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Studies of the Fine Structure of Ovarian Interstitial Tissue

2. The Ultrastructure of the Thecal Gland of the Domestic Fowl

Erik Dahl

Department of Anatomy, Dental Faculty, University of Oslo, Norway

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Summary. The fine structure of the thecal gland of the domestic fowl, is described for the first time. In the fowl, the glands are located as islets of lipid-laden cells in the theca interna and also in the interfollicular regions. They appear as well defined structures, organized like endocrine glands, quite different from the surrounding theca interna cells. Each gland is composed of two different cell types, the steroid-producing cell, and a cell type never described before, named the enclosing cell. Both cell types are surrounded by a common, distinct basal membrane. The steroid-producing cells are characterized by their content of organelles typical of steroid-producing cells in other organs. The enclosing cells are char cterized by their peripheral location within the gland and their membranous contact with the steroid-producing cells, long processes with desmosomes and their relation to the nerve fibers. They do not contain the organelles typically found in steroid-producing cells. So far, the real function of the enclosing cells is unknown. The following structures are demonstrated in ovarian steroid-producing cells of the fowl for the first time: cytoplasmic microtubules and filaments, intramatrical lipid-like droplets, attachment devices, the polarity of the steroid-producing cells of the thecal gland.

Key-Words: Ovary — Interstitial cells — Thecal gland — Cell types — Ultrastructure.

Although the ovarian stroma has been the subject of many investigations during the last century, uncertainty regarding the identity of the different cell types and their origin still exists. The main reason for this is a general acceptance of traditionally established misconceptions and a failure to identify and differentiate the ovarian tissues properly (Mossman, Koering and Ferry Jr., 1964).

Even though there are some reports concerning the fine structure of the oocyte (e. g. Krauskopf, 1968), the granulosa cells of the Graafian follicle (e. g. Bjersing, 1967), and the corpus luteum cells (e. g. Blanchette, 1966; Bjersing, 1967), the contribution of the electron microscope in this field has been surprisingly sparse.

The fine structure of the interstitial cells of the ovary has only been described in the mouse by Muta (1958) and recently in the rabbit by Davies and Broadus (1968). However, the ultrastructure of the thecal gland has, to the authors knowledge, never been described before, except for a preliminary report by Dahl (1970a). According to Mossman (1937), the theca interna of the pocket goopher, *Geomys bursaris*, forms a thick layer of tissue typical of endocrine gland tissue around both ripening normal follicles and large atretic follicles at, or near, the time of ovulation. This structure was designated the thecal gland by Mossman (1937).

The present investigation was undertaken in order to elucidate the fine structure of the thecal gland of the domestic fowl and establish a basis for subsequent studies of physiological and pathological alterations. As a preliminary study, a

comparative investigation of the rat and the hen was carried out (Dahl, 1970a). The study revealed differences as well as similarities of the fine structure of the ovarian tissues in the two species. Obviously, the interstitial tissue of the hen ovary was less complex than that in the rat, and possessed neither corpus luteum nor well developed interstitial gland as in the rat. However, the thecal gland was present in both species within the theca interna of the follicle.

Most of the difficulties regarding identification and classification of ovarian cell types met with in previous light microscopical work were avoided in the present study. This was made possible by choosing a suitable experimental animal and by using electron microscopy in connection with an improved fixation method (Kjaerheim, 1969; Dahl, 1970a).

Materials and Methods

Nine White Leghorn hens, 18—24 months old, with an average body of 1650 g (range 900—2,120), and three white Leghorn chickens, 3 months old, with an average body weight of 850 g (range 840—860), were used for this study. The animals were kept on a 12 hr light dark cycle in individual cages in a well ventilated air-conditioned room, with a constant temperature of 17° C and relative humidity of 60%. The hens were fed pelleted commercial chicken fodder, cabbage, sand grit, and water *ad lib.* The principal constituents of the fodder were proteins (17—19%), fats (2—4%), calcium (1.1—1.2%), phosphorus (0.7%), and sodium chloride (0.5%). Before sacrifice, the hens were kept for a minimum of 12 days for adaption to their new environment.

The hens were sacrificed between 10.00 a. m. and 1.00 p. m. to minimize possible structural changes due to diurnal variations in ovarial activity. Fixation was performed as an intraaortic perfusion with dextran (Intradex "Nyco", Nyegaard & Co., Oslo, Norway) under nembutal anesthesia (Nembutal sodium, 5%, Abbott laboratories S. A. Brussels, Belgium) followed by 1.7% glutaraldehyde (Fluka AG, Buchs SG, Switzerland) in either 0.1 M phosphate buffer (two hens and all the chickens), 1% Tyrode (four hens), and 0.1 M cacodylate buffer (three hens), all at pH 7.3. The perfusion technique was used according to specifications given in detail by Kjaerheim (1969).

After perfusion for 10 min the ovary was excised and cut into thin slices under the dissecting microscope. The left ovary was chosen in all animals. In all the hens samples were taken from the cortex, and efforts were usually made to get more than one follicle in a single sample. The size of the follicles could vary from 20 μ up to 5 mm. The tissues were then fixed for an additional period of 2 hr by immersion in the perfusion fixative. Twenty blocks were processed from each ovarium. Subsequently, the specimens were rinsed for 10 min in 0.15 M phosphate buffer at pH 7.3 and post-fixed in 1% osmium tetroxide at 4° C for 2 hr (Millonig, 1961). After fixation the blocks were rapidly dehydrated in graded series of acetone solutions and embedded in Vestopal W (Ryter and Kellenberger, 1958). Ultrathin sections were cut on an LKB Ultrotome III, equipped with glass knives, collected on formvar-coated copper grids, the formvar being backed with carbon. The sections were treated with uranyl acetate for 30 min, followed by lead citrate (Reynolds, 1963) for 5 min. The sections were examined in a Siemens Elmiskop Ia electron microscope operated at 80 kv, and equipped with 50 microns platinum objective apertures. From the same plastic blocks, sections one micron thick were cut for light microscopy. These sections were stained on a heating stage with an aqueous solution of 0.1% toluidine blue adjusted to pH 8.9 with 0.067 M Na_2HPO_4.

Nomenclature

The cells studied in the hen's ovary proved to correspond to the thecal gland of the rat both in location as well as structurally. Therefore, the designation "thecal gland" will be used also in the following description of the hen. Since this investigation also disclosed several similarities between the thecal gland cells and the adrenocortical cells in the domestic fowl, as described by Kjaerheim (1968a), some of the terms concerning the latter

cells are used according to his description. The pole of the cell facing the basal lamina is termed "basal", whilst the term "apical" is used to designate the opposite end of the cell.

The term "dense bodies" is used for the round or oval, electron dense, homogeneous bodies observed in the apical cytoplasm. Conglomerates containing a dense, homogeneous substance as well as lipid-like material are referred to as "complex bodies" or "intermediary stages between lipid droplets and dense bodies".

The thecal gland of the domestic fowl is composed of two cell types, here called "steroid producing cells" and "enclosing cells". The term "steroid-producing cells" refers to the vast majority of the thecal gland cells, characterized by their content of agranular endoplasmic reticulum, lipid droplets, mitochondria with tubular cristae, dense bodies, and Golgi apparatus, i. e. similar to cells involved in the production of steroidal hormones. The term "enclosing cells" refers to cells which are also supposed to be a component of the gland. They have a different morphology and are located at the periphery of the gland, "enclosing" the steroid-producing cells.

Observations

Macroscopical Observations. In the domestic fowl only the left ovary normally reaches a functional state, the right one remains rudimentary from an early stage in the embryo. The cortex of the left ovary possesses little stromal tissue and is subject to wide structural variations according to the reproductive state of the individual. The marked variation in size, coloration, and weight is largely due to the rapid growth of the oocytes during the breeding cycle. The rapid and extensive increase in size of the oocytes causes the follicles to bulge conspicuously from the surface of the entire ovary.

Light Microscopy. The thecal glands in the ovary of the domestic fowl were easily identifiable in toluidine blue-stained sections. They were seen as islets of epithelial cells located in the theca interna forming spherical or elongated glands, usually two layers in thickness. Several glands were distributed throughout the whole circumference of the follicle, sometimes almost at a regular distance from each other. The gland cells were in contact, devoid of intervening connective tissue, capillaries, or a glandular lumen. The nuclei were round or oval, and generally they contained a prominent nucleolus. The cytoplasm contained a large number of vacuoles from which the lipid had apparently been extracted during the preparation procedure, making the cells and the whole gland easily identifiable from the surrounding stroma. Differences in the amount of lipid content of various thecal glands could regularly be found within a single follicle. However, there was no significant difference in lipid content in follicles of varying sizes in animals of varying ages. Even small follicles in the chickens were sometimes surrounded by thecal glands with a great amount of lipid droplets. Enclosing cells were barely identified in the light microscope as a component of the thecal gland, because of their close resemblance to connective tissue cells of the theca interna.

Electron Microscopy. The fine structure of the thecal gland revealed it to consist of a well defined, compact organ with a periglandular basement membrane separating it from the interglandular connective tissue (Figs. 1, 17, 18). Two cell types could be distinguished within the gland (Figs. 17, 18). One type corresponded structurally to specific steroid-producing cells demonstrated in other organs. The second type is here referred to as enclosing cells.

The Steroid-Producing Cells. These cells formed the main part of the gland (Figs. 1, 3). They were irregular in shape with many processes (Fig. 2). In cross section 4—6 cells could usually be seen within a gland. The bipolar structure of

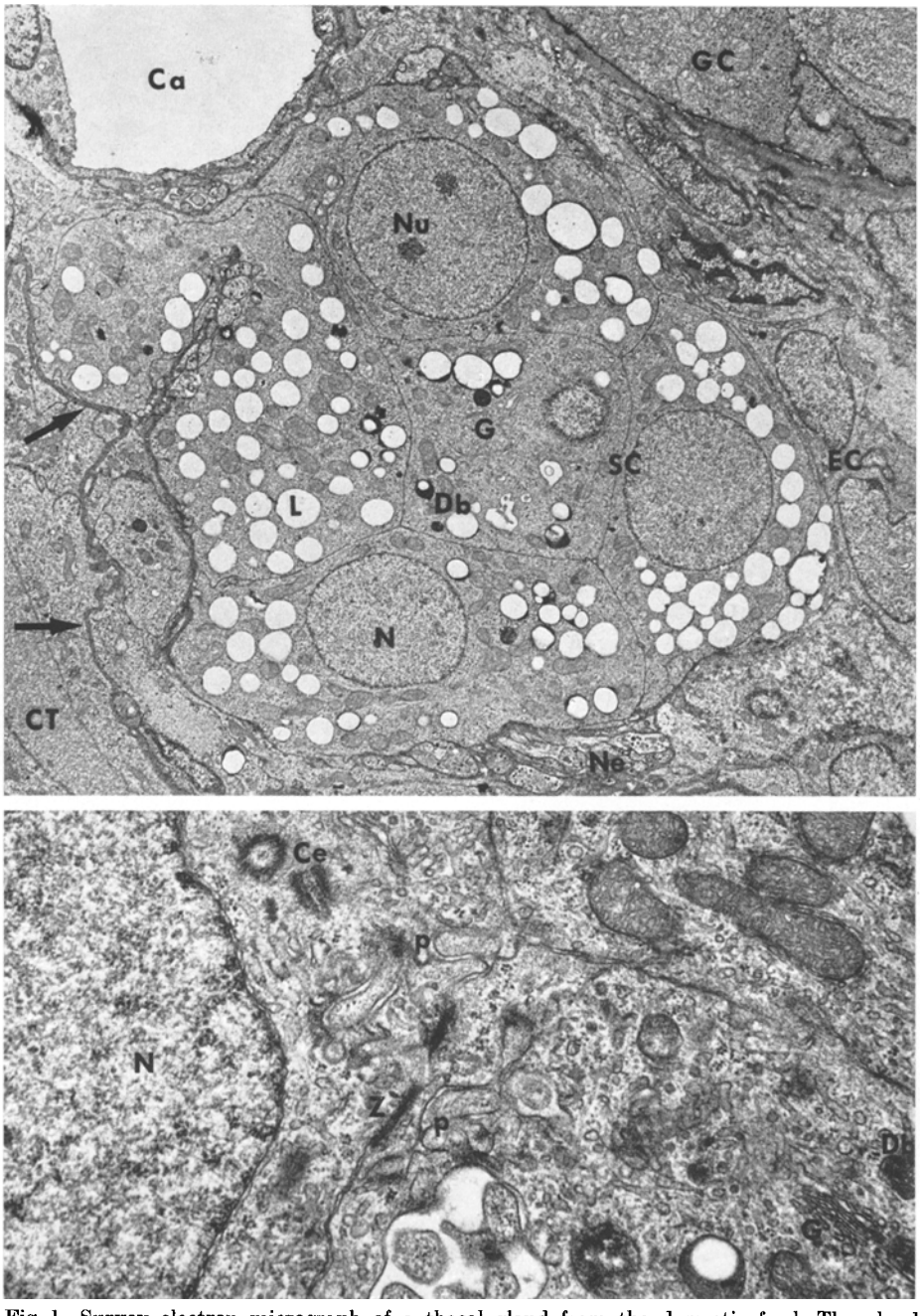


Fig. 1. Survey electron micrograph of a thecal gland from the domestic fowl. The gland is surrounded by a well-defined basement membrane (arrows). The major part of the gland is formed by the steroid-producing cells (*SC*) which are characterized by lipid droplets (*L*), dense bodies (*Db*) and a spherical nucleus (*N*) with nucleolus (*Nu*). The lipid droplets are located mainly in the basal parts of the cells, i. e. the end adjacent to the connective tissue (*CT*) or the enclosing cells (*EC*), whereas the dense bodies (*Db*) and the Golgi apparatus (*G*) are located in the opposite portions of the cells. The enclosing cells are seen at the periphery of the gland surrounding parts of the steroid-producing cells. Capillaries (*Ca*) and nerve fibres (*Ne*) are always seen adjacent to the gland. Granulosa cells (*GC*) are seen in the upper right corner. $\times 6{,}000$

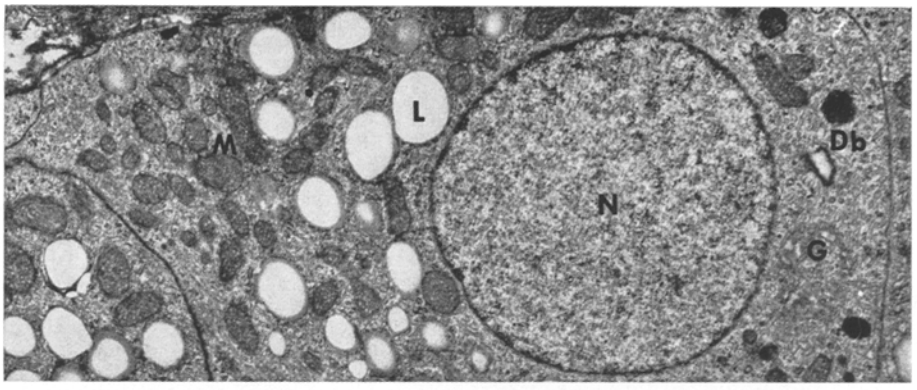
the steroid-producing cells and the intercellular distribution of the various organelles are demonstrated in Fig. 3. The Golgi apparatus and the dense bodies were found almost exclusively within the apical cytoplasm (Figs. 1—4), but the majority of the lipid droplets were observed in the basal part of the cell (Figs. 1, 3). The mitochondria were usually found in clusters, located more towards the basal portion and among the lipid droplets (Fig. 3). Typical "light" and "dark" cells, as reported in other steroid-producing cells in the ovary, were never observed (Davies and Broadus, 1968).

Mitochondria. The mitochondria varied in size as indicated by the disparity in their cross sectional area (Fig. 8). Most often they were round (Fig. 7) or oval (Fig. 8), but elongated forms occurred as well (Figs. 9, 13). Their internal structure consisted of numerous tubular cristae with an electron-lucent interior in contrast to the high density of the mitochondrial matrix (Figs. 7—9). Dense, homogeneous lipid-like globules were sometimes observed in the mitochondrial matrix (Figs. 9, 10). Tubules aggregated in a paracrystalline pattern were never encountered despite extensive scrutiny, but mitochondria of the cupped disc type were found relatively often.

Endoplasmic Reticulum and Ground Cytoplasm. The endoplasmic reticulum of the steroid-producing cells occurred in relatively large amounts and was almost completely of the smooth type. It usually consisted of tubules of varying length evenly distributed throughout the cytoplasm except for the Golgi region. It often formed several layers around the lipid droplets, and real "parasomes" (Bjersing, 1967) (Fig. 11) were occasionally found both in chickens and hens. Sometimes mitochondria could be seen surrounded by one or more layers of smooth endoplasmic reticulum, but never to the same extent as around lipid droplets. Reticulum of the rough variety was seldom encountered (Fig. 12), but when present it was mostly restricted to the Golgi region adjacent to the nucleus. Ribosomes were found in large numbers, scattered between areas of mitochondria and lipid droplets (Figs. 5—9). In areas in smooth endoplasmic reticulum, ribosomes were encountered only sporadically and were often completely absent (Fig. 11).

The Golgi apparatus. The Golgi complex occupied an extensive region of the apical portion of the cell, usually within the juxtanuclear position where the cytoplasm was relatively free of endoplasmic reticulum (Figs. 1—4). In cross section a curved or semi-circular area of three or four parallel cisternae were sometimes extended to outline a nearly round Golgi zone (Fig. 4). Varying numbers of vesicles, 400—800 Å in diameter, were observed, some of which were coated (Fig. 4). In the same areas membrane-bound dense bodies (Fig. 4), multivesicular bodies, microtubules, and centrioles (Fig. 4) were observed. In the apical cytoplasm, and rather often in association with the Golgi apparatus, dense bodies of varying sizes were found (Figs. 1—4, 14). Several of them were in close contact with the lipid droplets. Some of the lipid droplets in this area were more or less surrounded by a dense substance, presumably derived from dense bodies,

Fig. 2. Apical portions of steroid-producing cells of the thecal gland. Typical structures of this part of the cells are the Golgi apparatus (*G*), centrioles (*Ce*), attachment devices (*Z*), interdigitating processes (*P*) and dense bodies (*Db*). Note the paucity of lipid droplets as compared to the basal part illustrated in Figs. 1 and 3. Nucleus (*N*). ×24,000



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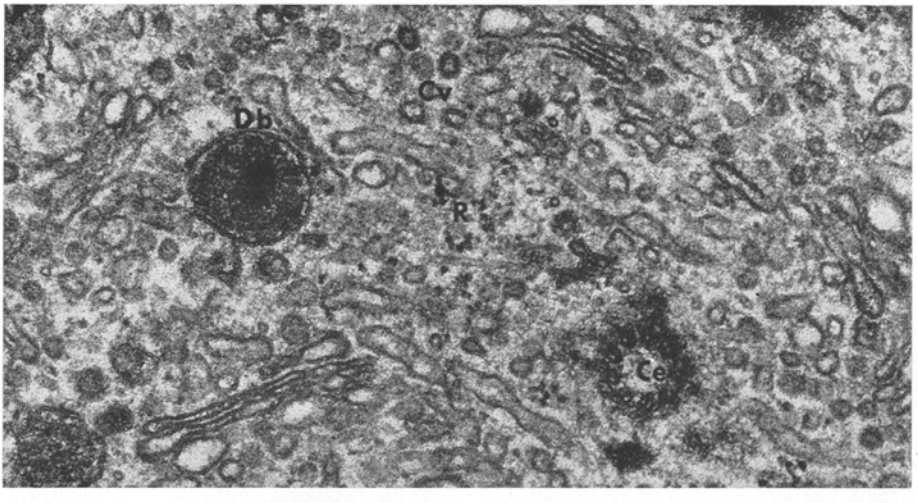

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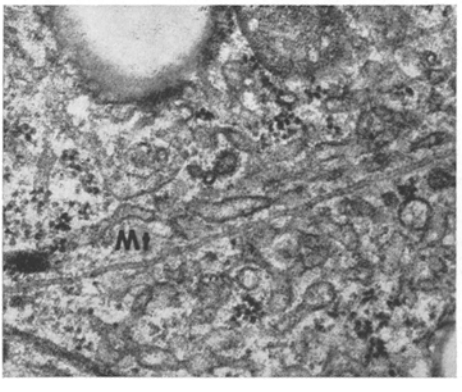

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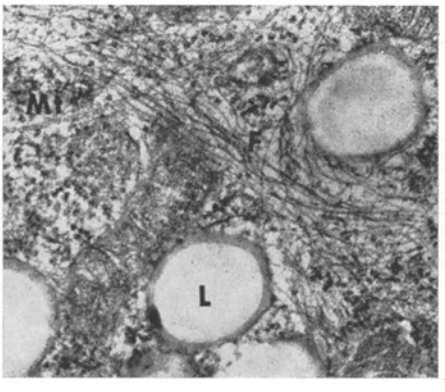

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Fig. 3. Portion of a steroid-producing cell with typical localization of the organelles. The apical portion is seen to the right with the Golgi apparatus (*G*) and dense bodies (*Db*). The basal portion is seen to the left with lipid droplets (*L*) and the majority of the mitochondria (*M*). The nucleus (*N*) is round and located towards the apical portion. Note the rather close relationship between the mitochondria and the lipid droplets. ×12,000

Fig. 4. High magnification of the Golgi apparatus in cross section. It forms a curved semicircular zone with centriole (*Ce*), membrane-bounded dense bodies (*Db*), coated vesicles (*Cv*) and free ribosomes (*R*). In some of the membrane-bounded dense bodies (bottom left) vesicles are seen. ×60,000

and forming intermediary bodies between lipid droplets and dense bodies, or complex bodies (Figs. 13, 14).

Lipid Droplets. One of the most striking features was the presence of abundant lipid droplets of varying size in the cytoplasm of all steroid-producing cells (Figs. 13, 18). Generally, one could not demonstrate any difference in the lipid content in the steroid-producing cells of the chickens and hens in follicles of the same size; neither were more lipid droplets demonstrable by increasing the size of the follicle up to 5 mm in diameter. Even very small follicles in the chickens possessed a theca interna with thecal gland heavily loaded with lipid droplets were round or oval with a regular outline, sometimes surrounded by a tiny brim of osmiophilic substance (Figs. 1, 14), but a surrounding triple membrane was never seen. The size was normally about the same as that of the mitochondria (Fig. 3), or slightly larger (Fig. 18), the largest ones usually being located in the basal area of the cell (Fig. 18).

Nucleus. In the normal gland the nuclei were positioned in the apical part of the cell (Figs. 1—3). They were round or oval with scarce chromatin and usually a single nucleolus (Fig. 15). Most often the nucleolus was composed of granules about 150 Å in diameter, located centrally in the nucleus, but, in addition, a filamentous part was also found in the nucleolus (Fig. 16). The two parts were often segregated so that granules and filaments did not intermingle.

Spherical nuclear bodies of different sizes and types were regularly encountered (Figs. 15, 16). They varied in size from 0.2 to 0.5 μ, as previously described by Dahl (1970 f) (Figs. 15, 16).

Filaments. Filaments, about 80 Å in diameter, were regularly found (Fig. 6). They were more numerous near the periphery of the cell, but were generally scarce compared with the amount found in the enclosing cell. They seemed, however, to increase by altered functional activity of the cell (Dahl, 1970 c—e).

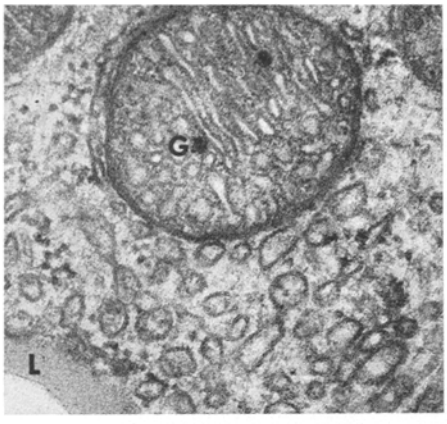
Microtubules. Microtubules, 200—250 Å in diameter, were found at random within the cytoplasm (Fig. 5). They were found singly, usually with a straight course, but were also forming angles. They were never found in bundles.

Cell Attachments. Specialized attachment devices, several microns long, were regularly found, most often within the apical part of the cells (Figs. 2, 18). Typical desmosomes were lacking, contrary to what was seen in the enclosing cells (Figs. 18, 20).

The Enclosing Cell. These cells were few in number as compared with the steroid-producing cells and were always located at the periphery of the glands where the peri-glandular basement membrane separated their outer aspect from the inter-glandular connective tissue (Figs. 1, 17, 18). They did not possess so much cytoplasm as the steroid-producing cell, nor did they contain the same organelles. The enclosing cells had a more elongated form and were characterized

Fig. 5. Cytoplasmic microtubules (*Mt*) of a steroid-producing cell. The microtubules are found dispersed at random within the cytoplasm. ×40,000

Fig. 6. Cytoplasmic filaments (*F*) of a steroid-producing cell, running in small bundles close to the lipid droplets (*L*). Note the microtubule (*Mt*) crossing the direction of the filaments. ×40,000



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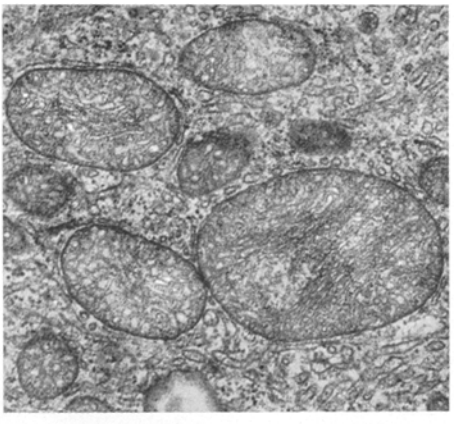

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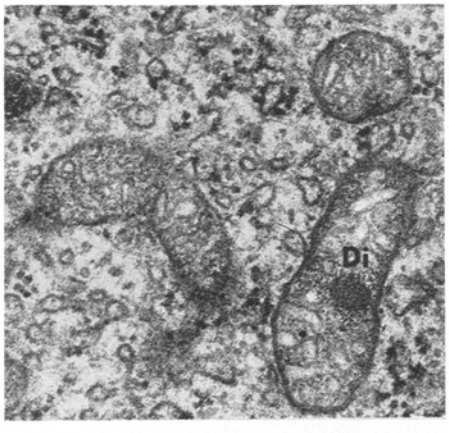

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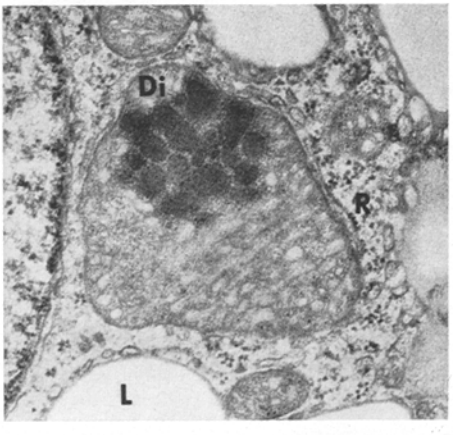

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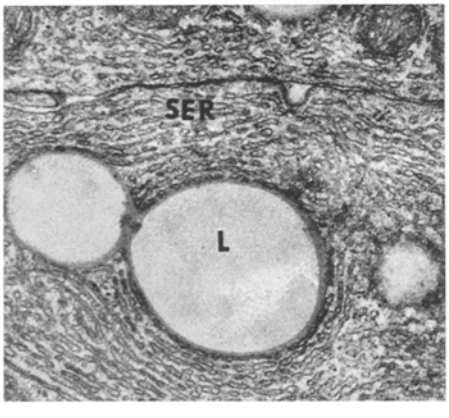

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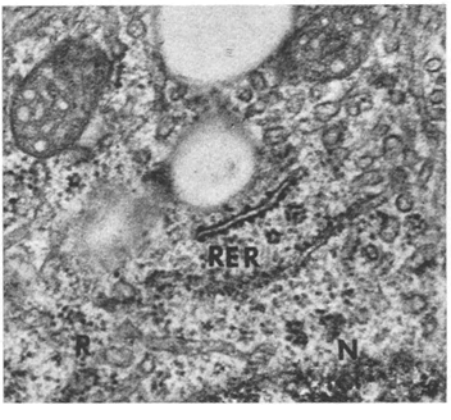

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Fig. 7. Mitochondrion of a spherical form from a steroid-producing cell. The content of the tubular cristae is electron-lucent. Note the close relationship between the outer membrane of the mitochondrion and the smooth endoplasmic reticulum. Small granules (*G*) of the size of glycogen are seen in the matrix. Lipid droplets (*L*). ×60,000

Fig. 8. Mitochondria of varying sizes from a steroid-producing cell. Note the dense matrix and the electron-lucent cristae. ×24,000

by their tenuous cytoplasmic extensions which partly or completely enclosed cell processes or portions of the steroid-producing cells. These cells also surrounded the nerve processes in a manner essentially similar to Schwann cells enclosing axons.

The bulk of the cytoplasmic organelles of the enclosing cell were in the perinuclear region. Sometimes the cytoplasm of these cells was characterized by an abundant amount of fibrils (Fig. 22). The endoplasmic reticulum was of the rough type (Fig. 21) and found in the vicinity of the Golgi complex which was located near the nucleus. Smooth endoplasmic reticulum was not encountered.

The mitochondria were elongated in shape and lacked typical tubular cristae (Fig. 21). They were smaller in size than those of the steroid-producing cells (Fig. 18). The mitochondrial matrix was not so dense as in the steroid-producing cells, and it was also without any lipid inclusions. Furthermore, dense bodies and lipid droplets were seen only infrequently.

Contrary to what was found in the steroid-producing cells, desmosomes were regularly encountered (Figs. 18, 20).

The nucleus was more elongated, the chromatin was more abundant and located at the periphery, in contact with the nuclear membrane (Fig. 17). Nucleoli were only exceptionally seen.

Thus, the differences between the two cell types of the thecal gland were not only spatial, but also qualitative and quantitative.

Nerves. The thecal gland was extremely well supplied with nerves of presumptive different function, and the complexity of this innervation will be described in a subsequent paper (Dahl, 1970b).

Blood Vessels. Capillaries with a partly fenestrated endothelium were always found either close to the glands (Fig. 1) or sometimes even in contact with the steroid cells without any basal membrane intervening (Dahl, 1970e).

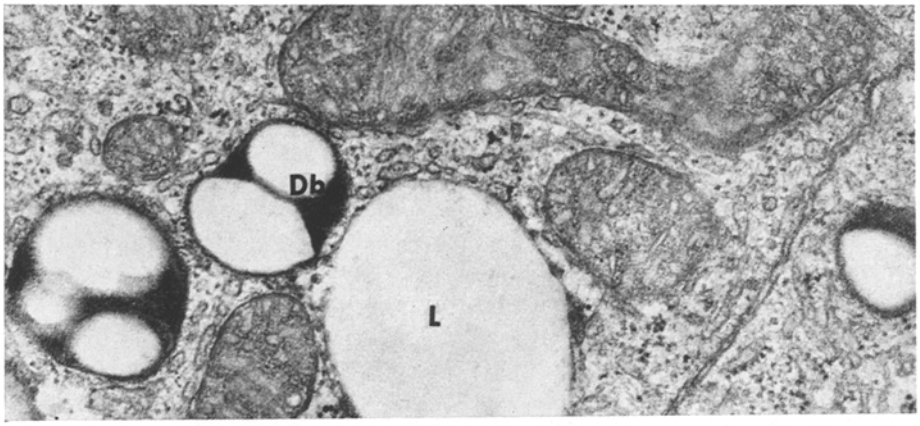
Interstitial Tissue. In addition to the thecal gland, the interstitial tissue of the theca interna contained some cells which appeared to be rather undifferentiated (Fig. 23). In general, the nuclei of these cells were more elongated, they contained granular endoplasmic reticulum, elongated mitochondria without tubules, occasional lipid droplets, but not a granular endoplasmic reticulum. They were suggested to be an intermediate cell type in the differentiation of the thecal gland cells.

Fig. 9. Mitochondrion of more elongated form. The micrograph illustrates the occasional observation of a dense lipid-like inclusion (*Di*) in the mitochondrial matrix. ×60,000

Fig. 10. In some large mitochondria the dense, lipid-like inclusions (*Di*) occupy a relatively large portion of the matrix. Note the free ribosomes (*R*) adjacent to the mitochondrion. Lipid droplet (*L*). ×30,000

Fig. 11. Smooth endoplasmic reticulum (*SER*) appears in relatively large amounts in the steroid-producing cells. Tubules of *SER* often surround lipid droplets (*L*) in one or several layers, occasionally forming "parasomes". Note the absence of free ribosomes. Lipid droplet (*L*). ×24,000

Fig. 12. Free cytoplasmic ribosomes (*R*) occur in relatively large amounts in steroid-producing cells, whereas rough-surfaced endoplasmic reticulum (*RER*) is observed only occasionally, and then adjacent to the nucleus (*N*). ×60,000



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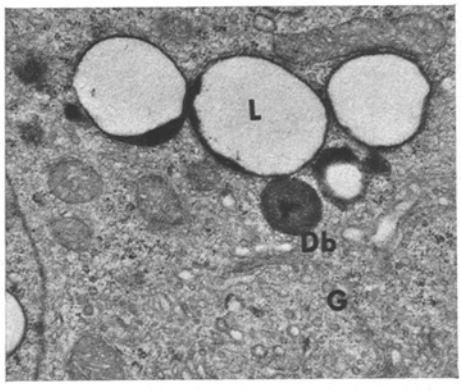

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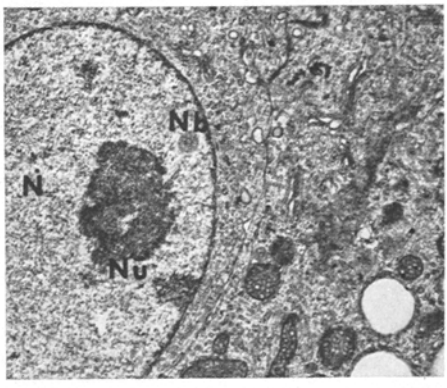

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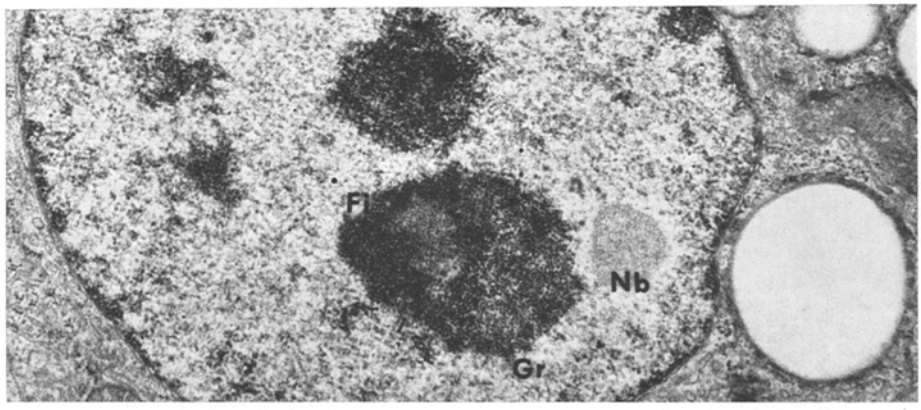

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Fig. 13. Portions of cytoplasm of steroid-producing cells with lipid droplet (*L*) and dense bodies (*Db*). Note the electron-lucent interior of the dense bodies which indicate that they are transformed to complex bodies, an intermediary form between lipid droplets and dense bodies. ×40,000

Fig. 14. Lipid droplets (*L*) which are more or less surrounded by a dense substance. These lipid droplets are presumably derived from dense bodies (*Db*) (cfr. Fig. 13). Golgi apparatus (*G*). ×18,000

Discussion

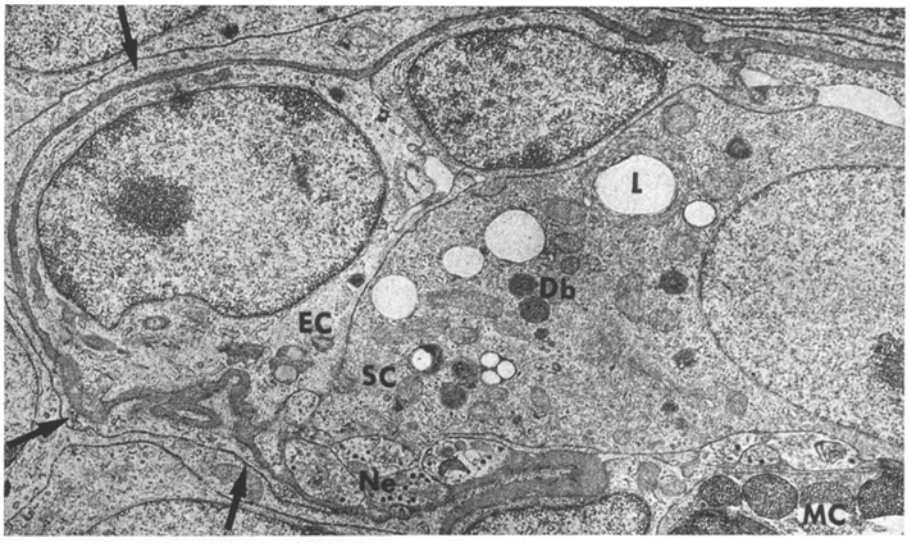
It is striking to notice the differences in opinion which exist among anatomists, physiologists, and pathologists concerning the ovarian stroma and its function (for review see: Watzka, 1957; Falck, 1959; Young, 1961; Brambell, 1962; Zuckerman, 1962; Mossman *et al.*, 1964; Appelgren, 1967; Bjersing, 1967). Some reasons for this disagreement are obvious. First, it is somewhat difficult to distinguish the different tissues and gland cell types in light microscopical sections. Secondly, there may be differences in the species examined. Thirdly, both in ordinary histological examinations, and even more so in electron microscopic investigations, insufficient fixation have posed severe difficulties. Fourthly, differences in terms used to classify the tissues have not minimized the problems. Most important is probably the confusion as to what is meant by interstitial tissue, interstitial gland, and the concept of the theca interna as a cytological and morphological unit. Even though Schroen (1863) and Pflüger (1863) described the interstitial gland already in 1863 (the term was coined by Limon in 1902), Schroeder (1930) apparently did not differentiate between the theca interna of growing follicles, the thecal gland of ripe follicles, and the interstitial tissue formed from the theca interna of atretic follicles. Mossman (1937), however, considered the hypertrophied theca interna to be a separate gland. Similar hypertrophy of the theca interna during oestrus has later been described in other mammals (Stafford *et al.*, 1942; Dempsey and Basset, 1943; Harrison, 1948, 1962). Watzka (1957), however, made no distinction between the thecal gland and interstitial cells. Corner (1938) concluded that the various cell types was so much alike that it was often difficult, and in many cases impossible, to distinguish one from another. There was, for example, no light microscopical cytological criterion for distinguishing theca interna from interstitial cells.

Mossman *et al.* (1964), based on their own investigations and the literature, proposed the following three types of steroid secreting cells in mammals: 1) Interstitial gland cells formed from the theca interna cells of degenerating (atretic) follicles, hence present from infancy to old age. 2) Thecal gland formed from the theca interna of ripening follicles, hence present only at or near the term of ovulation. 3) Luteal cells formed from the granulosa cells of ovulated follicles and from undifferentiated stroma cells surrounding these.

There seems to have been disagreement as to the existence of the thecal gland as a histologic structure *per se*, different from the rest of the theca interna or the interstitial cells. However, no comprehensive comparative study of the fine structure of the ovarian stroma has, so far, been undertaken in order to identify the morphology and possible function of the different tissues in the theca interna. As demonstrated in a preliminary study (Dahl, 1970a), both the

Fig. 15. Portion of a steroid-producing cell with the nucleus (*N*) which is spherical and surrounded by an even nuclear membrane, and with a distinct nucleolus (*Nu*). A nuclear body (*Nb*) is visible between the nucleolus and the nuclear membrane. ×12,000

Fig. 16. Portion of a steroid-producing cell with a nucleolus which consist of darker and lighter areas, indicating granular (*Gr*) and filamentous (*Fi*) parts. A nuclear body (*Nb*) surrounded by a relatively electron-lucent halo is seen. ×24,000



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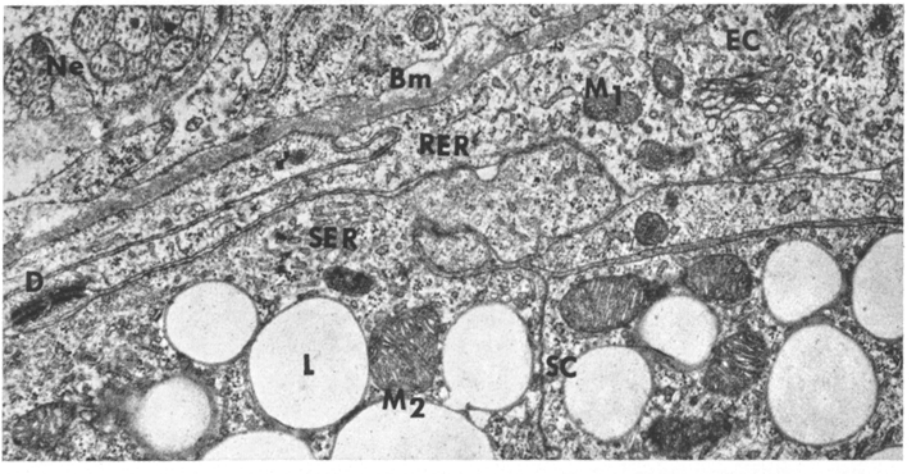

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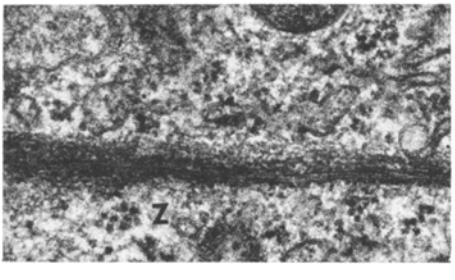

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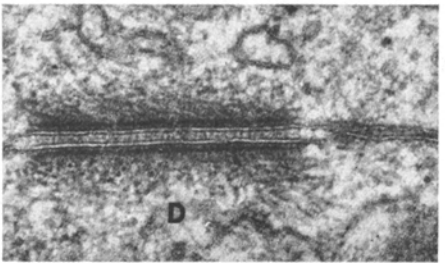

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Fig. 17. Portion of a thecal gland with the peripherally located enclosing cells (*EC*) to the left and the steroid-producing cells (*SC*) with their characteristic organelles to the right, surrounded by the basement membrane (arrows). Note the membranous contact between the two cell types without any intervening basement membrane and the difference in content of chromatin in the nuclei. Nerves (*Ne*) are seen in contact with the steroid-producing cells. Dense bodies (*Db*). Lipid droplet (*L*). Bottom right: Part of a mast cell (*Mc*). ×12,000

rat and the hen possess lipid-laden cells in the theca interna. In the domestic fowl, the present study has revealed compact gland consisting of two cell types surrounded by a basement membrane which separated them from the surrounding tissue. The vast majority of these cells could easily be identified from their form, size, characteristic cytoplasm, mitochondria, endoplasmic reticulum, lipid droplets, dense bodies, microtubules and filaments organized in a distinct pattern with organelles quite different from the cells of the rest of the theca interna. Cells of the second type of this gland, the enclosing cells, were few in number and were always located near the periphery of the glands. These cells have apparently never been described before. Morphologically they seemed most likely to be supporting elements, both for the steroid-producing cells and the nerve fibers and probably also for the axon terminals. However, since they seemed to be influenced by stimulation and inhibition of the steroid cells (Dahl, 1970c, d, e), it cannot be denied that their function, in addition to being a mechanical one, may also be more specific with close functional interrelationship to the steroid cells.

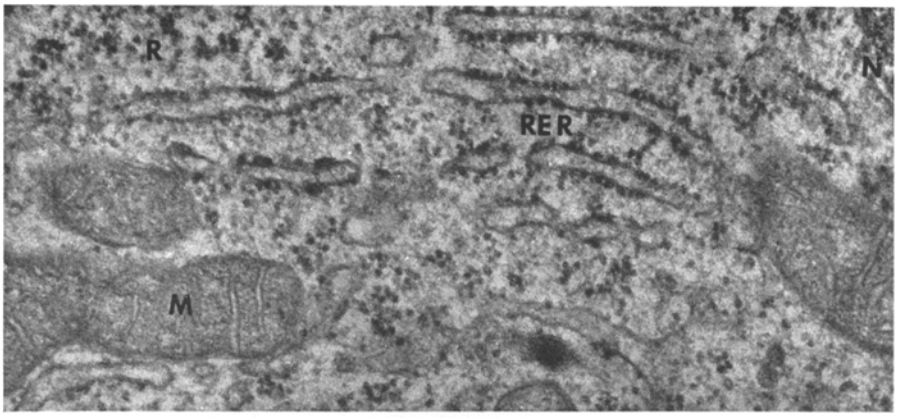
The present study leaves no doubt that in the theca interna of the domestic fowl, there are well defined cells which are organized like an endocrine gland, the thecal gland.

According to Bjersing (1967), little is known about the formation of steroid hormones in the different types of normal cells of the ovary. Corpus luteum is generally accepted as the source of progesterone, but there has been some divergence of opinion about the site of the production of estrogen. Histochemical as well as biochemical studies seem to favour the theca interna as an estrogen-producing site. However, even though several attempts have been made to correlate ovarian hormome formation with cytology, contradictory results have been obtained. Some investigators (Deane and Barker, 1952), found the theca interna, others (Freed and Soskin, 1937; Hernandez, 1943) found both the theca interna and the granulosa, or the granulosa alone to be the site of estrogen formation (e. g. Claesson, Claesson *et al.*, 1947—1954; Falck, 1959; Mossman *et al.*, 1964; Bjersing, 1967; Appelgren, 1967). Although these investigations point to the theca interna and interstitial gland as the main source of estrogen production in the ovary, the evidence obtained is indirect and thus allows other interpretations. This is perhaps best illustrated by the statement of Allen (1941): "I think there

Fig. 18. Portion of the thecal gland with the surrounding basement membrane (*Bm*), the enclosing cell (*EC*) and the steroid-producing cell (*SC*). Note the desmosome (*D*) between processes of the enclosing cells, and the differences in the organelles in the two cell types. Lipid droplets (*L*), smooth endoplasmic reticulum (*SER*) and the mitochondria (M_2) with typical tubular cristae are seen in the steroid-producing cells, while smaller mitochondria (M_1) and rough endoplasmic reticulum (*RER*) are seen in the enclosing cell. Upper left: Nerve fibres (*Ne*). ×20,000

Fig. 19. This illustration demonstrates a typical attachment device (*Z*) which can be followed over considerable distances between steroid-producing cells. Desmosomes were never encountered between these cell types. ×45,000

Fig. 20. This illustration demonstrates a typical desmosome (*D*) between two enclosing cells. Attachment devices of the type illustrated in Fig. 19 were never seen between enclosing cells. ×70,000



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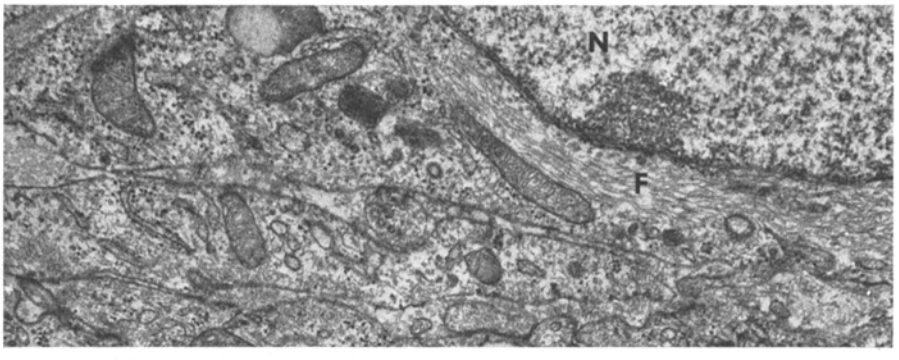

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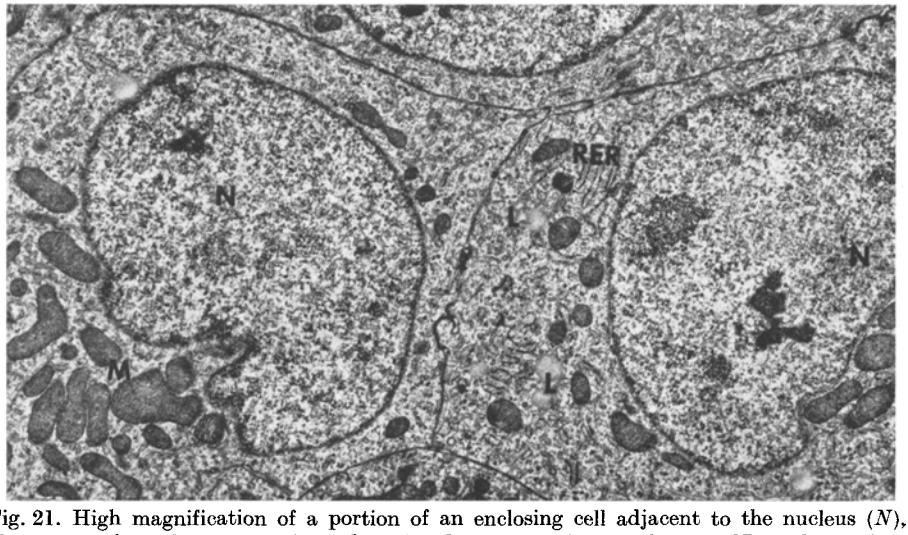

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Fig. 21. High magnification of a portion of an enclosing cell adjacent to the nucleus (*N*), illustrating the rich amount of rough-surfaced endoplasmic reticulum (*RER*) and the elongated mitochondria (*M*) without the electron-lucent tubular cristae, typical for the steroid-producing cell. Ribosomes (*R*). ×51,000

Fig. 22. A portion of an enclosing cell with a rather large amount of filaments forming fibrillary bundles (*F*) adjacent to the nucleus (*N*). ×24,000

is now evidence that all ovarian tissues may secret estrogen, granulosa, theca, interstitial and luteal cells, but that the follicular epithelium is probably normally the primary source", and as late as in 1967, the studies of Bjersing reveal that no definite conclusion can be drawn as to the production site of estrogen.

As demonstrated by Marshall and Coombs (1957) and confirmed in the first part of this investigation (Dahl, 1970a), there is no structure in birds that may be regarded as homologous with the mammalian corpus luteum. The production of estrogen, progesterone, and androgen in this animal has been demonstrated with the use of different technical approaches (van Tienhoven, 1961). Histochemical and histological investigations have suggested that in the theca interna there are cells containing lipids which may be responsible for the synthesis of steroids. Narbaitz and De Robertis (1968) have recently examined the postnatal evolution of steroidogenic cells in the chicken ovary and found that, from hatching to sexual maturity, cytochemical techniques demonstrated steroid synthesis in groups of cells loaded with lipids. Because of the growth of the cortex of the ovary in the course of the postnatal morphogenesis, these cells shifted in location to the theca interna and interfollicular spaces. Their study also revealed that in all stages investigated, there was steroid synthesis going on in lipid-containing cells.

In the present study of the theca interna it has been demonstrated that the thecal gland is a definite histological entity. Since this gland is present in immature as well as sexually mature animals, and even in small follicles, it seems to be proven that his gland 1) exists as a morphologic entity before sexual maturity and 2) is not confined to the pre-ovulatory stage of the follicle. There seems to be no doubt that the thecal gland is the only structure within the theca interna, which, from a cytological point of view, possesses the cells capable of secreting steroids, since these cells are the only ones containing the organelles required for steroid-production (Kjaerheim, 1968b). Finally, the results obtained in this study, combined and compared with previous cytochemical studies (Narbaitz and De Robertis, 1968) seem to indicate that these glands are responsible for the steroid-production even before sexual maturity.

In conclusion it may be stated that the present investigation has revealed the existence of ultrastructural differences in the various tissues of the theca interna, even in small follicles. Furthermore, it has been convincingly demonstrated that the thecal gland is an anatomically well-defined structure with cytological characteristics similar to those of other steroid-secreting cells (adrenal cortex and Leydig cells).

Investigations to be described in subsequent papers have demonstrated that these glands are supplied with many nerves and their fine structure can be changed by the influence of hormones and drugs (Dahl, 1970b, c, d, e).

Fig. 23. Rather undifferentiated cells from the theca interna of a follicle in the chicken. The mitochondria (*M*) have a dense matrix without typical electron-lucent tubular cristae, the endoplasmic reticulum is of a rough-surfaced type (*RER*) and the nuclei (*N*) are elongated with indentations. Small lipid droplets (*L*) with a semi-translucent interior are seen adjacent to the *RER*. ×12,000

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Dr. Erik Dahl
Department of Anatomy
Dental Faculty
University of Oslo
Oslo, Norway