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The ultrastructure of Leydig cells in the testis of the domestic fowl

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INTRODUCTION

The presence of steroid biosynthetic activity as evidenced by the demonstration of 3β-hydroxysteroid dehydrogenase has been shown in the intertubular tissue of the fowl embryo (Chieffi, Manelli, Botte & Mastrolia, 1964; Boucek, Gyori & Alvares, 1966; Scheib & Haffen, 1967, 1969) and the adult cockerel (Arvy, 1962; Woods & Domm, 1966; Tingari, 1973).

The fowl testis is also known to be capable of metabolizing progesterone to testosterone (Subhas & Edwards, 1968; Nugara & Edwards, 1970) and the presence of steroids has been demonstrated in tissue extracts (Delrio, di Prisco & Chieffi, 1967; Hohn & Cheng, 1967). Testosterone has also been shown to be produced by the chicken testis *in vitro* (Connell, Connell & Eik-Nes, 1966).

Leydig cells are considered to be the principal steroid-secreting cells in the intertubular tissue of the testis, and an extensive review of their fine structure in mammals has been presented by Christensen & Gillim (1969). They are described as polygonal in shape and are characterized by an abundant smooth endoplasmic reticulum (SER), many mitochondria with tubular cristae, and usually lipid droplets in their cytoplasm.

Leydig cells of similar ultrastructure are found in the testis of the Japanese quail (Nicholls & Graham, 1972).

In the chicken Narbaitz & Adler (1966) studied the embryonic differentiation of the gonads and observed vesicles of SER and lipid droplets in interstitial cells in the testis of 9 day embryos. They noticed an increase in number and size of the SER vesicles and lipid droplets and they related this to age, especially after the sixteenth day, and hence to a progressive differentiation of Leydig cells. Connell (1972) identified Leydig cells according to the criteria of Christensen & Gillim (1969) in normal two day old chickens, and compared them with cells from birds injected with luteinizing hormone (LH), indicating the possible cytological changes associated with steroid hormone production.

The present investigation was carried out to identify the ultrastructural features of Leydig cells in developing and adult testes.

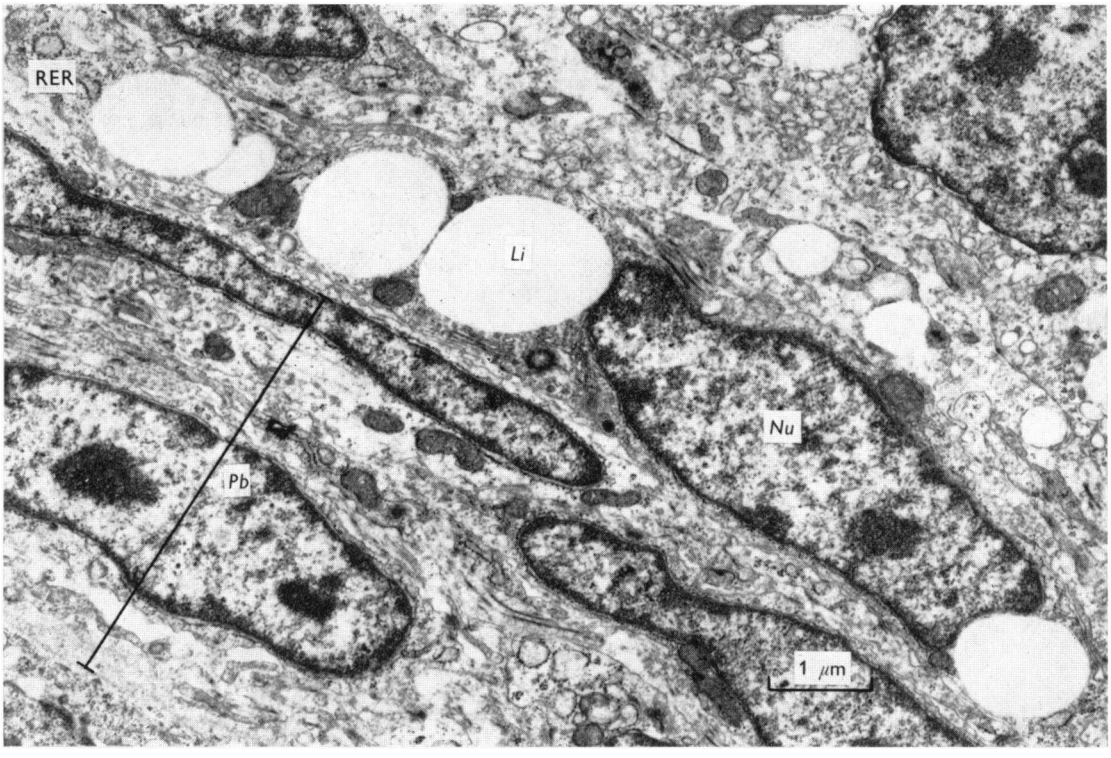

Fig. 1. Elongated transitional Leydig cell, *Nu*, nucleus; *Pb*, peritubular boundary layers; *Li*, lipid droplet; RER, rough endoplasmic reticulum.

MATERIALS AND METHODS

Male birds of a lightweight laying strain (Shaver) of various ages were killed by a lethal dose of pentobarbitone sodium (Nembutal, Abbot Laboratories). Their testes were excised and fixed in a modified Dalton's osmium fixative (3 % potassium dichromate, 2·6 % sodium chloride, 320 m-osmole), modified Karnovsky (4 % paraformaldehyde, 1 % glutaraldehyde) or 2·4 % glutaraldehyde in 0·1 M Millonig phosphate buffer. They were then dehydrated in ethanol and embedded in Araldite. Silver/silver grey sections were cut, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in a Philips EM 300 microscope.

RESULTS

Leydig cell differentiation in the chicken is not a simultaneous process, and therefore, as in mammals, a heterogeneous population of cells is found at all ages except extreme youth.

Transitional cells or primitive mesenchyme cells described by Connell (1972) are elongated cells similar in appearance to fibroblasts except for the presence of lipid droplets and some SER in their cytoplasm. Cells of this kind are generally spindle

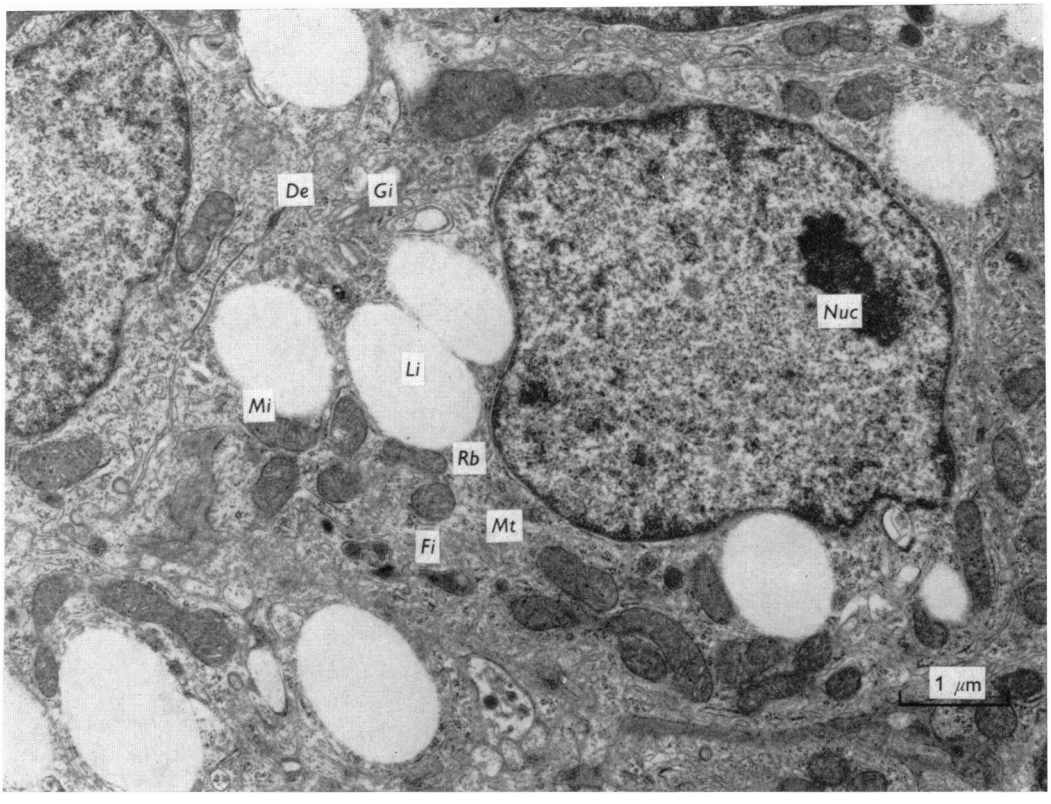

Fig. 2. Polygonal Leydig cell. Note that the mitochondria (*Mi*) have transverse cristae. *De*, desmosome; *Fi*, cytoplasmic filaments; *Gi*, Golgi apparatus; *Rb*, ribosomes, *Li*, lipid droplet; *Mt*, microtubule; *Nuc*, nucleolus.

shaped with an elongated nucleus, a distinct nucleolus and some chromatin condensation along the inner nuclear membrane (Fig. 1). Patches of rough endoplasmic reticulum (RER) are filled with a finely granular, electron-dense material, and in favourable sections a connexion is seen between RER elements and the perinuclear cisterna on the one hand and the scattered elements of SER on the other. Circular to oval mitochondria with a dense matrix and lamellar cristae are found and distinct Golgi elements and a centriole are also seen. Several large clear cytoplasmic vacuoles indicative of extracted lipid droplets occupy large areas of cytoplasm. These cells are found in all ages of birds sandwiched between boundary-tissue elements and intertubular tissue, particularly more mature Leydig cells. In some images cytoplasmic fibres and interfilamentous dense bands are seen, similar to those described by Rothwell & Tingari (1973) in peritubular myoid cells. Irregularly polygonal cells found singly or in clusters within the intertubular tissue show the more typical Leydig cell images. The plasma membrane is generally unfolded, and where cells are in clusters areas of membrane fusion and desmosomal adhesion are evident (Fig. 2). Where an appreciable extracellular space is found the cell surface is

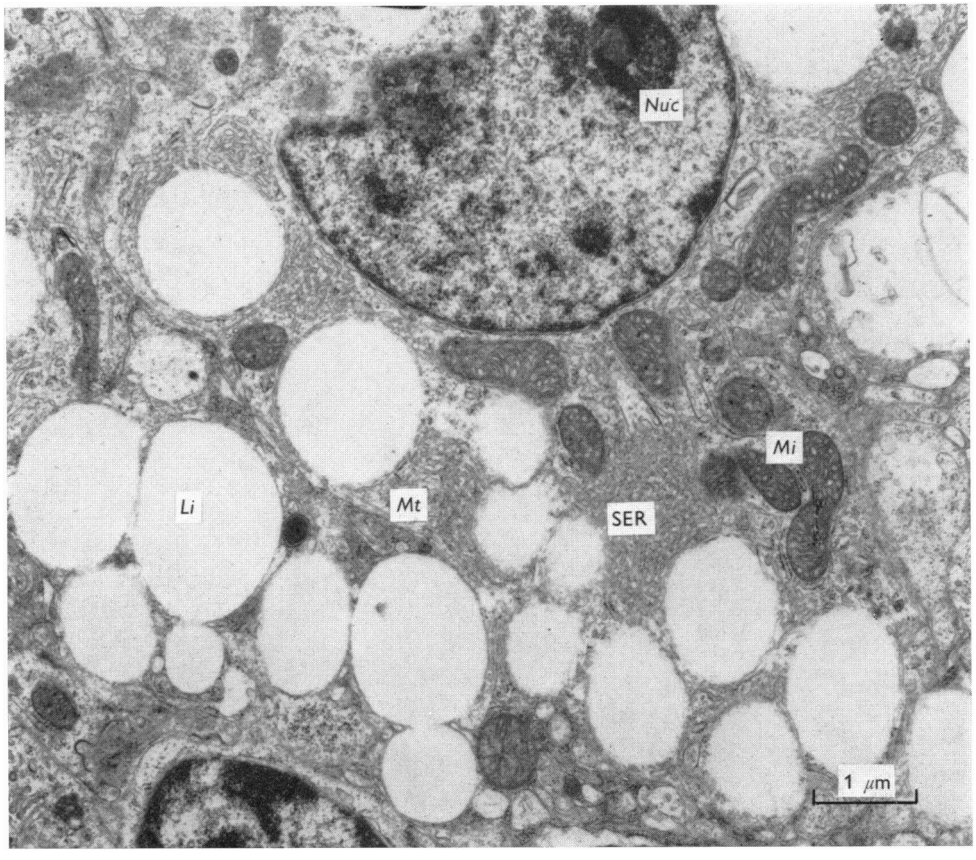

Fig. 3. Cytological features of a more typical Leydig cell. *Li*, lipid droplets; *Mi*, mitochondria with dense intramitochondrial granules and tubular cristae; *Mt*, microtubule; *Nuc*, nucleolus; *SER*, smooth endoplasmic reticulum.

ruffled and extended into microvillous projections. Coated pits in the plasma membrane and dense-walled cytoplasmic vesicles as well as smooth pits and vesicles indicate micropinocytotic activity.

The nuclei of these cells are generally large, circular to oval, with an even granular chromatin pattern except for some margination, and a prominent nucleolus. In favourable sections the nucleoli appear dark peripherally, with a lighter central *pars amorpha*. A honeycomb of dense nucleonemata is another common configuration (Fig. 3). This nucleolar configuration is considered indicative of a synthetically active cell (Fawcett, 1966).

Two clearly evident cytoplasmic features are a population of large circular or oval mitochondria and a series of clear cytoplasmic vesicles of various sizes. The mitochondria, although generally round or oval and between 0·3 μm and 0·6 μm in diameter, show some rod-shaped images. Their most significant feature is the tubular configuration of their cristae. A moderately dense mitochondrial matrix and dense intramitochondrial granules are present but no intramitochondrial lipid

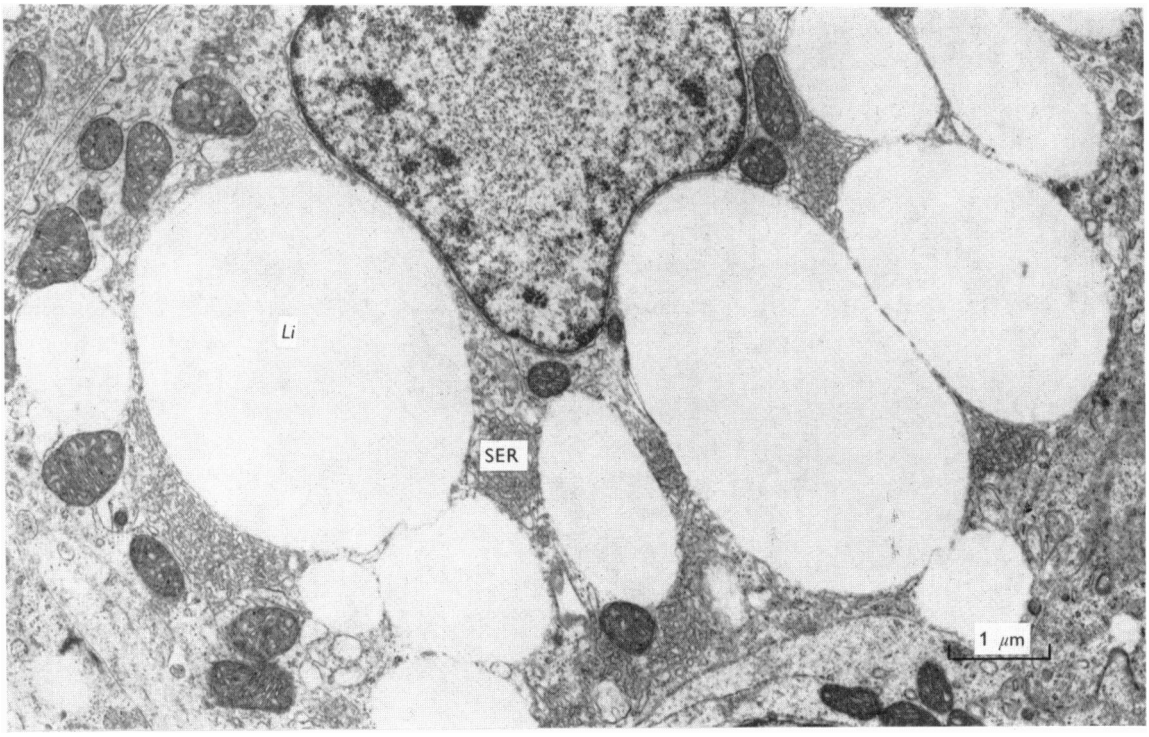

Fig. 4. Leydig cell showing maximum development of lipid droplets and SER.

is found (Figs. 3 and 4). The smaller mitochondria in some cells show distinct transverse lamellar cristae (Fig. 2).

Partially or completely extracted lipid droplets account for the clear cytoplasmic vesicles. A definite margin to these droplets is not always evident, because of the often close association of elements of endoplasmic reticulum and mitochondria. In some instances the lipid droplets are many times the size of a mitochondrion and occasionally approach the size of a nucleus (Fig. 4).

Endoplasmic reticulum is found predominantly in the form of the typical smooth-surfaced random tubular configuration of smooth endoplasmic reticulum (SER). Cisternae of rough endoplasmic reticulum (RER) are occasionally seen, but more commonly vesicles with only a few attached ribosomes, typical of transitional endoplasmic reticulum (TER), are found (Fig. 3). A distinct Golgi complex is evident, consisting of stacked cisternae and numerous round and smooth vesicles. Free ribosomes and polysomes are scattered throughout the cytoplasm and in favourable sections microtubules and microfilaments can be seen. Occasionally membrane-bound electron-dense bodies resembling primary lysosomes are found.

Often one cell or a whole group of cells appears darker than others although their morphology is essentially the same. Christensen & Gillim (1969) consider this an artefact of immersion fixation in glutaraldehyde, although Ladman & Young (1958) are of the opinion that the dark and light cells are modifications of a single cell type at different phases of metabolic activity.

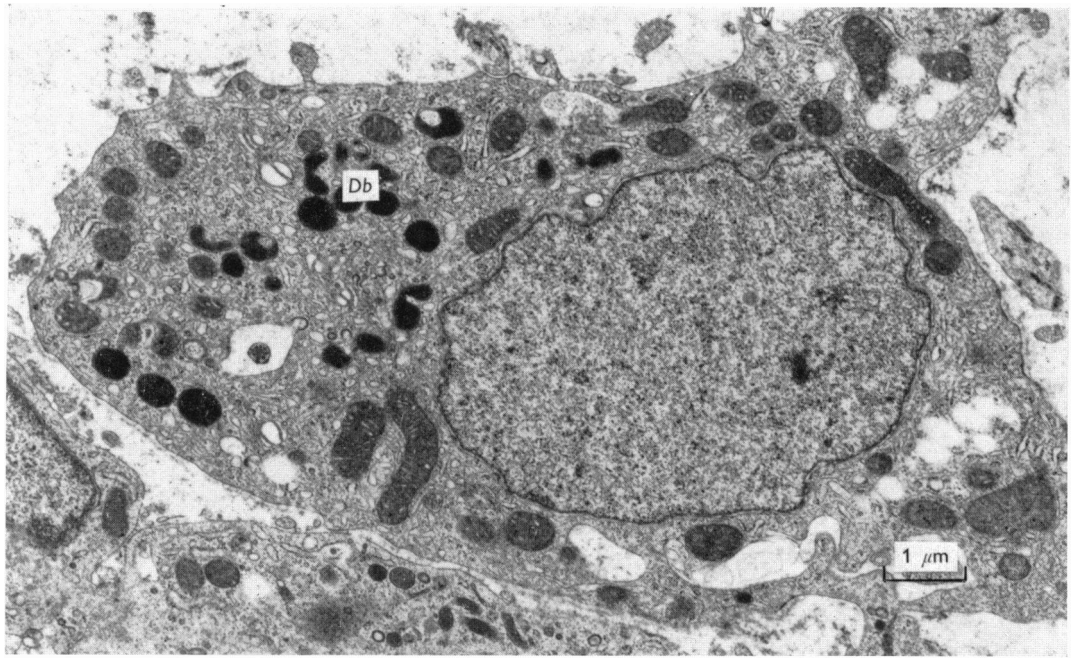

Fig. 5. Leydig cell. Note the reduction in size of the lipid droplets and the development of dense osmiophilic bodies (*Db*).

DISCUSSION

The Leydig cells of the fowl are similar to those of mammals (Fawcett & Burgos, 1960; Crabo, 1963; Christensen, 1965; Christensen & Gillim, 1969; Burgos, Vitale-Calpe & Aoki, 1970), showing fine structural features suggested by Fawcett, Long & Jones (1969) to be indicative of cells involved in steroid biosynthesis, namely large amounts of SER, a prominent Golgi complex, mitochondria with tubular cristae, lipid droplets and numerous lysosomal elements.

The presence of 'fibroblast-like' precursors of Leydig cells has been reported in the testes of different mammals (Fawcett & Burgos, 1960; Christensen & Fawcett, 1961; Crabo, 1963; Black & Christensen, 1969) as well as in those of the Japanese quail (Nicholls & Graham, 1972) and the fowl (Connell, 1972). The transitional cell image presented is essentially the same as that described in two day old chickens by Connell (1972), who considered the variation in ultrastructure seen in these cells as consistent with a transformation from a fibroblast-like form through a transitional form to a more mature Leydig cell. Some images had transitional cells with remnants of cytoplasmic filaments and interfilamentous dense bands indicating their possible origin from peritubular myoid cells or from a common precursor. A similar situation is found in man (Fawcett & Burgos, 1960; de Kretser, 1967). Because mitotic figures are rarely seen in the intertubular tissue, and since a cytological continuum exists between the fibroblast-like cell and the interstitial cell, Connell (1972) suggested that the transitional cell seemed to be the most likely

precursor of the Leydig cell in the two day old chicks. The present study supports this interpretation in fowls of various ages up to full sexual maturity.

It is possible to identify trends with respect to certain cytological features of the Leydig cells. Transitional cells have a large RER component in lamellar or cisternal elements. In other more polygonal cells some cytoplasmic vesicles are entirely smooth, representing SER, although the majority bear some ribosomes and can be described as TER. As more and more SER is found within a cell its configuration changes from the vesicular to a random tubular form and elements of TER and RER particularly are very sparse. This pattern of cytoplasmic membranes in the fowl follows the classic differentiation pattern described by Dallner, Siekevitz & Palade (1966). Cells in which the complement of TER is most developed, with areas of SER and RER in scattered patches, show an image indicative of a rapid production of SER which can be correlated with its implication in steroid biosynthesis (Christensen & Gillim, 1969).

Mitochondria range from about 0·3 μm in diameter, with a moderately dense mitochondrial matrix and predominantly lamellar cristae, to about 0·5 μm in diameter; in the latter the cristae are reduced in number and distinctly tubular. Intramitochondrial dense granules are seen which are considered in other cell types to be associated with divalent cation storage (Greenwalt, Rossie & Lehinger, 1964).

Lipid droplets are a major feature of Leydig cells in the fowl, but in the quail none are found (Nicholls & Graham, 1972). There is a build-up of lipid droplets from the transitional cell type to a stage where much of the polygonal Leydig cell cytoplasm is packed with them. This build-up is achieved concomitantly with the differentiation of SER and mitochondria with tubular cristae, to which the lipid is intimately related. These droplets probably represent a pool of steroid precursors and not storage of newly synthesized steroid (Applegren, 1967).

A diminution of this lipid appears to take place when one considers cells in which all the cytoplasmic features are well developed but in which only remnants of the lipid residues can be found (Fig. 5). This in mammals is considered indicative of Leydig cell functional maturation, as stored precursor is utilised in the production of new steroid. The increased deposition of dense osmiophilic bodies in these cells may also indicate an increase in metabolic debris of lipid metabolism, as in mammals (Christensen & Gillim, 1969).

Although a prominent Golgi complex is found in all Leydig cells no specific secretory function has yet been ascribed to it.

Various types of dense osmiophilic granules are observed in chick Leydig cells. Similar granules have been reported in other Leydig cells (Christensen & Fawcett, 1961, 1966; Belt & Cavazos, 1967; de Kretser, 1967; Nicholls & Graham, 1972) and although their nature is uncertain they are considered as different types of lysosomes (Frank & Christensen, 1968; Christensen & Gillim, 1969).

Amongst the cytological images presented, the pattern of cytoplasmic membrane differentiation, the build-up of lipid droplets concomitant with this differentiation and the development of mitochondria with tubular cristae, followed by the subsequent apparent diminution of the lipid, are trends that are similar to those suggested from studies on mammals (Black & Christensen, 1969; Christensen & Gillim, 1969;

Burgos *et al.* 1970), particularly those involving hormone stimulation known to increase androgen production (Merkow, Acevedo, Slifkin & Caito, 1968*a*, *b*; Aoki, 1970; Russo & Sacerdote, 1971) and are considered to be indicative of Leydig cell functioning. They also closely parallel the studies of Connell (1972) on LH-stimulated fowl testis and Nicholls & Graham (1972) on photoperiodically stimulated quail testis. Cells possessing morphological criteria indicative of Leydig cell functioning are therefore present in the fowl testis intertubular tissue from immediately after hatching through to sexual maturity.

SUMMARY

Leydig cells of the domestic fowl have ultrastructural characteristics similar to those of the quail and mammals. An elongated transitional cell with well-developed RER and lipid droplets, and a polygonal series of cells possessing mitochondria with tubular cristae, smooth endoplasmic reticulum and lipid droplets are found. Trends within the cell types associated with these and other cytological features indicate the possible functional similarity of the fowl Leydig cell to such cells in mammals.

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