Histological Studies on the Prenatal Development of the Thyroid gland in the Guinea Pig

By

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Introduction

The development of the thyroid gland of higher vertebrates has been extensively studied along pure morphological and histological lines. Recently, the embryonic differentiation of this organ has been often studied in combination with its functional activity, biochemically (Rankin, '41, pigs; Wolff et al., '49, calf), histochemically (McAlpine, '55, rats), by quantitative histometrical measurements (Togari, Sugiyama and Sawasaki, '52, rabbits; Kraicziczeck, '56, chicks), by radioautography and by radiochromatography (Gorbman and Evans, '43, rats; Hansborough and Khan, '51, chicks; Hansborough and Seay, '51, hamsters; Waterman and Gorbman, '56, rabbits). Taki ('58) studied histologically the prenatal development of the human thyroid gland, and suggested the time of onset of functional activity by the occurrence of glycogen and its disappearance in the thyroid primordium. Following previous studies on the embryonic thyroid gland (Sugiyama, '41, albinorats and mice; Togari, Sugiyama and Sawasaki, '52, rabbits; Taki, '58, man) at our Laboratory, the author undertook a study histologically of the prenatal development of the same gland in guinea pigs, in combination with radioautography of I\textsuperscript{131}. The embryonic thyroid gland of guinea pigs has been histologically studied by Rabl ('13; '22) and recently radioautographically by Logothetopoulos and Scott ('56).

Materials and Methods\textsuperscript{1}

A total of 175 embryos of guinea pigs, ranging in crown rump

\textsuperscript{1} Some of the specimens used here were made available by courtesy of Prof. Dr. Hara, Gifu Prefecture University Medical School and assistant Prof. Dr. H. Muto, Nagoya City University Medical School.
length from 5 to 105 mm were used. In embryos under 50 mm, the upper half of the body was fixed. In embryos over 50 mm, the thyroid gland was removed with the trachea and esophagus from the neighboring organs, and then fixed. The fixatives were Zenker, Zenker-formol, Carnoy and Bouin. The materials were embedded in paraffin, sectioned at 6μ transversely or sagittally and mounted serially. The sections were stained chiefly with hematoxylin and eosin, sometimes with Heidenhain's iron hematoxylin. Furthermore, Weigert's resorcin fuchs in was used for demonstrating elastic fibers, azan stain for connective tissue fibers and Bie1schowsky's silver impregnation for argyrophilic fibers. Some of the sections fixed in Zenker-formol fluid and Carnoy's fluid were stained by periodic acid-Schiff's method and Best's stain for demonstrating glycogen and other substances, in combination with saliva digestion.

A single tracer dose of 30 to 40μ of radioactive iodine (NaI131) was injected into pregnant guinea pigs and after 24 hours the animals were killed. Developing thyroid glands were fixed in Bouin's fluid and examined by use of the dental film method and the stripping
film method to determine the presence of accumulated radioactive iodine and its distribution.

Investigations of growth change of the thyroid gland were made by measuring the transverse, ventrodorsal and cephalocaudal diameters of the lateral lobes and the isthmus by the usual method (fig. 1). Furthermore, the average size of the large follicles was measured and shown as an indication of the development of the follicles in figure 2.

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Fig. 2 Development of dimensions averaged of large follicles representative of each embryonic stage. O: long diameter; ●: short diameter.

**Observations**

The Growth of the Thyroid Gland.

In the 5 mm stage the thyroid primordium appeared as several solid epithelial cords arising from the mesobranchial area of the primitive pharynx. The cords were directed ventrad and surrounded the branches of the Truncus arteriosus. Some of them were thickened at their free ends and some were branched. The area, from which the thyroid primordium was derived, showed a shallow depression and suggested the Foramen caecum. In this stage, the thyroid primordium was 205μ in the ventrodorsal diameter and 186μ in the
cephalocaudal diameter (fig. 3).

In the 7 mm stage, the primordium was completely separated from the pharynx, and found as an irregular-shaped structure consisting of cell cords and plates. It was located near the bifurcation of the Truncus arteriosus, at a distance of 335µ ventrad from the Foramen caecum (fig. 4). In the 9 to 10 mm stages, the primordium grew laterad and formed an arched plate consisting of cell cords, plates and conglomerates of various sizes. Its middle part surrounded the ventral wall of the Truncus arteriosus and its lateral parts grew along the external carotid artery (figs. 5 and 7). In the 11 mm stage, the primordium became a mandible-shaped structure, suggesting the most primitive type of the grown gland (fig. 8).

In the 13 mm stage, the primordium moved caudad and was found between the heart and trachea, at a distance of 222µ from the trachea. In the 14 mm stage, the lateral lobe grew to an ovoid structure. In the 15 mm stage, the lateral lobe preceded the isthmus in growth. The parathyroid (III) was lateral to the lateral lobe, and the parathyroid (IV)-ultimobranchial complex dorsal to it (figs. 9 and 10). In the 16 to 19 mm stages, the lateral lobe grew cephalad as a long ellipsoid lying between the trachea and the external carotid artery. The ultimobranchial body was found within the dorsal part of the lateral lobe and the parathyroid (IV) dorsal to the lateral lobe.

After this stage the thyroid primordium grew gradually with some variability in size (fig. 1). The cephalocaudal diameter of the lateral lobe reached near term about 11-fold and the ventrodorsal about 8.3-fold in size, of the 16 mm stage, while the transverse diameter decreased 5.2-fold. In comparison with the lateral lobe, the isthmus was rather large in the early stages, but later was retarded in growth. It was sometimes absent.

Follicle Formation.

The thyroid primordium, which was separated from the pharyngeal floor in the 7 mm stage, consisted of cell plates and cords. In them, epithelial cells were arranged generally in two-cell thickness (fig. 4). This two-cell thickness pattern was maintained until the later stages (figs. 9 and 10). The primordium grew further by increase in cell plates and cords of various sizes. Additionally, cell conglomerates were produced in the 11 mm stage. In these conglomerates, the epithelial cells were arranged regularly perpendicularly to the surface at the periphery, and irregularly in the central zone, where some of them were densely packed and others loosely scattered to form a wide cytoplasmic zone
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(fig. 8). Subsequently, radial cell groups with central intercellular splits were formed in these cell plates, cords and conglomerates. The radial groups suggested the most primitive type of follicle (primitive follicle), and a few appeared already in the 7 mm stage (fig. 4). The plates, cords and conglomerates, therefore, represented basic cell arrangements for producing the primitive follicles during a short period of the early stages. The formation of spaces within these three basic arrangements in the 10 to 11 mm stages (fig. 8) and their opening to the outside by ingrowth of mesenchymal tissue and capillaries in the 13 mm stage served as an additional increase of the three basic arrangements.

In the 13 to 15 mm stages, the primitive follicles increased evidently in number in three kinds of basic arrangement. Their central intercellular splits stained with eosin but were negative to periodic acid-Schiff's stain (fig. 10). In the 16 mm stage, a considerable number of the primitive follicles found in the central zone were transformed into transitional follicles with the formation of distinct cavities. The cavities contained foamy secretory substance faintly stained with eosin and feebly red purple with periodic acid-Schiff's stain. Most of the cell cords, plates and conglomerates were replaced and masked by primitive and transitional follicles which connected with each other (fig. 11).

Transitional follicles grew gradually and were on the average 17.6μ x 15.6μ in diameter in the 25 mm stage. They were regularly round to oval in shape. In the 30 mm stage, some of them became irregular in shape and showed a tubular form. In this stage, the thyroid primordium was composed in major part, of transitional follicles. Only a small number of primitive follicles and cell cords remained in the peripheral zone of the lateral lobe and in the isthmus.

In the 33 mm stage, the majority of transitional follicles were converted into definitive follicles, with the production of typical colloid. They were 27μ x 25μ in diameter and round to oval in shape. Immature follicles still remained in the peripheral zone of the lateral lobe and in the isthmus (fig. 12). After this stage, follicles grew further producing primitive follicles through a budding process.

Follicles.

Follicles were always large and well-developed in the central zone of the lateral lobe, but small in the peripheral zone and in the isthmus (fig. 12). The follicles of the central zone became developed earlier in the 50 mm stage, those of the intermediate zone in the 60
to 85 mm stage (fig. 14), and finally those of the peripheral zone in the 95 to 105 mm stages. This may suggest the centrifugal differentiation of the thyroid parenchyma.

The follicles were associated with each other without open communication of their cavities and formed chains and conglomerates. Silver impregnation showed this clearly and further that these associated follicles separate (dissociate) from each other with age by ingrowth of perifollicular connective tissue and capillaries (fig. 15). The dissociation took place chiefly in the central zone of the lateral lobe and prevailed later over the peripheral zone.

The irregular change in shape of follicles increased in degree from the 50 mm stage on. Some of the large follicles formed nipple-like epithelial projections from their wall, and the nipple sometimes almost came in contact with a nipple of the opposite wall. Others formed hollow protrusions of their wall towards the surrounding connective tissue and the hollow protrusions were almost constricted off by connective tissue (fig. 14).

Epithelial cells.

In the 5 mm stage, the epithelial cells of the thyroid primordium showed no feature different from those of the pharyngeal floor (fig. 3). They showed no distinct cell boundaries. The cytoplasm was small in amount, faintly eosinophilic and little granular. The nuclei were vesicular and of various sizes and shapes. The chromatin reticulum was delicate and loose, and a few chromatin blocks were distributed here. The nucleoli were generally indistinct. Metachromatic chromatin particles and pyknotic nuclei were often found in the boundary between the primordium and the pharyngeal floor (fig. 3). This may suggest an initiation of separation of the primordium from the floor.

From the 7 mm stage, the epithelial cells formed cell plates and cords, rarely primitive follicles, but indicated no feature different from those found in previous stages. Periodic acid-Schiff's stain showed that they contain glycogen granules in the cytoplasm (fig. 6). In the 11 mm stage, the epithelial cells formed another kind of cell arrangement and cell conglomerate (fig. 8).

In the 13 and 15 mm stages, a considerable number of the epithelial cells came to form primitive follicles (figs. 9 and 10). Their cell boundaries were not yet distinct. Best's and periodic acid-Schiff's stains indicated that the epithelial cells contain a small amount of glycogen granules. The nuclei were vesicular, of
various sizes and less chromatic (fig. 10). In the 16 mm stage (transformation into transitional follicles of primitive follicles), the epithelial cells became cubical or low columnar with differentiation of the cell boundaries. The cytoplasm became abundant, more granular and still contained glycogen. The nuclei were smaller.

In the 33 mm stage (the first appearance of typical colloid), the epithelial cells became follicle cells. In large follicles they were low columnar and in small follicles low cubical. They contained somewhat small nuclei with moderately dense chromatin reticulum. The cytoplasm was finely granular, slightly eosinophilic, and contained no glycogen granules. It was often loaded with secretory droplets which stained red purple with periodic acid-Schiff's stain. The positivity remained unchanged by saliva digestion. The epithelial cells of the primitive follicles found in the peripheral zone contained a small amount of glycogen in the cytoplasm (fig. 12).

From the 41 mm stage on, a small number of colloid cells began to appear. The cell body was slenderly pressed by adjacent follicle cells and stained strongly eosinophilic and red purple by periodic acid-Schiff's stain. The positivity remained unchanged by saliva digestion. The nuclei were small, somewhat irregular in shape, more chromatic and sometimes pycnotic.

Mitoses of the epithelial cells were numerous in the early stages. They were more often found in the middle part of the primordium, but little later in the lateral lobe. In late embryonic life they were found often in the walls and buds of the definitive follicles. The mitoses contributed to an increase in size and number of follicles.

Colloid.

In the 7 mm stage, central intercellular splits of primitive follicles contained no stainable substance. The central splits, rather represent apical portions of the epithelial cells and stained strongly with eosin, suggesting probably an initial sign of secretory activity (fig. 4). Later, the central splits became tiny intercellular spaces, but did not contain yet any substance stainable with periodic acid-Schiff's technique (fig. 10). In the 16 mm stage (appearance of transitional follicles), the intercellular spaces grew to small but distinct cavities, containing faintly eosinophilic substance. The substance stained faintly red purple by periodic acid-Schiff's technique and remained unchanged by saliva digestion. The substance was foamy. In the 19 mm stage, the substance became homogeneous but sometimes foamy (fig. 11). Radioautography revealed that radioiodine is collected for
the first time in this stage, probably within this substance. The collection, however, was very small in amount (fig. 17b), but increased in degree later (fig. 17c).

In the 33 mm stage, the substance became typical colloid. The colloid became stained better with eosin and deeply red purple with periodic acid-Schiff's stain, and contained a small number of marginal vacuoles. With age the colloid became concentrated, especially in the follicles of the central zone. In the 50 to 60 mm stages, numerous vacuoles were found (fig. 14). Radioiodine was further accumulated in the colloid, especially in that of the central large follicles (figs. 17 a and b).

Capsule and Interstitial Connective Tissue.

In the 7 mm stage, the mesenchymal tissue around the thyroid primordium was loosely and irregularly arranged without showing any capsular arrangement (fig. 4). In the 15 to 19 mm stages, the mesenchymal tissue suggested an initial form of the capsule, and at the same time represented the outer surface of the neighboring organs. The mesenchymal tissue, later, was transformed into young connective tissue. With the first appearance of the definitive follicles, the capsule became well-developed, and represented at the same time the adventitia of the trachea and the perimysium of neighboring muscles, and further the capsule of the thymus. Fat tissue appeared first in the dorsal and medial parts of the capsule in the 55 mm stage.

The intraprimordial mesenchymal tissue appeared in the 7 to 11 mm stages between cell cords, plates and conglomerates. The mesenchymal tissue surrounded these arrangements with primitive capillaries and later was transformed into young interstitial connective tissue. In the 28 mm stage, a part of it assumed septm-like or interlobular arrangements to form primitive lobules. This interlobular connective tissue was better developed in the central zone of the lateral lobe, and diverged towards the periphery (fig. 13). With the first appearance of definitive follicles, the interlobular and perifollicular connective tissues became mature and densely arranged. They contained argyrophilic fibers in abundance (fig. 15) but no elastic fibers. The argyrophilic fibers surrounded the follicles as a perifollicular layer and further circularly the perifollicular capillaries (fig. 15).

No striated muscle fibers were found in the interstitial connective tissue, but rarely a piece of hyaline cartilage was found here (fig. 16). An islet of thymic tissue was often found.

Blood Vessels.
In early stages, a few primitive capillaries already appeared around the primordium (fig. 4). In the 11 mm stage the capillaries increased in number by repeated mitoses of the endothelial cells and contributed, together with the mesenchymal tissue, to the separation of the growing parenchyma into cell cords, plates and conglomerates. From the 12 to 15 mm stages on, the primitive capillaries became engorged and filled the spaces between various cell arrangements. Some of these capillaries appeared sinusoidal (figs. 9 to 11). The sinusoidal capillaries occurred most markedly during the 28 mm and 42 mm stages. They formed a rich plexus around the follicles and further were distributed numerously in the peripheral zone of the lateral lobe (figs. 13 and 15). After the 42 mm stage, the sinusoidal capillaries decreased in number, and some of them were transformed into the veins. The new formation of capillaries continued still further by repeated mitoses of the endothelial cells.

The arteries of the primordium appeared for the first time in the medial part of the primitive capsule in the 16 mm stage. Then afterwards, the arteries grew and supplied a few branches to the center of the lateral lobe, running along the primitive interlobular connective tissue. After the 41 and 42 mm stages, these branches ran further, producing smaller branches, towards the periphery of the gland. In the 70 to 85 mm stages, some of the arteries came to have a distinct lamina elastica interna, but others were still in development showing mitotic figures of the endothelial cells and muscle fibers. The veins appeared in the medial part of the primitive capsule in the 18 mm stage. Then afterwards, the vein grew and ran with the arteries in the same manner. Near term, some of the veins came to have an elastic layer.

Discussion

Certain stages in the functional differentiation of the embryonic thyroid gland have been elucidated by biochemical, radioautographic, radiochromatographic, histometrical and histochemical methods. These methods have suggested that the differentiation of the thyroid gland occurs in a step-wise manner. The present observations also render it possible to divide histologically the embryonic development of the thyroid gland of guinea pigs into the following three stages in relation to its glandular activity. (1) Early differentiation stage (until 10 mm CRL), (2) Preparatory differentiation stage (about 11 to 30 mm sub-
divisible into the first (11 to 15 mm) and the second parts (16 to 30 mm), and (3) Follicle stage (from about 33 mm to full term). This is in general agreement with the data of Togari, Sugiyama and Sawaasaki ('52, rabbits) and Taki ('58, man).

In the early differentiation stage, the thyroid primordium presents no characteristic endocrine architecture, but growth through repeated mitotic division of the epithelial cells, including attainment of its definitive form and topographical location, proceeds with apparent relation with the vascular system. Investigations have been extensively made on the early development corresponding to this stage (Kölliker, 1879, rabbits; Born, 1883, pigs; Grosser, '11, man; Rabl, '13, guinea pigs; Sotomura, '33, mice; Takashima and Hara, '34, man; Crisan, '35, mice; Selle, '35, bats; Fujita, '50, man, bats, mice and rabbits; Terada, '54, cats; Taki, '58, man). These results showed agreement in principle, except for some slight difference, that the thyroid primordium arises as a single or a few epithelial evaginations of various shapes from the pharyngeal floor, is constricted off from here, moves caudad towards the bifurcation of the Truncus arteriosus, and finally is located at the front of the trachea as an U-shaped structure. The present results also agree with these results. The thyroid primordium arises as several solid epithelial cords from a shallow depression (Foramen caecum) of the pharyngeal floor in the 5 mm stage, is separated from here and becomes situated near the bifurcation of the Truncus arteriosus in the 7 mm stage. The primordium moves further caudad with the development of the initial lateral lobes, and reaches the definitive location (anterior to the trachea) (figs. 3 to 7). But the initial pattern of development is a little different from most of the data, but in agreement with the data of Rabl ('13, guinea pigs), that the primordium occurs as a group of several “Schläuache”.

The isthmus is well developed in comparison with the lateral lobe in the early differentiation stage. The thyroglossal duct is not found and this is in agreement with the results of Kingsbury ('15, man), and Sugiyama ('41, rats and mice). However, the rare occurrence in this stage of small cell cords arising from the middle part of the primordium may suggest a transient and inconstant formation of the thyroglossal duct (cf. Norris, '18, man).

The primordium, even at the end of this stage, presents a little differentiation and consists of a number of cell plates with few cell cords and radial cell groups (most primitive type of follicle).
The next stage is the preparatory differentiation stage which includes the preparatory process for the formation of the definitive follicles. The first part contains the formation of basic cell arrangements (cords, plates and conglomerates) for producing the primitive follicles and the production of the primitive follicles in the basic cell arrangements (figs. 8 to 10). The second part (16 to 30 mm CRL) contains the increase of the primitive follicles, their conversion into transitional follicles and the beginning secretion in the transitional follicles (fig. 11). This preparatory differentiation appears to proceed from the central zone of the primordium towards the peripheral zone (centrifugal differentiation). This result is in general agreement with that of Wölfler (1880). The primordium comes to have an intimate relation with the developing vascular system. Cell cords and plates are surrounded by ordinary and sinusoidal capillaries, and later primitive and transitional follicles are enclosed by them (figs. 9, 10, 11 and 13).

There are two opposite views concerning the initial process of the follicle formation. Kölliker (1879, rabbits), Streiff (1897, man), Hertwig ('10, man, vertebrates), Broman ('11, man), Hammer ('25, man), Pulaski ('29, man) and Stefko ('34, man) stated that anastomosing epithelial cylinders become tubular, in which varicose dilatations occur and then are constricted off into primary follicles. Contrary to these investigators, Grosser ('11, man), Sobotta ('15, man), Rabl ('13, guinea pigs), Norris ('16, man), Sotomura ('33, mice) and Sugiyama ('41, rats and mice) observed the direct formation of the primitive follicles in the cell cords and plates without the formation of the tubules. The present result is also in agreement with those of Grosser and others. Recently, Fujü ('50, man, bats, mice and rabbits), Terada ('54, cats) and Takí ('58, man) described similar results.

The mechanism of formation of the follicle cavity has been differently discussed. One is that the cavity is made by disintegration of the cells centrally located in the follicle primordium (Wölfler, 1880, man; Bozzi, 1895, man and mammals; Lobenhoffer, '09, man; Krause, '14, man). The other is that it is formed by accumulation of precolloidal substance between the cells of the follicle primordium (Norris, '16, man) and still another that it originates from central intercellular splits arising in the cell mass or in the radial cell group (Rabl, '22, guinea pigs; Sugiyama '41, rats and mice; Takí, '58, man). The present result shows that the cavity is
derived from the central intercellular split in the primitive follicles. The split is well dyed with eosin, but completely negative with periodic acid-Schiff's stain (fig. 10). This may suggest that the secretory polarity of the epithelial cells occurs already before the initiation of secretion, and the secretion is not previous in time relation to the formation of the cavity.

The initiation of secretion in guinea pig embryos is found as a faintly eosinophilic substance in the small cavity of the transitional follicles in the 16 mm stage. The secretory substance also stains feebly with periodic acid-Schiff's technique and corresponds with the precolloidal substance (Norris, '16, man; Togari, Sugiyama and Sawasaki, '52, rabbits) and with the half-mature colloid (Taki, '58, man). The epithelial cells of the transitional follicles still contain glycogen granules (fig. 11). This agrees with Taki's result.

Notwithstanding this histological half-maturity, the primordium indicates an initiation and persistence of a collection of radioiodine from the 19 mm stage on (figs. 17b and c). This phase is included in the second part of the preparatory differentiation stage.

Logothetopoulos et al. ('56) found positive autoradiographs in the 28-day-old fetal thyroid gland of guinea pigs and described that the first positive photographic images correspond to the small colloid accumulation. According to Waterman and Gorbman ('56), organic binding of radioiodine from the blood in the fetal rabbit thyroid gland begins at least as early as the 17th day of gestation, which is several days prior to the appearance of colloid and follicles. Jost et al. ('49) found in rabbit embryos that the fixation of radioiodine occurs at 20 days of gestation and appears more active at 22 days when follicles contain colloid. Earlier, Gorbman and Evans ('43) reported that the functional ability to store radioiodine in rats is correlated with the first differentiation of follicles with lumina. The human fetal thyroid gland does not collect radioiodine in the first 12 weeks of life and the onset of function as measured radioautographically is associated with the appearance of definitive follicles with colloid (Chapman and others, '48). Hodges and others ('55) found the functional activity to collect radioiodine around the 12th week in the human fetal thyroid gland by radioautography. Avanzini et al. ('55) found that the human fetal thyroid gland can store radioiodine as early as the beginning of the third month.

Just before the end of the second part (28 mm CRL), the capillary
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Engorgement occurs and persists towards the beginning of the next stage (fig. 13). It is supposed from this result that the engorgement may have some relation with the secretion of typical colloid.

The 30 to 33 mm stages may be suggested as a turning point. The follicle stage starts at about the 33 mm stage (31 days of gestation) with the first appearance of definitive follicles (fig. 12). The time of first appearance may depend upon the animal species, the length of gestation period and furthermore on the difference of the methods used (Aron, '26, sheep-9 cm; Kull, '26, rats-20 to 21 days; Bardertscher, '18, pigs-75 cm; Abbott and Pendergast, '37, cows-2 months; Ramsay, '38, cats-40 to 41 mm; Sugiyama, '41, rats-25 mm and mice-18 mm; Fujù, '50, man-73 mm, mice-19 days, rabbits-32 mm; Terasa, '54, cats-33.5 to 37.5 mm; Togari, Sugiyama and Sawasaki, '52, rabbits-46 mm, 21 to 22 days; Jost, '53, rabbits-22 days).

With persistence of the same pattern of differentiation as that of the previous stage (centrifugal differentiation), the thyroid gland indicates that the follicles are larger in the center of the gland than at the periphery (figs. 12 and 14). It may suggest the so-called two different zones of Wölfier (1880), medullary and cortical. This pattern is in marked contrast with that of other mammals (Sugiyama, '41, rats and mice; Gorbman and Evans, '43, rats; Taki, '58, man). Bargmann ('39) described in newborn puppies a distribution of follicles similar to that of guinea pig embryos.

The follicles grow gradually in size (fig. 2) and increase in number. Several different opinions concerning the formation of new follicles have been presented. 1. Epithelial cell buds or sprouts produced from the follicle wall form new follicles (Ribbert, 1889, dogs; Müller, 1896, man; Norris, '16, man; Weggelin, '26, man; Yamashita, '27, rats; Wilson, '27, man; Florentin and Grujic, '29, guinea pigs; Spöttel, '29, sheep; Cowdry, '34, man; Wetzel, '36, man; Sugiyama, '41, rats and mice). Norris ('16, man) described additionally the formation of hollow buds from the follicle walls, from which they later are constricted off into new follicles. Florentin ('26) found in guinea pigs that the plasmodia originating from hypertrophic nuclei through amitosis separate from the follicle walls and are converted into new follicles. Florentin ('26) found in guinea pigs that the plasmodia originating from hypertrophic nuclei through amitosis separate from the follicle walls and are converted into new follicles. 2. Collapsed follicles are rearranged into a number of new follicles (Andersson, 1894, mammals; Zechel, '31, dogs; Herrmann, '33, pigs). 3. Fusion of follicles to a new follicle (Streiff, 1897, man; v. Ebner, '02,
Division of follicles by the formation of nipple-like septums of the follicle walls (Isenschmid, '10, man; Yamashita, '27, rats; Spöttel, '29, sheep; Norris, '16, man). The present result indicates that the formation of new follicles proceeds chiefly by a budding process through mitotic division, from the follicle walls. The nipple-like septums may be supposed to take only a minor role in producing new follicles (fig. 14) and it may be suggested rather as a sign of increased thyroid activity.

An exocrine glandular pattern has been reported to exist in the thyroid lobule. Central large follicles in the lobule and peripheral small follicles have been considered to represent collecting ducts and terminal portions, and further their cavities have been described to communicate with each other (Hammer and Loeschcke, '34). The development of this pattern is not found both in the preparatory differentiation stage and in the follicle stage. A number of irregular-shaped follicles resemble partly in appearance the exocrine glandular pattern (fig. 14), but represent not always a constant usual pattern of the normally developing parenchym.

The thyroid parenchym of guinea pigs in late embryonic life resembles in structure those of rats and mice (Sugiyama, '41) and rabbits (Togari, Sugiyama and Sawasaki, '52). This data is in agreement with some of the data of Heidenhain ('21). The follicles of various sizes and primitive follicles connect with each other without communication of their cavities and form the follicle groups. The parenchym of guinea pigs present an association type of follicle, but later a dissociation type to some extent (fig. 15). This association pattern may suggest a sign of proliferative activity through budding process of the follicles, and, on the other hand, may be interpreted as a sign of the remnants of the pattern of cell cords and plates.

In the follicle stage, the epithelial cells become the follicle cells, and most of them lose their glycogen granules, except for the cells of the immature follicles placed peripherally (figs. 12 and 14). The same fact has been confirmed by Takei ('58) with human fetal materials. A small number of colloid cells appear for the first time from the 41-mm stage on. Some of them have pyknotic nuclei. Rabl ('22) also found them in the 50 mm stage of the same animals. They may be interpreted as a kind of degenerating or
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underfunctioning cells. It is very difficult to find interfollicular cells. Sugiyama ('41) found them in mouse fetuses near term but none in rat fetuses. Takî ('58) found the so-called interfollicular cells to be merely a tangential section of small follicles or primitive follicles.

The typical colloid found in the follicle stage is often vacuolated, stained moderately eosinophilic, and deeply red purple by periodic acid-Schiff's technique. The colloid stained like this has been interpreted as showing glycoprotein reactions (Dempsey, '55).

Radioiodine is collected within the colloid (figs. 17 a and d). The desquamation of follicle cells into colloid (Hesselberg, '10, human fetuses; Stämmleer, '15, human fetuses) may be considered as a kind of post mortem change in general agreement with the results of Wegelin ('26) and Pulaski ('29). No desquamation of follicle cells is found in guinea pig embryos.

With completion in differentiation of the definitive parenchym in the follicle stage, the vascular system also reaches a status almost completed in structure. The capillaries forming a rich plexus about the follicles appear. The capillary engorgement is also found usually in the follicle stage, especially at its beginning (fig. 15).

Summary

A total of 175 guinea pig embryos were used for this study, and certain phases of glandular activity in prenatal life were elucidated histologically and radioautographically. The thyroid development is divisible into the following three stages: (1) early differentiation stage, (2) preparatory differentiation stage, and (3) follicle stage.

The early differentiation stage (until 10 mm CRL) includes the following. In the 5 mm stage the thyroid primordium appears as several epithelial projections from the pharyngeal floor. Separated from here in the 7 mm stage, it moves ventrocaudad growing into a mandible-shaped structure and finally lies anterior to the trachea in the 10 mm stage. During this stage the epithelial cells are immature and contain glycogen granules. The nuclei are vesicular and of various sizes.

The preparatory differentiation stage is subdivisible into two parts: The first part (11 to 15 mm) includes the formation of basic cell arrangements (cell plates, cell cords and cell conglomerates) for producing the primitive follicles and the production of the primitive
follicles. The second part (16 to 30 mm) includes increase of the primitive follicles and their conversion into the transitional follicles.

From the 19 mm stage onwards, an ability to store radioiodine is established with a slow increase of secretory substance in the transitional follicles. The secretion within the cavities is positive to periodic acid-Schiff's stain. The epithelial cells are still immature and contain glycogen granules. The capillary engorgement occurs from the end of this stage and persists until the beginning of the next stage.

At the 33 mm stage, the follicle stage starts with the formation of definitive follicles with typical colloid. The colloid is stained red purple by periodic acid-Schiff's stain. Most of the follicles associate with each other and form follicle groups without open communication of their cavities. Later, a considerable number of them separate from each other by ingrowth of connective tissue. The epithelial cells become the follicle cells and lose their glycogen granules. The follicle cells contain often periodic acid-Schiff positive secretory granules. Some of the follicle cells are converted into colloid cells, whose cytoplasm is intensely positive to the same stain. The collection of radioiodine is gradually increased in this stage.

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Explanation of Plate Figures

3 to 17: Photomicrographs of transverse sections of thyroid glands of guinea pig embryos.

3. 5 mm CRL stage. The thyroid primordium appears as several solid epithelial cords arising from the floor of the primitive pharynx, and surround the dorsal part of the bifurcation of the Truncus arteriosus. Epithelial cells are found without distinct cell boundaries. The nuclei are vesicular, less chromatic and of various sizes. Zenker-formol. Hematoxylin-eosin. ×400.

4. 7 mm CRL stage. The thyroid primordium is situated on the bifurcation of the Truncus arteriosus, separated from the pharyngeal floor, and forms an irregular-shaped structure consisting of cell cords and plates. The epithelial cells are arranged in two-cell thickness and some form radial arrangements with central intercellular splits (primitive follicles). Zenker-formol. Hematoxylin-eosin. ×220.

5. 9 mm CRL stage. The thyroid primordium grows laterad, and is composed of cell cords and plates. The epithelial cells are arranged in two-cell thickness and radially in the cords and plates. Zenker. Hematoxylin-eosin. ×140.

6. 9 mm CRL stage. The epithelial cells contain a small amount of glycogen. Zenker-formol. Periodic acid-Schiff's stain. ×280.

7. 10 mm CRL stage. The thyroid primordium grows further laterad to form an arched structure. Cords, plates and radial arrangements (primitive follicles) of the epithelial cells are seen. Zenker-formol. Periodic acid-Schiff's stain. ×70.

8. 11 mm CRL stage. The thyroid primordium becomes a mandible-like structure consisting of the lateral lobes and isthmus, and is situated ventral to the trachea. Cell cords, plates and conglomerates are seen. Some of them contain intraepithelial spaces (arrows). Zenker-formol. Hematoxylin-eosin. ×70.

9. 15 mm CRL stage. The cell cords form anastomosing networks, in which the epithelial cells are arranged in two-cell thickness or radially as primitive follicles. Within the networks, sinusoidal capillaries are found. Bouin. Hematoxylin-eosin. ×70.

10. 15 mm CRL stage. The cell cords and primitive follicles are seen. The central intercellular splits contain no secretory substance that stain with periodic acid-Schiff's stain. The epithelial cells of the thyroid primordium contain a small amount of glycogen, while those of the ultimobranchial body contain a great amount of it. Zenker-formol. periodic acid-Schiff's stain. ×280.

11. 19 mm CRL stage. Transitional follicles and primitive follicles connect with each other directly and form anastomosing beaded chains. Transitional follicles contain foamy substance, which is faintly positive to periodic acid-Schiff's stain. The positivity is not changed by saliva digestion. The epithelial cells contain a small amount of glycogen. Zenker-formol. Periodic acid-Schiff's stain. ×280.

12. 33 mm CRL stage. Definitive follicles make their first appearance at this stage, and contain typical colloid which stains deeply red purple with periodic acid-Schiff's stain. Definitive follicles are larger in the central zone of the lateral lobe than in the peripheral zone. The cells of peripheral small and immature follicles still contain a little amount of glycogen. Carnoy. Periodic
13. 28 mm CRL stage. Numerous transitional follicles are seen. Numerous sinusoidal capillaries are seen in the peripheral zone of the gland. Connective tissue forms interlobular arrangements. Zenker-formol. Hematoxylin-eosin. ×70.

14. 60 mm CRL stage. Large follicles are seen in the central zone and small follicles in the peripheral zone. Nipple-like projections of the follicle wall are sometimes seen. Zenker-formol. Periodic acid-Schiff's stain. ×70.

15. 35 mm CRL stage. Argyrophilic fibers are seen in the interlobular and capsular connective tissues. They form further a perifollicular layer around the follicles. Follicles still remain connected with each other. Zenker. Bielschowsky's silver impregnation. ×70.

16. 100 mm CRL stage. A piece of hyaline cartilage is seen in the center of the lateral lobe. Bouin. Hematoxylin-eosin. ×400.

17. Radioautographs of the thyroid glands at different stages. a: 85 mm CRL stage. ×70. b: 19 mm CRL stage. ×20. c: 23 mm CRL stage. ×20. d: 33 mm CRL stage. ×20.

Abbreviations

car ........................................ carotid artery
es ........................................ esophagus
h ............................................. heart
par .......................................... parathyroid
pf .......................................... primitive follicle
ph ............................................ pharynx
sc ........................................... sinusoidal capillary
sp ........................................... intraepithelial space
thy .......................................... thyroid primordium
tra ........................................... trachea
trb ........................................... truncus arteriosus branch
trf .......................................... transitional follicle
ult .......................................... ultimobranchial body
Plate II

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