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Cytochemical Studies on the Follicles in Sheep Parathyroid Gland

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Summary

Follicles observed in the parathyroid gland of one sheep were examined by histochemical and ultrastructural methods. The follicles were irregularly distributed in the gland parenchyma. They consisted of chief cells, but not of water-clear cells and oxyphil cells. Two types of the follicles, dark and light, were observed. The dark follicles contained glucoprotein of non-amyloid type, and the others were negative to all histochemical methods used. The dark and light follicular cells had higher NADPH dehydrogenase activity than the chief cells of parathyroid gland devoid of the follicles.

In electron microscopy, the light follicles were empty-looking, but sometimes a few cilia were observed. The follicular cells were composed of the light cells in which zonula adherences with membrane densification, abundant secretory granules, bundles of filaments and microvilli were observed. The follicular cells consisted of various cell types. Some were similar to the light follicular cells, while others were rich in filaments. Some dark cells had well-developed Golgi aparatus and RER. The morphogenesis of the various parathyroid follicles was suggested.

It is well known that follicles sometimes occur in the parathyroid glands of various species. In human parathyroid glands, they appear in hyperplastic-, adenomatous or normal glands and are most frequent in old age (1–11). The follicles contained amyloid with some other materials detected by histochemistry. In electron microscopy, the follicles contained amorphous materials, blood corpuscles, desquamated and often degenerated epithelial cells, and bundles of filaments and fine dense granules (4, 5, 7). Intrafollicular amyloid deposit may be a marker for secretion of a peptide closely related to calcitonin (5).

As far we are aware, there is no report on the occurence of parathyroid follicles in the sheep. This report represents a combined histochemical and ultrastructural study on sheep parathyroid gland follicles, to clarify their structural as well as functional significance.

Experimental Procedure

The outer parathyroid glands of 15 sheep under various feeding and various ages were examined.

Histochemistry

The parathyroid glands were collected immediately after slaughter and were frozen in a mixture of dry ice and acetone. Frozen sections were cut with a cryostat microtome, mounted on clean glass slides and dried at room temperature for 30 minutes. For histochemistry the sections were stained with hematoxylin-eosin, periodic acid Schiff (PAS), congo red, crystal violet, methyl violet, acrolein-Schiff, ninhidrin-Schiff, and tryptohan (DMAB) methos (12).

The enzymes demonstrated by histochemistry were NADH dehydrogenase (NADH-DH), NADPH dehydrogenase (NADPH-DH), succinate dehydrogenase (SDH), α-glycerophosphate dehydrogenase (α-GDH), β-hydroxy butyrate dehydrogenase (β-HDH), alkaline phosphatase (ALPase), and acid phosphatase (ACP ase) (12–14).

Electron microscopy

The outer parathyroid glands were fixed for 2 hours in a cold 3% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2). The tissues were then washed in 4 changes of the same buffer and then were post-fixed for 2 hours with 1% osmium tetroxide in the same buffer. They were then dehydrated in a graded series of acetone and embedded in Epon 812 routinely. Ultrathin sections cut with a LKB 4800–A ultrotome were stained with uranyl acetate and lead citrate and were viewed by a JEM 100-B electron microscope.

Results

Light microscopy

The follicles were observed in the parathyroid gland of one out of 15 sheep examined. They were irregularly distributed in the parenchyma of the gland. The follicles were round in shape in sections and consisted of a few or several epithelial cells. The contents of the follicles varied. Some were light and empty-looking, while others were dark and contained homogeneous materials in the lumen (Fig. 1). The parathyroid gland with the follicles consisted of lobules of the chief cells, separated by fibrous stroma. The glands had few lipid droplets. Water-clear cells and oxyphil cells were not present. The parathyroid glands devoid of such follicles consisted of ordinary chief cells, exclusively.

Histochemical study

As present in Table 1, the dark follicles were positive to PAS, acrolein-Schiff, and ninhidrin-Schiff reactions and were stained by eosin (Fig. 2). This showed

TABLE 1. *Histochemical reactions of light and dark follicles.*

Reaction or staining	Light follicle	Dark follicle
PAS reaction	−	⫲
Arcolein Schiff	−	⫲
Ninhidrin Schiff	−	+
Eosin	−	⫲
Tryptophan (DMAB)	−	−
Congo red	−	−
Cristal violet	−	−
Metyl violet	−	−

TABLE 2. *Enzymehistochemical reactions of follicular cells, and ordinary chief cells.*

	follicular cell	ordinary chief cell
NADPH-DH	⫲	+
NADH-DH	⫲	⫲
SDH	⫲	⫲
α-GDH	⫲	⫲
GDH	±	±
β-HDH	±	±
ALPase	⫲	⫲
ACPase	⫲	⫲

that the follicles were composed of glycoprotein, but contained no amyloid. The light follicles were negative to all the histochemical reactions tested.

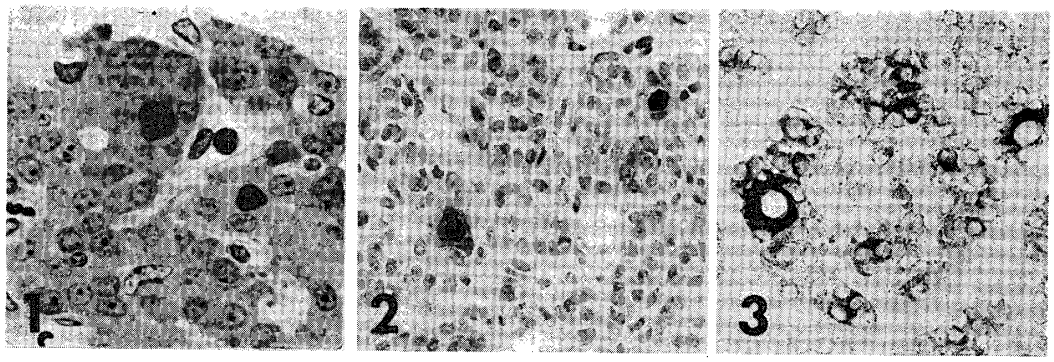
Enzyme-histochemistry

As seen in Table 2, the follicular cells showed a few distinct differences from ordinary chief cells. A high NADPH-DH activity was noted in the former cells, in contrast to the weak activity in the latter cells (Fig. 3). The activities of other enzymes of follicular cells was similar to those of ordinary chief cells.

Electron microscopy

The parathyroid follicles in sheep consisted of lobules of glandular cells. The parenchymal cells of the gland consisted of cheif cells. Lymphoid cells were often found in the center of the lobules (Fig. 4). The intercellular spaces between the glandular cells were enlarged with or without any distinct zonula adherence. The cells had a moderately dense matrix, well developed Golgi apparatus and RER, and few lipid droplets. Secretory granules were also observed in their cytoplasm close to intercellular spaces. The lymphoid cells in the center of the lobules were in contact with surrounding cheif cells by simple apposition (Fig. 4).

A few cilia were present in the lumen of the light follicles. The follicular cells had distinct zonula adherences with membrane densification near the luminal surface of plasma membrane (Fig. 5). The follicular cells had many microvilli,



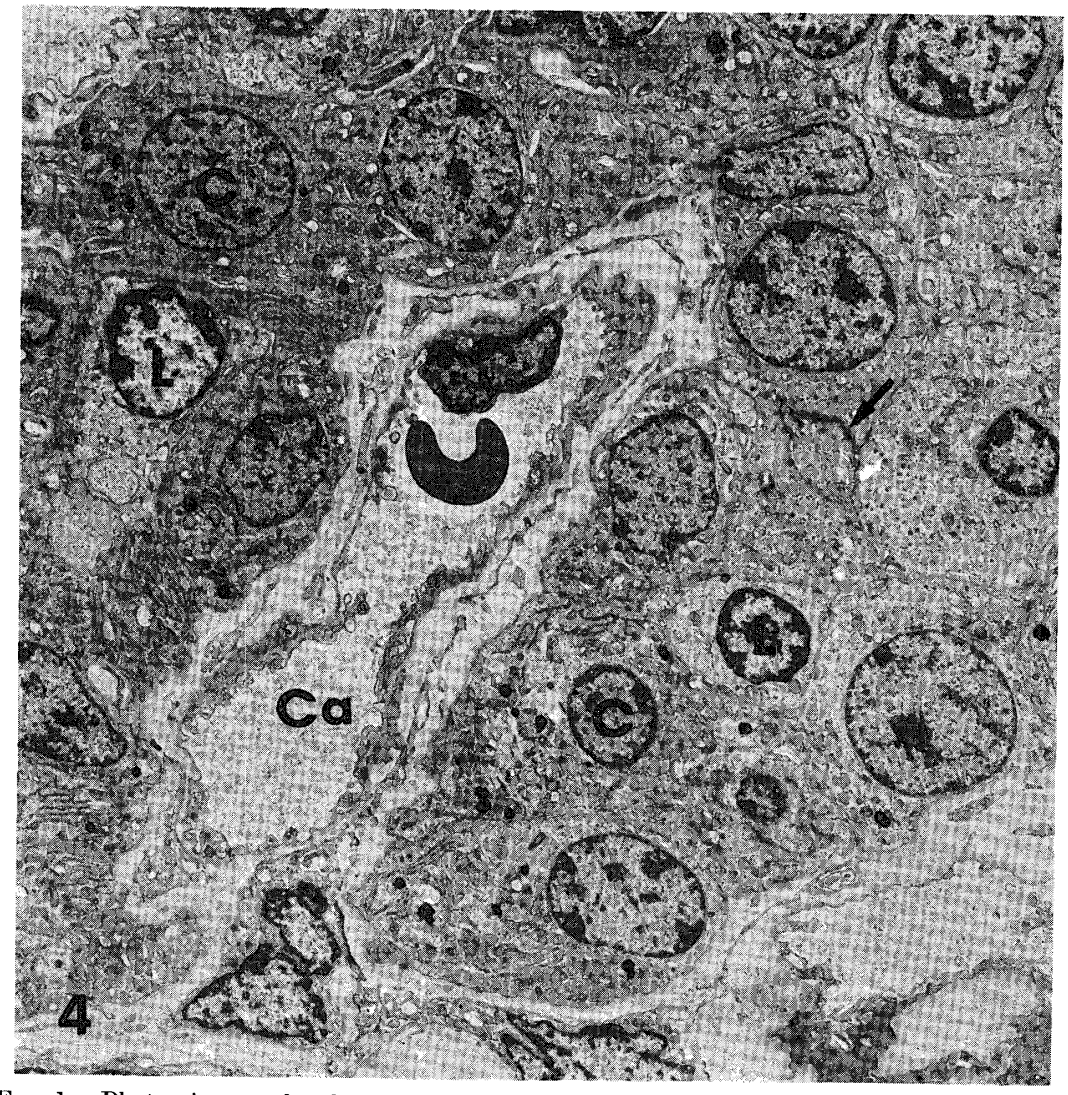
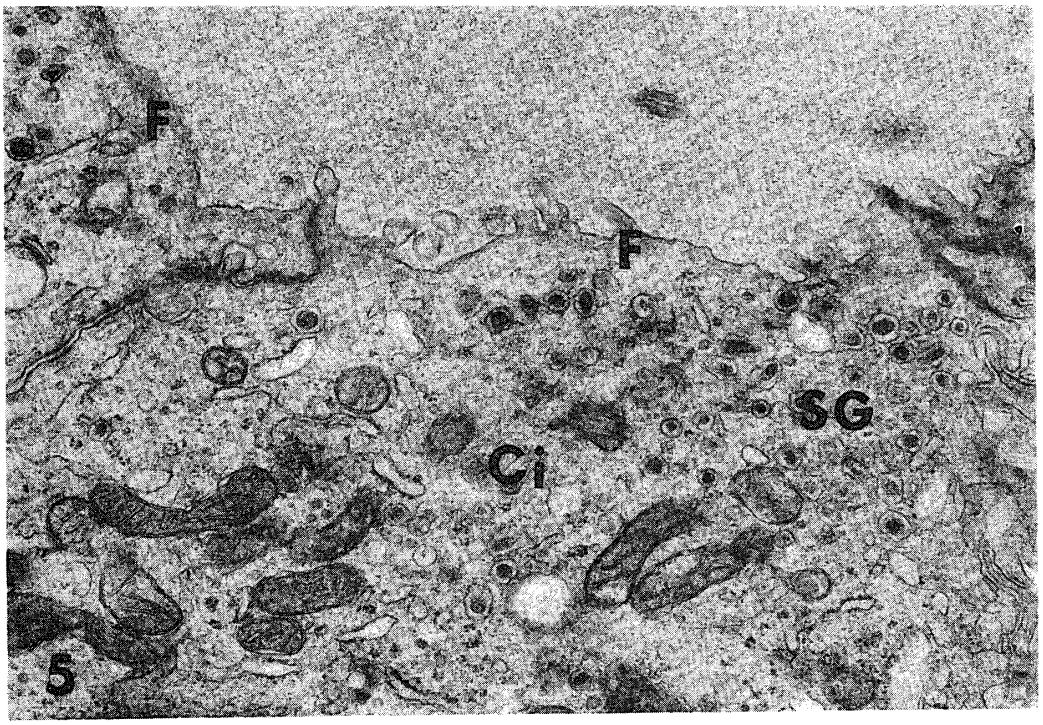


FIG. 1. Photomicrograph of dark and light follicles of sheep parathyroid gland. One micron epon section. Toluidine blue staining. × 480.

FIG. 2. PAS positive dark follicles. PAS staining. × 240.

FIG. 3. NADPH dehydrogenase reaction of parathyroid gland. Follicular cells exhibit intense reaction. × 240.

FIG. 4. Electron micrograph of sheep parathyroid gland consisted of the lobules of chief cells and lymphoid cells. Lymphoid cells are found in the center of the lobules. Arrow indicates zonula adherences with membrane densification of the plasma membrane in intercellular space. C: Chief cell, Ca: Capillary, L: Lymphoid cell. × 3120



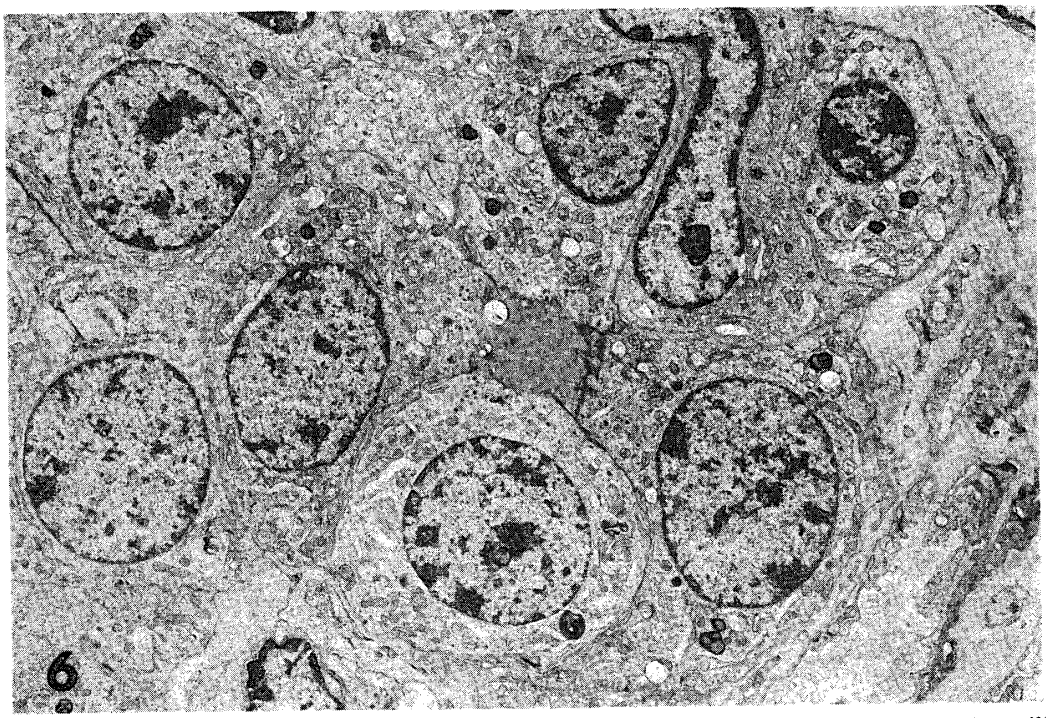


FIG. 5. Light follicle. The follicular cells exhibit many secretory granules and microvilli, Ci: Cilia, F: Fibrils. SG: Secretory granules. × 29,000

FIG. 6. A type follicle. Follicle is filled with amorphous dense material. × 6480

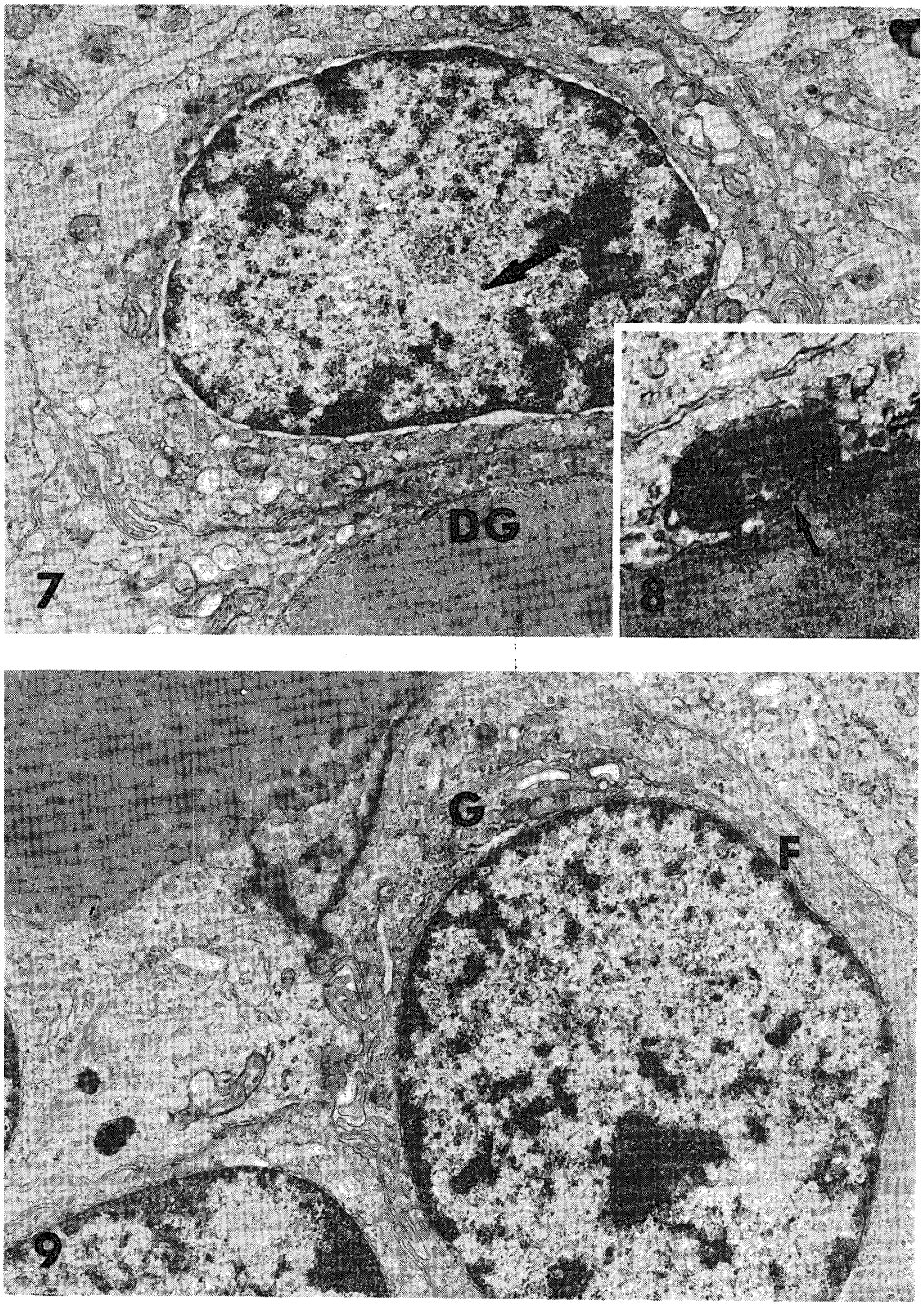


FIG. 7. B type follicle. Follicle is filled with amorphous material and fine desne granules. Arrow indicates electron lucent area in the nucleus. DG: Fine dense granules. × 16080

FIG. 8. The luminal surface of B type follicular cell. Multivesicular body is secreted from cytoplasm to follicular lumen. Arrow indicates fine dense granules. × 48,000

FIG. 9. C type follicle. Follicular cells are filled in cytoplasm with aboundant fibrils. G: Golgi apparatus, F: Fibril. × 16,080.

secretory granules and microtubules and some multivesicular bodies in the peripheral cytoplasm. RER and Golgi apparatus were not as well-developed as in ordinary chief cells (Fig. 5). Some degenerating mitochondria were also observed. Bundles of electron-dense fibrils, often running parallel to the luminal surface, were observed (Fig. 5). The lymphoid cells were often found near the follicular cells.

Dark follicles were filled with fine dense granules and amorphous dense materials. Three types of follicular cells could be differentiated as follows:

A type

The follicles were filled with moderately dense amorphous materials. The follicular cells were similar to the light follicular cells. Towards the peripheral cytoplasm, the number of microvilli and secretory granules decreased, while those of prosecretory granules and Golgi apparatus increased (Fig. 6).

B type

The follicles were filled with amorphous dense materials, and with fine dense granules of approximately 30 nm in average diameter (Fig. 7). They had abundant fine filaments close to the zonula adherence, well-developed Golgi apparatus and many degenerating mitochondria. Some multivesicular bodies, approximately 200–500 nm in diameter and similar to luminal fine dense granules, were also observed in apical cytoplasm (Fig. 8). In the nuclei of the follicular cells, one or two electron lucent areas were observed (Fig. 7).

C type

The follicles were filled with amorphous dense material with no fine granules. The follicullar cells were lighter than ordinary chief cells, and a few Golgi apparatus, RER and prosecretory granules were present in the cytoplasm. On the contrary, these cells were filled with fibrils near the free surface of plasma membrane and zonula adherence, and in the perinuclear cytoplasm (Fig. 9).

Discussion

Follicles and dilated intercellular spaces occur frequentlly in hyperplastic and adenomatous parathyroid glands of human with hyperparathyroidism (1–4). Setoguti reported that in senile dog parathyroid, follicular structures were occasionally observed (11). In sheep parathyroid we observed that follicles and intercellular spaces were enlarged with few lipid droplets in the parenchymal cells. This finding suggests that the follicular parathyroid gland of sheep may exhibit hyperfunction.

Some of the follicular contents of human parathyroid glands consisted of amyloid (1, 2, 3, 5, 6). Setoguti reported that in parathyroid glands of senile dogs, colloidal follicles were stained pink with eosin (11). Boquist showed that

follicular contents were PAS-positive after diastase digestion and were negative to amyloid staining. Leedham and Pollock suggested that amyloid and fiblilar materials in the follicular contents may be markers for secretion of peptides, closely related to clacitonin (5). We found that the follicles of sheep parathyroid gland consisted of glucoprotein which did not contain amyloid and fiblilar materials, and that the follicular cells had many secretory and prosecretory granules and some multivesicular bodies. This suggested that the follicular contents were the materials secreted from the cytoplasm to the lumen.

The activity of NADPH dehydrognease is higher in the oxyphil and adenomatous cells than in normal chief cells, though the significance of this enzyme in human parathyroid glands is unknown (16). Hellman and Larsson suggested that NADPH, which provided G–6–P dehydrognease and 6–PG dehydrognease, was related to the production of some amino acids necessary for the hormone synthesis (17). In sheep parathyroid glands, the follicular chief cells with well-developed Golgi apparatus and RER had high NADPH-DH activity. These findings suggest that NADPH dehydrognease may be related to the protein biosynthesis in sheep follicular cells (17).

According to Boquist, the follicles of human parathyroid glands are an active rearrangement of the parenchymal cells to create the regular epithelium (4). Sheep parathyroid follicles morphogenesis may be stated as follows:

1. The first contacts between glandular cells occur following the prolification of glandular cells or the enlargement of intercellular spaces. Establishment of zonula adherence occurs and membrane densification increases as a result of the establishment of the light follicles.

2. The formed light follicles are filled with secretory granules and pro-secretory granules to become A type dark follicles.

3. The A type follicles are filled with fine dense granules of multivesicualr bodies, which are degraded to amorphous dense materials rsulting in the appearance of B type dark follicles. Nuclear electron-lucent areas were often found in the follicular cells.

4. Finally the follicular cells decrease in the amount of cytoplasmic organellae and become filled with fibrilar materials at the surface of plasma membrane, at the zonula adherence and in surrounding nuclei resulting in the appearance of C type dark follicles.

The morphogenesis of sheep parathyroid follicles just stated seems to be similar to that of thyroid follicles (18). One difference between sheep parathyroid and thyroid follicle morphogensis is the relation of lymphoid cells to the chief cells. The close relation between lymphoid cells and chief cells in the former may be related in some ways to follicular formation in sheep parathyroid glands.

Boquist reported that fibrilar bundles were observed near the plasma membrane (4). He suggested that these fibrilar materials were useful for binding between

follicular cells and the stock of luminal materials (4). The same fibrils observed in the follicular cells in this study are at the suface of plasma membrane and zonula adherence in good agreement with that of Boquist.

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