primary follicle in which secondary follicles were about to form. These structures will be mentioned later in the discussion of the secondary follicles. It has been seen that there is no sharp line of demarcation between the prefollicular and follicular periods. Similarly, in the origin of follicles, the period of primary follicle formation is not sharply marked off from that of secondary follicle formation.

The number of thyroid follicles is apparently not absolutely established, or finally limited at any stage of development (as is the case, for instance, with the glomeruli of the kidney). In the earlier stages, the number of follicles increases by the formation of additional primary follicles. Soon, however, these primary follicles begin to give rise to secondary follicles (at 56 mm.). In later stages the formation of primary follicles apparently ceases, although their occurrence even in the adult has been claimed, e.g. Hürthle ('94), Sobotta ('15), the new-formed follicles being all secondary in character. Various methods of secondary follicle formation have been described.

1. Origin from buds or sprouts. Ribbert ('89), L. R. Müller ('96), Streiff ('97), Isenschmid ('10) and others have described this process. The bud is usually described as a local thickening of the follicle wall, which continues to increase in size by the proliferation of cells, until a solid bud, projecting into the stroma is formed. Directly, through the concentric rearrangement of the cells, the form of the lumen can be made out, even while the young follicle is still in contact with the mother follicle.

2. Origin from collapsed follicles. Biondi ('89), Anderson ('94), and others have described the process as follows. After filling, the vesicle discharges its contents, collapses and finally

rearranges itself in the form of a number of small acini which repeat the process.

3. Origin by fusion of follicles. Streiff ('97), v.Ebner ('02) Isenschmid ('10) and others have observed follicles which are apparently formed by the secondary fusion of two or more preexisting follicles.

4. Origin by division of follicles. Isenschmid ('10) has described the formation of daughter-follicles by the growth of an epithelial spur across the lumen of the mother follicle; and Peremeschko ('67) has noted the formation of secondary follicles by the constriction of the parent follicle.

In discussing my observations concerning the formation of secondary follicles in comparison with those of earlier observers, it may be said at the outset that nothing to support either the second or the third methods just outlined has been noted. These, however, have been described chiefly by previous observers upon postnatal material. The other two methods (budding and division of follicles), however, are in general agreement with the findings of the present investigation.

There appear to be three general methods by which secondary follicles arise in the fetal thyroid, the third of which might be regarded as a modification of the second. But each of these types is subject to a wide degree of variation, so that many intermediate and modified forms are found.

1. Solid epithelial buds may develop on the follicle wall (figs. 19, 22, 23). These may become separated from the parent follicle while in the solid state, or they may develop lumina while connected with the wall of the mother follicle, and subsequently be constricted off. This method is essentially that advanced by

Ribbert ('89), L.R. Müller ('96), Streiff ('97), and Isenschmid ('10). It is difficult, especially in the earlier stages, to distinguish these solid buds from the "epithelial tags" representing persistent portions of the original epithelial plates remaining attached to the earlier primary follicles.

2. Hollow buds whose cavities are continuous with that of the mother lumen are also found (figs. 14 and 15). It might be suggested that these were originally solid buds whose lumen established a secondary connection with the lumen of the parent follicle. While it is difficult to disprove such an occurrence, it would tend to reduce rather than to increase the number of follicles and seems improbable. ~~But stages in such a process have not been observed.~~ The parent follicle apparently sends off extensions or branches, which represent both the wall and the lumen and gradually become constricted off to form new follicles. All possible stages in such a process, from the slightest outpouching of the wall to the finally separated follicle are easily observable (figs. 14, 15, 19, 21 and 23).

3. The third method of secondary follicle formation is the simple division of the parent follicle somewhat as described by Peremeschko. The process appears similar to that of hollow bud formation. The follicle first takes on the form of an elongated ellipse which becomes constricted about its center. No cases of division by the ingrowth of epithelial spurs as described by Isenschmid (for postnatal stages) have been observed in my fetal material.

Having considered the primary and secondary follicles separately it remains to consider them in their relations to each other and to the gland as a whole.

The formation of the secondary thyroid follicles begins when the fetus has reached a length of about 56 mm. From the 70 mm. stage on the formation of secondary follicles progresses with such rapidity that the total number of follicles is very greatly increased within a short period of time, and the relative number of primary follicles becomes progressively smaller.

It might be thought that such elongated, irregular, branched and budding forms as appear in large numbers in certain of the older members of the series (especially in the 86 mm. fetus, No. 43) are derived from irregular, branching bands (fenestrated plates) retained from the prefollicular period. But the evidence disproves that view. After the appearance of the first follicles, the bands and plates are being progressively broken up until the gland parenchyma in a fetus of 65 mm. is practically devoid of such structures, and is almost entirely made up of isolated solid or hollow masses of cells. So closely does this resolution of the plates parallel the increasing length of the embryo up to 65 mm. that it is very improbable that individual variations could explain the presence of these structures in such great numbers at the relatively late stage of 86 mm. Further, as shown in figure 30, forms of all degrees of complexity may be found from simple spheroidal follicles to those of extreme complexity. The conclusion is therefore reached, that these structures, which are better described as follicular complexes than as follicles, are developmentally only follicles which have grown excessively and attained a high degree of complexity.

Such a rapid increase in the number of thyroid follicles as occurs in fetuses between 65 and 158 mm. in length might be expected to produce a marked increase in the size of the gland. But according to Jackson ('09) the growth curve for the prenatal thyroid gland

shows no remarkable increase in the size (weight) of the gland during this period. These two observations, which at first may appear contradictory, are readily explained when the size of the follicles is taken into account. The secondary follicles formed by budding and division of the primary follicles are very small and arise from follicles which are in most cases relatively of much greater size. So that while the number of follicles is greatly increased during this period, the gland mass is not correspondingly larger. As previously described, the formation of secondary follicles becomes less rapid before the fetus has reached 163 mm. in length. From this point on, the number of thyroid follicles apparently increases but slowly, the subsequent growth of the gland being due rather to the increase in the size of the individual follicles, than to a further increase in their number.

The significance of the large cyst-like follicles described in four of the fetuses is uncertain. Kürsteiner ('99) has described the presence of similar follicles in four fetuses. The remarkable regularity with which they were found, in his cases as well as in those of the present series, located in the lower and posterior part of the lateral lobe of the gland, is a striking fact. Possibly they may be related to the cysts of the thyroid gland, frequently met in pathological conditions of postnatal life.

VI. Summary.

By methods of reconstruction (both graphic and wax-plate), the complicated process of morphogenesis of the follicles of the pre-natal human thyroid gland has been worked out and several mooted points definitely established.

1. The so-called "cords" forming the anastomosing network in sections of the thyroid (median anlage) in the later prefollicular stages represent chiefly sections of epithelial bands, two cells in thickness, and forming irregular fenestrated plates.

2. The frequently described stage in which the "cords" are transformed into an anastomosing set of epithelial tubes from which the follicles are derived does not exist. The process of follicle formation gives no evidence or indication that the thyroid has been derived from a branching tubular gland.

3. The primary thyroid follicles arise directly as isolated and independent structures from the epithelial plates of the pre-follicular period, by the rearrangement of cells, cell proliferation, increase in the size of the cells, and lumen formation.

4. The primary follicles appear in fetuses slightly above 20 mm. in length. The epithelial bands (fenestrated plates) have practically disappeared in a fetus of 65 mm., but a few solid inter-follicular epithelial masses are still present in fetuses 163 mm. in crown-rump length.

5. Secondary thyroid follicles are formed from preexisting follicles by three methods: by solid buds; by hollow buds; and by constriction of the parent follicle.

6. The first secondary follicles appear in fetuses about 56 mm. in length, but are formed most rapidly in stages when the fetus

is between 80 and 158 mm. long. After 163 mm., the growth of the gland probably takes place largely by the increase in size of the individual follicles, rather than by increase in their number.

7. Large cystic follicles were observed in the lower and posterior parts of four glands from the older fetuses. Their significance is uncertain, as is likewise the apparent involution of the follicles with desquamation of epithelium observed in the later fetal and newborn stages.

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Fig. 4. A late stage in the breaking up of a plate into follicles. Note the epithelial tags (Ep.T.) attached to the walls of the first, second, and fourth follicles (counted from above downwards). Also note at either extremity of the chain the solid epithelial masses (interfollicular epithelium, Inf.Ep.). B.V., blood vessel; Mes., mesenchyme. X 600.

Fig. 5. A small portion of the thyroid gland of a human fetus 30 mm. long (No.30) highly magnified to show the microscopic structure of the plates. Note the rearrangement of nuclei and the indentations (In.) on the surface of the plate indicating that cells have shifted away from these points. Note the expansive central cytoplasmic areas (C.Cy.A.) in those regions where the cells are beginning to rearrange themselves and where no lumen (L.) has appeared. Cap., capillary; Mes., mesenchyma. X 600.

Fig.6. Semi-diagrammatic drawing (camera lucida outlines) of a small portion of the thyroid gland of a human fetus 24 mm. long (No. 20), to show the number and position of the mitotic figures (M.F.) in the plates which are in the process of follicle formation. B.V., blood vessel; R.Ep.Pl., section through a rough epithelial plate; S.Ep.Pl., section through a smooth epithelial plate. X 600.

Fig. 7. Semidiagrammatic drawing (camera lucida outlines) of a section through the right lateral lobe of the thyroid gland and neighboring structures of a human fetus 30 mm. long (No.30). Shows epithelial plates developing rough surfaces; also early follicles in various stages of separation from the plates. Note the general increase in width of the plates as compared with those shown in figure 1. Cap., capillary; Fol.L., follicle lumen; S.Ep.Pl., smooth epithelial plate; R.Ep.Pl., rough epithelial plate; Mes., mesenchyme; C., cartilage; L., larynx. X 200.

Fig. 8. A graphic reconstruction of parts of two fenestrated epithelial plates from the thyroid gland of a human fetus 30 mm. long (No.30) to show the relative position of the lumina (Fol.L.) as they appear in the plate. Note that in all cases the lumina are quite distinct and never connected with one another. Perf., perforation. X 400.

Fig. 9. Microphotograph of the left lateral lobe of the thyroid gland from a human fetus 60 mm. long (No.40). Note the large irregular and budding follicles in the peripheral regions. Note the smaller follicles and the large amount of solid epithelial material in the central part of the lobe. Colloid in some follicles. A portion of the trachea is shown. X 69.

Fig. 10. Microphotograph of the postero-medial portion of the left lateral lobe of the thyroid gland from a human fetus 86 mm. long (No.44). Note the very numerous large irregular and budding follicles in the peripheral regions and the smaller follicles in the central parts. X 69.

Fig. 11. Microphotograph of a section through the left lateral lobe of the thyroid gland from a human fetus 115 mm. long (No.46). Note the general distribution of the follicles, the larger ones being located in the periphery. The section is taken through the entire lobe so that peripheral regions of the gland are shown both at the upper and lower part of the figure. X 69.

Fig. 12. Microphotograph of a section taken from the periphery into the center of the left lateral lobe of the thyroid gland from a human fetus 163 mm. long (No.50). Note the general distribution of the follicles, their relatively large number and uniform size. X 69.

Fig. 13. Model (reconstructed by Burn's wax-plate method) of a single follicle from the peripheral part of the lateral lobe of the thyroid gland of a human fetus 60 mm. long (No. 42) to show the appearance of buds. X 200 (slightly reduced in photographing).

Fig. 14. Graphic reconstruction of the same follicle shown in figure 13. This figure is reconstructed viewed from the opposite side to that which is shown in figure 13. Note the connection of the lumen of the buds with that of the parent follicle. X 200.

Fig. 15. Drawing of a section through the follicle pictured in figures 13 and 14. Section taken at the level marked a-b on figure 13.

Fig. 16. A graphic reconstruction of a number of epithelial masses from the thyroid gland of a human fetus 60 mm. long (No. 40). Note the various degrees in the breaking up of the plates which are indicated, and the relative positions of the lumina.

Fig. 17. Microphotograph of a portion of the thyroid gland of a human fetus 64 mm. long (No. 44). Note the numerous branching follicles, also the elongated follicles in various stages of division by constriction. The lobule shown in the lower left hand corner is the first section in the reconstruction shown in figure 18.

Fig. 18. A graphic reconstruction of the elongated follicular complex shown in the lower central part of figure 17. Lumina outlined in red. X 400.

Fig. 19. Drawing of a section through "d" of figure 18. Note the irregular form of the follicle, both the lumen and the wall. X 400.

Fig. 20. Drawing of a section through the epithelial plate shown in figure 16 (No. 9). taken at the level marked a-b. X 600.

Fig. 21. Drawing of a small part of the thyroid gland of a human fetus 60 mm. long (No.40) to show the practical impossibility of distinguishing between solid buds from the wall of a follicle and epithelial tags. Also note the tendency shown for the follicle lumen to branch. X 600.

Fig. 22. Drawing of a section of a follicle from the thyroid gland of a human fetus 158 mm. long (No.49). Note the large number of solid buds. X 600.

Fig. 23. Drawing of a small portion of the thyroid gland of a human fetus 163 mm. long (No.50). The drawing shows early stages in the formation of solid buds. X 600.

Fig. 24. Model (reconstructed by Born's wax-plate method) of the left lateral lobe of the thyroid gland from a human fetus 22 mm. long (No.18). Antero-lateral view to show the perforated (fenestrated) plates. Plates present relatively smooth surface. ^{Line} a-b indicates the level of the section shown in figure 1, and the level at which the model was divided to show the relations pictured in figure 25. The carotid arteries are shown in the right hand side of the figure. X 300.

Fig. 25. The lower section of the model pictured in figure 24. The upper portion has been removed in order to demonstrate the relations to the gland mass of the appearances found in cross section. The separation of the two portions is made at the level of the section shown in figure 1 and indicated (a-b) in figure 24. X 300.

Fig. 26. Model (reconstructed by Born's wax-plate method) of the upper portion of the left lateral lobe of the thyroid gland from a human fetus 30 mm. long (No.30). Plates are more broken up than in figure 24 and present surfaces which are relatively rough. The upper parathyroid (P.) is shown. Lateral view. X 200.

Fig. 27. Model (reconstructed by Born's wax-plate method) of a part of a plate from the thyroid gland of a human fetus 30 mm. long (No.30) at a higher magnification, to demonstrate more exactly than shown in figure 26, the appearance and relations of the mounds forming on the surface of the plate. ^{The plane} a-b indicates the position of the section shown in figure 3. Lateral view. X 300.

Fig. 28. Epithelial plate similar to that shown in figure 27. ^{Plane} a-b indicates the position of the section shown in figure 2.

Fig. 29. Wax reconstruction of a portion of a peripheral lobule of the thyroid gland of a human fetus 60 mm. long (No.40).

Fig. 30. A series of follicles reconstructed by Born's wax-plate method to show the varying degrees of complexity. Taken from the thyroid gland of a human fetus 60 mm. long. (No.40).

Fig. 31. Wax reconstruction (Born's method) of a peripheral lobule of the thyroid gland of a human fetus 158 mm. long (No.49).

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