Zeitschrift für Zellforschung 61, 587—604 (1963)

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FINE STRUCTURE OF THE INTERSTITIAL CELLS OF THE RABBIT TESTES

By

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With 17 Figures in the Text

(Received July 22, 1963)

Bouin and Ancel (1903) were the first to ascribe hormonal activity to the interstitial cells. To-day these cells are considered to be the main androgen source of the body. The morphology of the Leydig cells as seen with the light microscope is well-known from a large number of works most of which have been surveyed by Rasmussen (1932). The interstitial cells are generally considered to develop from mesenchymal cells. The relative number of interstitial cells, as well as the amounts of lipids and pigments in the cell, however, vary greatly from species to species. The Leydig cells of the rabbit occur in moderate numbers and contain comparatively many lipid droplets (Williams, 1950). Williams described them as being almost identical to those of the wood-chuck *(Marmota monax)*, as observed by Rasmussen (1917, 1918), but noted that they differed slightly in that they contained no pigments. When transplanted to a transparent chamber in the ear, the rabbit cells showed cyclical changes similar to those of the cells of the wood-chuck, but in the rabbit these changes were not seasonal.

Nothing seems to have been published about the ultrastructure of the interstitial cells of the rabbit. However, during the last few years investigations have been carried out on the fine structure of the testicular interstitial cells of man (Fawcett and Burgos 1956, 1960), rat (Wilke and Scheuhardt 1958, Christensen 1959, and Christensen and Chapman 1959), opossum (Christensen and Fawcett 1961), and a fish, *Lebistes reticulatus*, (Follenius and Porte 1960). A common feature to all is a well-developed agranular endoplasmic reticulum, but in other points, species variations do occur. With the use of new techniques, it was thought that it would be rewarding to examine the ultrastructure of the interstitial tissue of the rabbit testes and to compare the findings thus obtained with the results published by Williams (1950) in his transplantation experiments.

Materials and methods

Fourteen sexually mature rabbits were euthanised by a blow on the neck. Immediately after death, very small tissue pieces from the testes were fixed for one hour in an ice-cold, 1 per cent buffered osmium tetroxide solution with the addition of sucrose according to Caulfield (1957). Testicular tissue from two animals was fixed in chromatedichromate-buffered 1 per cent osmium tetroxide solution adjusted to approximately pH 7.6 and containing 5 per cent sucrose. After fixation for two hours, these bits were washed briefly in distilled water and were then postfixed in two five-minute changes of 10 per cent formol (procedure of Dalton, cf. Christensen and Fawcett 1961). Small pieces of testicular tissue from three animals were fixed in 2 per cent unbuffered potassium permanganate for two hours at about 20° C according to Mollenhauer (1959). After fixation, the specimens were washed briefly

in distilled water, dehydrated in alcohol, and embedded either in a mixture of methyl- and n-butylmethacrylate (1:9), or in Epon epoxy resin (Luft 1959). Thin sections were cut on an Ultrotome (LKB) or a Porter-Blum microtome and were picked up on formvar coated copper grids. Some of the sections were stained with uranyl acetate before examination which was performed in a Siemens Elmiscope I. For low magnification electron microscopy, thick sections (about 0.25 μ) were used as recommended by Björkman (1962).

Material for light microscopy was fixed in Bouin's or Helly's fluids, or in a 10 per cent neutral formaldehyde solution and was stained with either haemalum and eosin, periodic acid-Schiff's reagent, or Sudan Black B.

Observations

Generals description. The interstitial spaces are limited towards the seminiferous tubules by the basement membrane. This is composed of three to five parallel lamellae of medium electron density

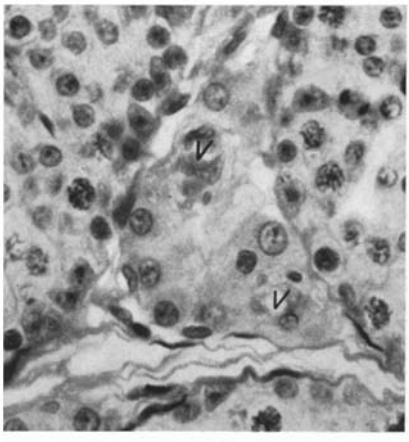

Fig. 1

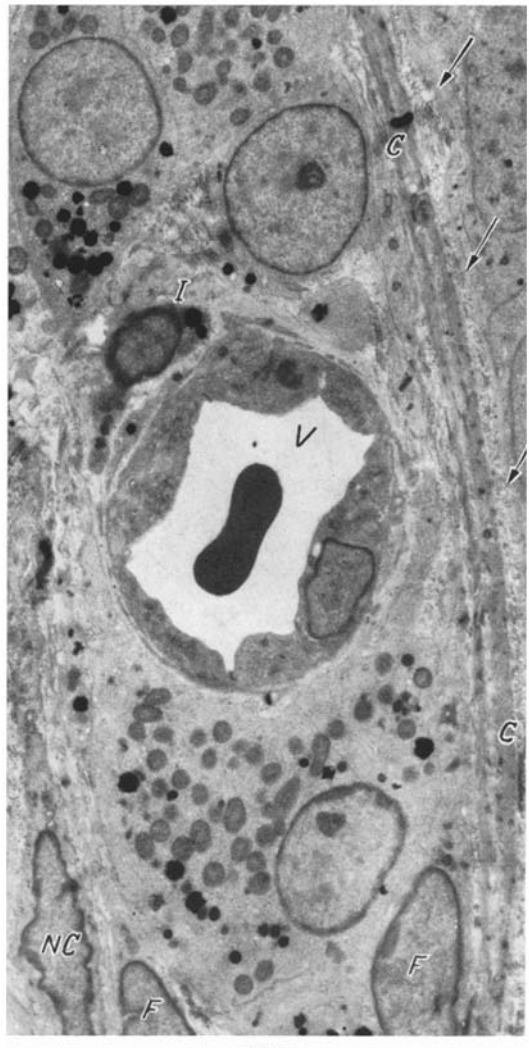

Fig. 2

Fig. 1. A group of typical interstitial cells enclosing vessels (*V*) and surrounded by fibroblasts. Hemalum and eosin. 450 ×

Fig. 2. Osmium tetroxide fixation and Epon embedding as in all the following pictures unless otherwise stated. Low magnification electron micrograph showing light and immature (*I*) interstitial cells. Vessel (*V*) with an erythrocyte in the middle. Note the contractile cells (*C*) to the right between the interstitial cells and the basement membrane (arrow). Nuclei of contractile cell (*NC*) and fibroblast (*F*) left bottom. 2300 ×

(Figs. 3, 4). The basement membrane is intimately connected with a regular plaited work of fibres. Outside these fibres, a layer of elongated cells occurs, surrounded by a layer of a homogenous material. The cytoplasm of these cells looks condensed and the organelles lie in the centre of the cells (Figs. 3 and 4). Along

the cell margins, there are a few small vesicles remniscent of pinocytotic vesicles. Occasionally it is possible to discern fibrils directed lengthwise in the cytoplasm. According to CLERMONT (1958), the corresponding cells in the rat are contractile. The structure of these cells in the rabbit is similar to that of smooth muscle cells. As a rule, one or two layers of fibroblasts are observed between this "tubule wall complex" and the interstitial tissue proper. This tissue consists mainly of interstitial cells dispersed among the common connective tissue elements.

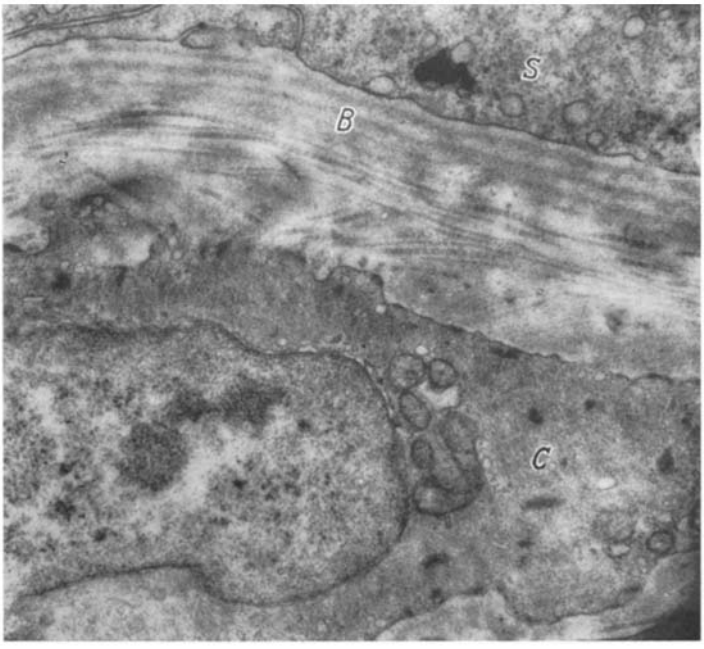

Fig. 3. Sertoli cell (*S*), lamellar basement membrane (*B*) and contractile cell (*C*). Note the fibres between the basement membrane and the contractile cell. 28000 ×

The mature interstitial cells are large, polygonal, and sometimes elongated. They are usually arranged in clumps which are perforated by one or two small vessels (Figs. 1 and 2). These cellgroups are separated from each other by an envelope of fibroblasts. This arrangement seems to be the rule in many species (cf. RASMUSSEN 1932, ROOSEN-RUNGE and ANDERSON 1959). The Leydig cells either lie close together or are separated by spaces of varying widths. Now and then, bundles of collagenous fibres are seen in these spaces (Figs. 2, 11). There is usually a space of varying width between the Leydig cells and the vessels. There may be both bundles of fibres and connective tissue cells within this space (Figs. 2, 12, 13, 16).

The capillaries (Figs. 2, 12), which are thus invested in connective tissue, are surrounded by a multilayered basement membrane which is complete and continuos, although it is faint. The endothelium forms a complete wall without fenestrations. Between adjacent cells, the connection is loose towards the perifery, where the cytoplasm forms irregular microvilli. Towards the lumen, however, the narrow intercellular spaces are sealed by desmosomes. Aside from the features

pointed out above, the capillaries belong to the A-1-α-type (Bennet et al., 1959). The endothelium is relatively thick with a fairly high content of mitochondria and a well developed endoplasmic reticulum with granular membranes.

Each interstitial cell contains one nucleus with one or two large nucleoli. The nuclei are round or ovoid and are relatively large. They are most often light with a diffuse peripheral rim of chromatin. Certain nuclei are, however, smaller and show varying degrees of pycnosis. The cytoplasm is more or less acidophilic. In preparations fixed in Helly's fluid, it is possible to see differences in acidophilia

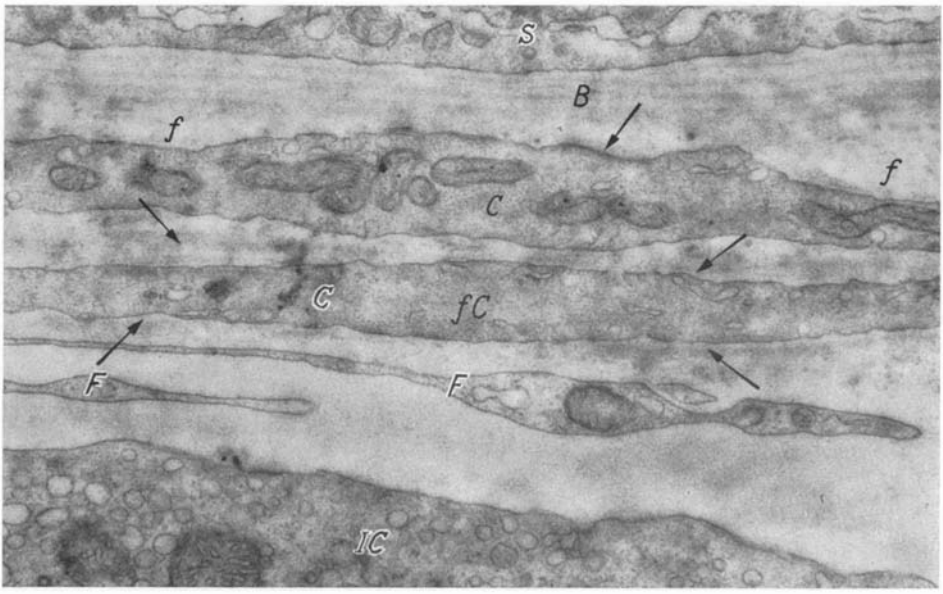

Fig. 4. Sertoli cell (*S*), basement membrane (*B*), contractile cells (*C*), fibroblasts (*F*), and light interstitial cell (*IC*). Note the basement membrane like substance (arrows) surrounding the contractile cells, the cross sectioned fibres (*f*) occuring at regular intervals between the basement membrane and the contractile cell, and the filaments in the lower contractile cell. (*fC*). 23000 ×

between adjoining cells. Those cells which contain a pycnotic nucleus show the strongest acidophilia. The interstitial cells are diffusely sudanophilic and contain a great number of lipid droplets which are generally localized near the poles.

With the electron microscope, it is possible to distinguish four different types of interstitial cells. Some cells are thought to be mature interstitial cells owing to their size and high content of well-developed organelles. Among these are both light and dark types. Transitional forms between these two types are seen regularly. Other cells, with an ultrastructure intermediate between that of light interstital cells and indifferent mesenchymal cells, have been classed as a third type. They have been regarded as immature interstitial cells. A fourth cell type is similar to the preceeding one but contains abundant osmiophilic inclusions of varying structure ("pigment cells"). This cell type is not common. Cells within each group very often show a uniform structure; however, there may be a mixing of dark and light interstitial cells as well as light and immature interstitial cells.

Light interstitial cells. This is the most common cell types in rabbits. They are large, light, polygonal cells which are sometimes provided with long protrusions.

Their borders are relatively smooth. The nuclei are light and most often round. After osmium tetroxide fixation, the cytoplasm of the light cells is crowded with small, sometimes interconnecting vesicles measuring 70—200 mμ in diameter. Their contents are somewhat opaque. There are also small areas with another

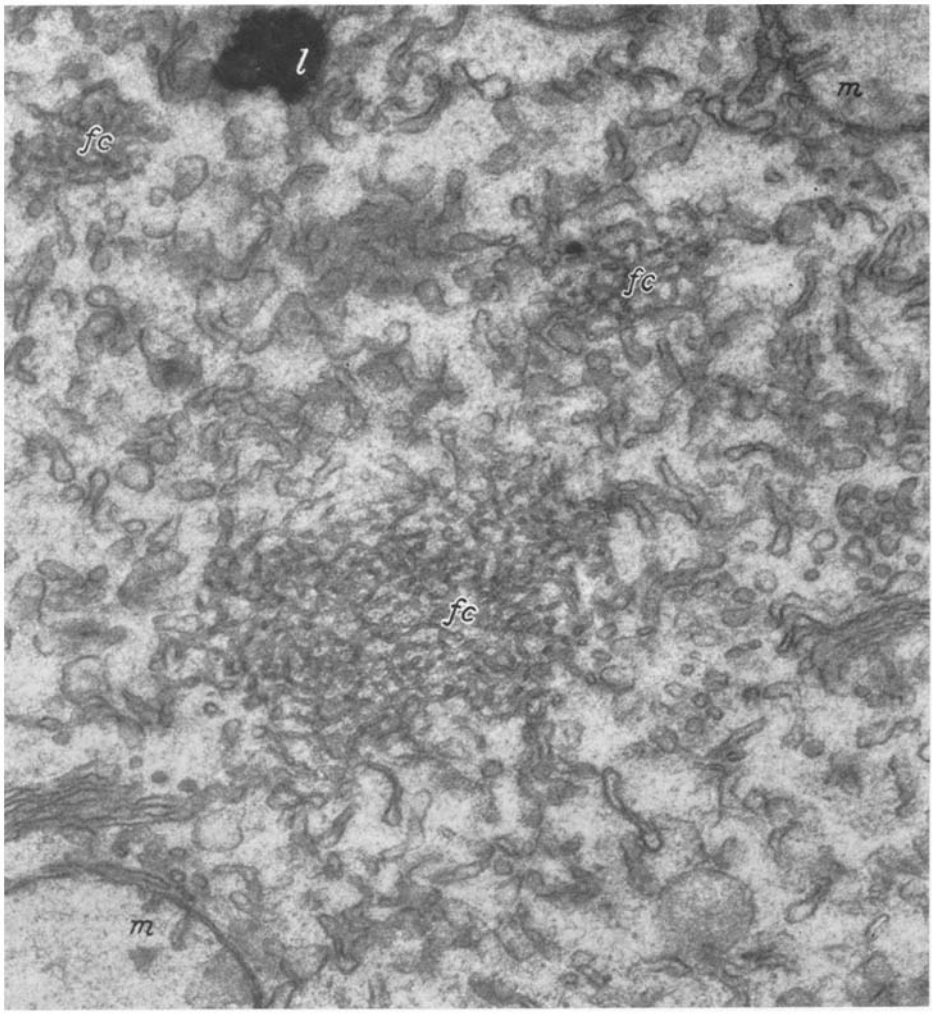

Fig. 5. Part of an interstitial cell showing the two modifications of the endoplasmic reticulum. Permanganate fixation. The fine calibre system occurs in nests (*fc*). Mitochondria (*m*), lipid droplet (*l*). 37 000 ×

membrane system, the units of which in the osmium material seem to consist of densely packed intercommunicating vesicles or tubules with a diameter of 15—40 mμ.

After permanganate fixation, the large vesicles seem to be collapsed and look like elongated, curved or branched, sometimes circular profiles with a diameter varying between 30 and 100 mμ. Their contents are always denser than the surrounding cytoplasm (Fig. 5). The fine calibre membrane system appears as branched tubules after this fixation. Their diameter is about 15 mμ and is

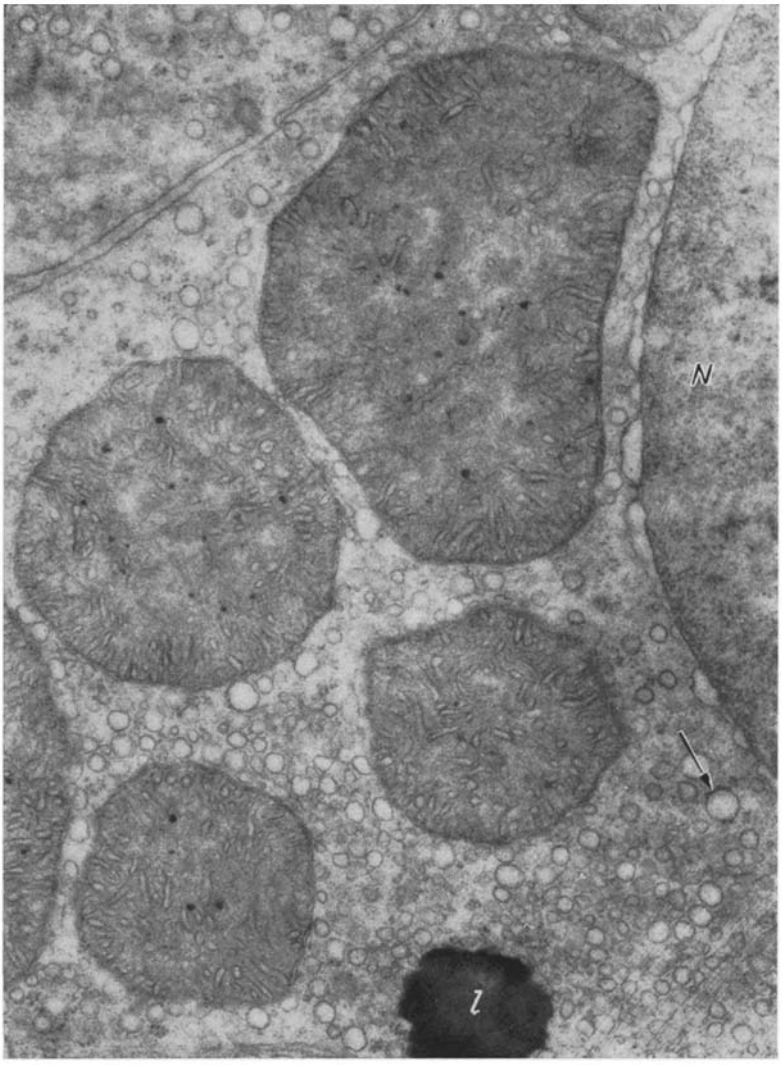

Fig. 6. Light interstitial cell containing "giant mitochondria". At the arrow, a few ribosomes associated with the endoplasmic reticulum. Nucleus (*N*), lipid droplet (*l*). 22000 ×

relatively constant. In the periphery of such areas, some tubules are seen to widen, giving the impression that these two membrane systems communicate. They probably represent two modifications of the *endoplasmic reticulum*.

Free *ribosomes* are common in the cytoplasm. They are most often assembled into rosettes. They are rarely related to the membranes of the endoplasmic reticulum.

The *mitochondria* are large and abundant (Figs. 6, 7). They are rounded or ovoid, seldom elongated or branched. Their transverse sections, as measured over the smallest diameter, vary somewhat but are at least 0,3 μ and most often about 0,6—0,9 μ. In some cells, large mitochondria were observed with a diameter exceeding 2 μ (Fig. 6). The inner membrane of the mitochondrion projects into the relatively dense matrix as fine tubules with a diameter of about 18 mμ. These

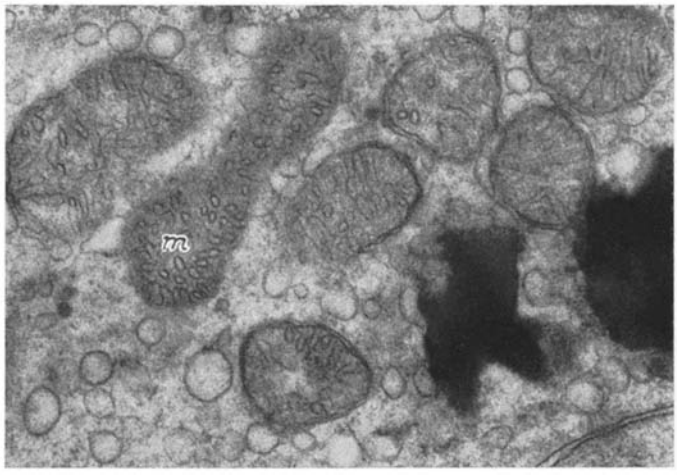

Fig. 7. Mitochondria of light interstitial cell. The tangentially sectioned mitochondrion (*m*) clearly show a tubular inner structure. 28 000 ×

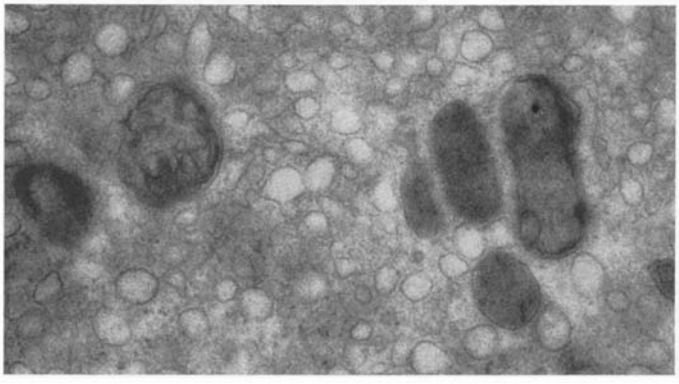

Fig. 8. Dense bodies (microbodies) with internal structure in a very young dark cell. To the left, a microbody which may have reached a more advanced stage of development. 29 000 ×

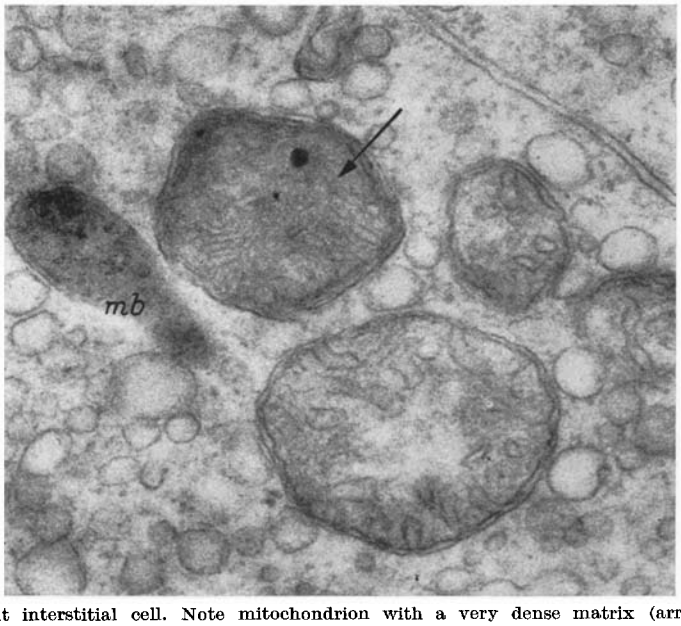

Fig. 9. Light interstitial cell. Note mitochondrion with a very dense matrix (arrow) and the microbody (*mb*). 50 000 ×

tubules often show a tendency to run radially towards the centre of the mitochondrion. As they are sometimes relatively short, the centres of many mitochondria look emty. The "giant mitochondria", on the other hand, are filled with curling tubules. One or more dense internal bodies are observed regularly in the matrix of the mitochondria. A few scattered mitochondria show a denser matrix (Fig. 9). In permanganate fixed material, the mitochondria are not well preserved.

The *Golgi apparatus* is most often observed in a juxta-nuclear position but is sometimes situated in the middle of the cytoplasm far from the nucleus. It con-

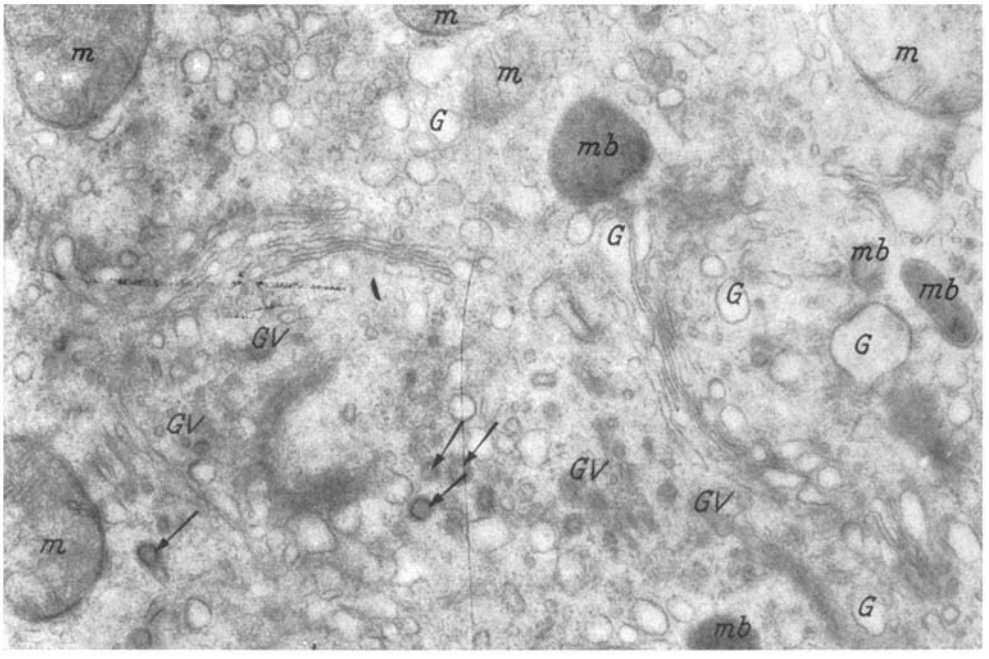

Fig. 10. Light interstitial cell Golgi apparatus surrounded by mitochondria (*m*) and microbodies (*mb*). Golgi vesicles (*GV*), Golgi vacuoles (*G*). Note the dense vesicles (arrows). 28000 ×

tains four to five pairs of curved parallel membranes. Outside these membranes small Golgi vesicles are assembled together with a few light vacuoles (Figs. 10 and 11.) It is impossible to distinguish the latter ones from the vesicles of the endoplasmic reticulum, but some of the vesicles are thought to be Golgi vacuoles because of their larger size (250—350 mμ). The Golgi vesicles have a varying size, from 35 to 180 mμ in diameter. Their periphery is more electron dense than their homogenous central parts and is often quite blackened.

The *centrioles* were sometimes observed. They are occasionally seen to lie perpendicular to each other. A small flagellum was once seen protruding from one of them (Fig. 11).

Lipid globules are always present in the light interstitial cells, often in large amounts. They sometimes lie in groups and have surfaces which are smooth or slightly irregular.

Dense bodies, which measure about 0.2—0.4 × 0.4—0.7 μ, are spread thoroughout the cytoplasm, but seem to be especially abundant around the Golgi apparatus

(Fig. 11). They have a very dense, somewhat granular matrix and are bounded by a single membrane. As a rule, the matrix is homogenous, but within some of the dense bodies, simple membrane structures or osmiophilic dots have been observed (Fig. 8). A light slit is observed between the surrounding membrane and the matrix

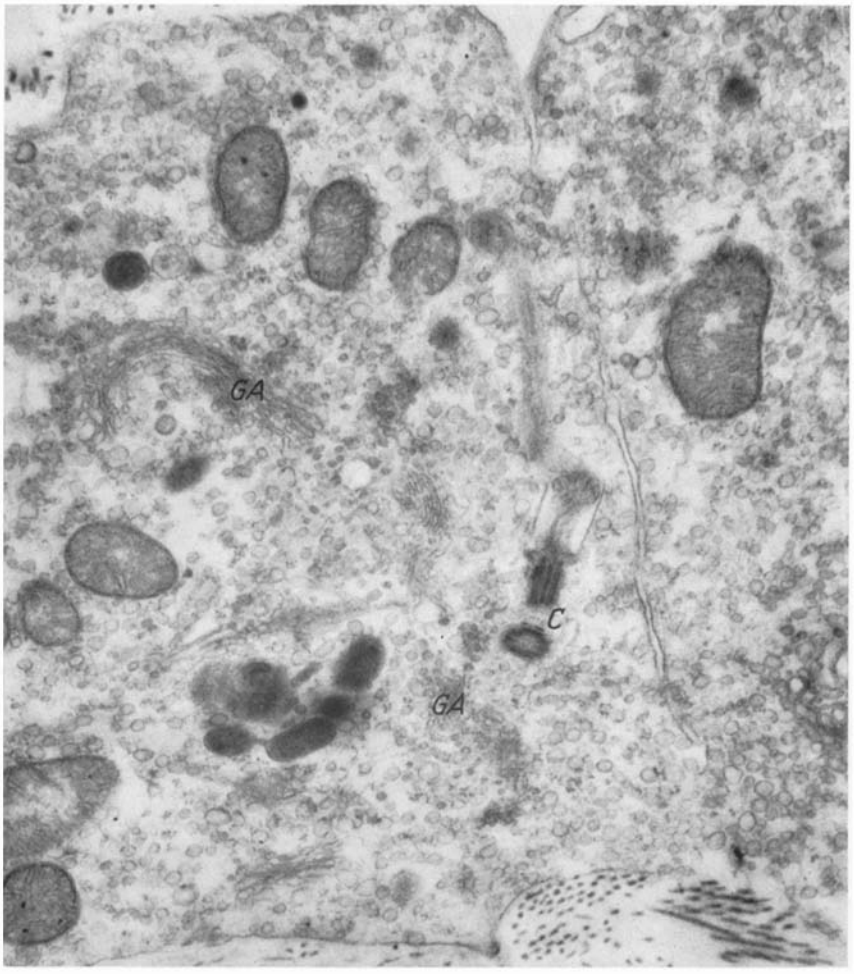

Fig. 11. Light interstitial cell with Golgi apparatus (*GA*) and the two centrioles (*c*) perpendicularly arranged and with a flagellum sectioned tangentially. Note the clump of microbodies in the Golgi area. 18000 ×

in the material fixed in osmium tetroxide. After permanganate fixation, the contents are greyish and no light slit is observed inside the membrane.

Dark interstitial cells. On the whole, the dark interstitial cells show the same structure as the light ones. The cytoplasm is more osmiophilic, but generally not of such a high degree as in the dark interstitial cells of the opossum (CHRISTENSEN and FAWCETT 1961) or the bull (CRABO 1963). There are also differences other than the stronger osmiophilia. The outlines of the dark interstitial cells are very often irregular with projections remniscent of microvilli (Fig. 13). There are many small peripheral vesicles which communicate withthe intercellular spaces. The

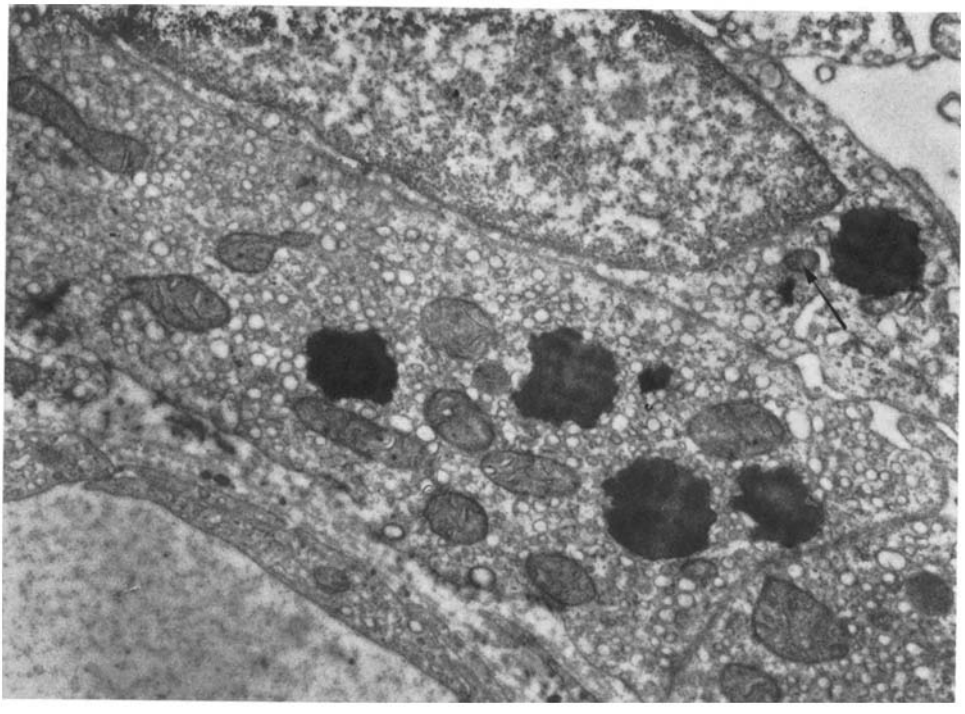

Fig. 12. Immature interstitial cells. Methacrylate. Note the very small mitochondrion (arrow) in the least developed cell. Capillary wall bottom left. 22000 ×

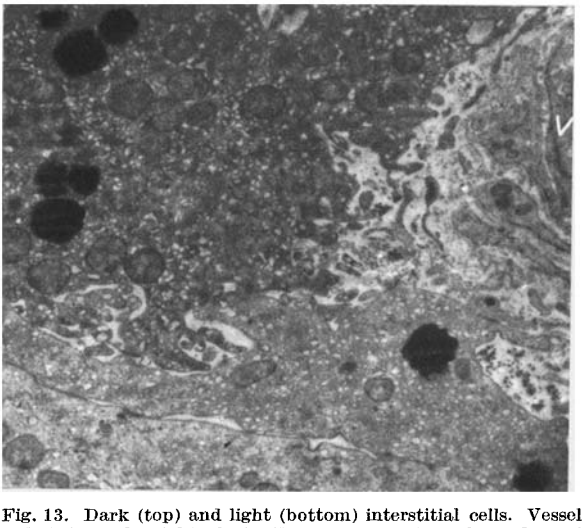

Fig. 13. Dark (top) and light (bottom) interstitial cells. Vessel wall (*V*) to the right. Note the very irregular cell borders of the dark cell. 6000 ×

nuclei look slightly creased compared to those of the light interstitial cells, as do also the mitochondria and the cisternae of the endoplasmic reticulum. The fine calibre component of the endoplasmic reticulum occupies a larger area than in the light cells (Fig. 14). Sometimes, it even occupies the main part of the cytoplasm. The dark appearance of these cells is, to a certain extent, dependent on the abundant occurrence of these membranes, but the ground cytoplasm is also dark and granular.

Those cells which have been classified as dark interstitial cells do not show a perfectly uniform picture. Some of them resemble the light interstitial cells in ultrastructure, while others show signs of degeneration (Fig. 15). Now and then,

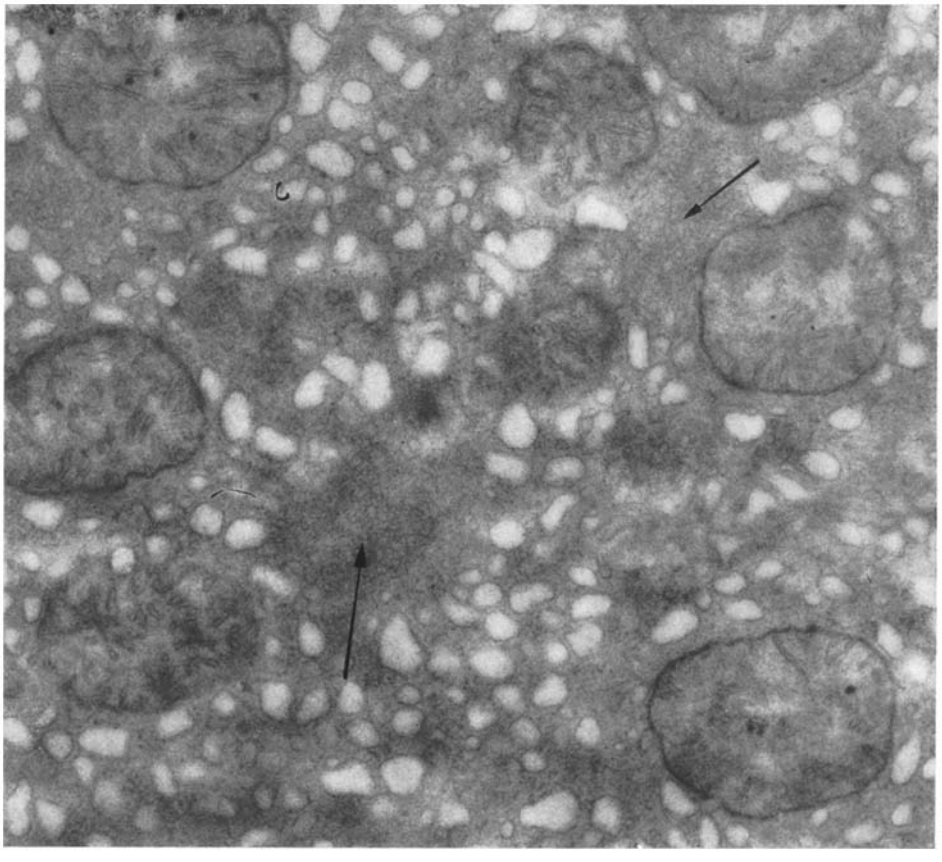

Fig. 14. Dark interstitial cell. Note the shrunken mitochondria and the nests formed by the fine calibre membrane system (arrows). 37 000 ×

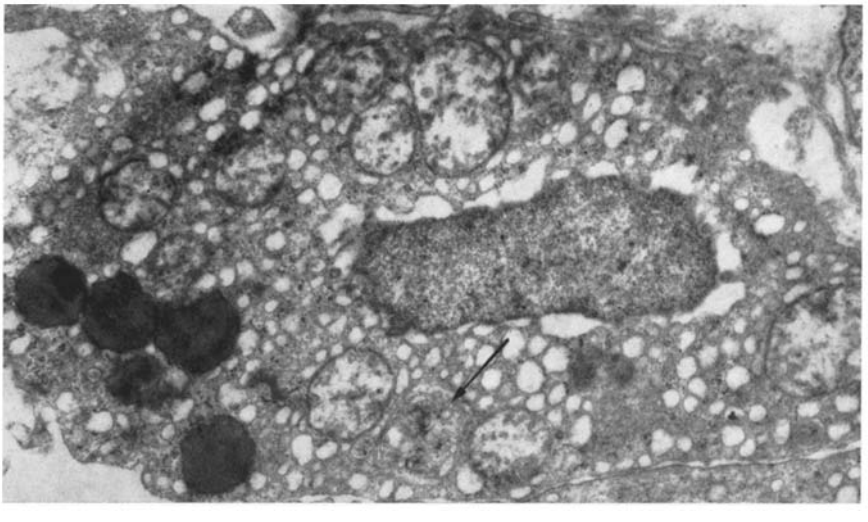

Fig. 15. Degenerating dark interstitial cell. At the arrow, a mitochondrion with osmiophilic inclusions and four membranes. 12 000 ×

one or two cells with small and dense nuclei are seen within a cell group. The perinuclear space is dilated and the vesicles or cisternae of the endoplasmic reticulum are enlarged and seem to be empty. The mitochondria are swollen and often contain dark membranes or osmiophilic inclusions. These cells probably correspond to the cells with pycnotic nuclei as seen with the light microscope.

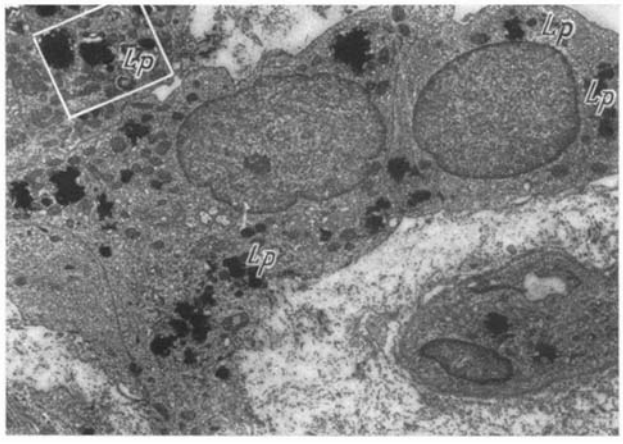

Fig. 16. Cells containing lipopigments (*Lp*). Methacrylate. 3500 ×

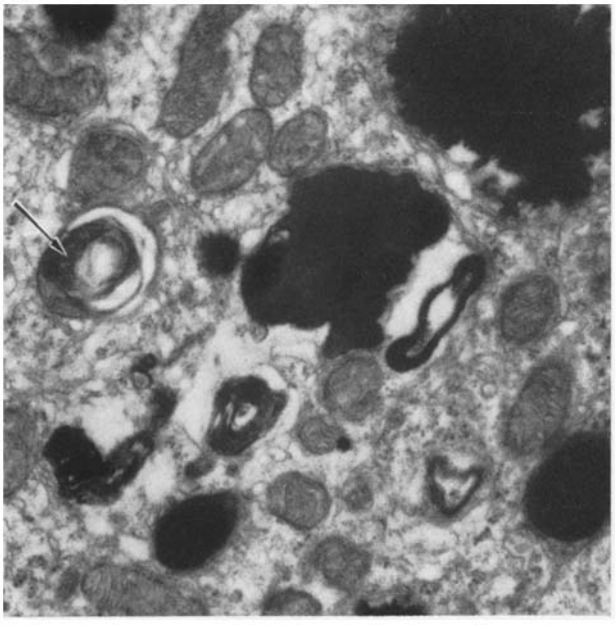

Fig. 17. Enlargement of the area marked in Fig. 16. At the arrow, a mitochondrion closely attached to lipopigments. 22000 ×

Indifferent mesenchymal cells. These are elongated slender cells with little cytoplasm. Their nuclei are also long and similar to those of the fibroblasts. The endoplasmic reticulum is represented by a few, most often agranular vesicles. A small undeveloped Golgi apparatus lies near the nucleus. The few mitochondria have a diameter of about 0.1—0.2 μ and show an ordinary internal structure with a few parallel cristae.

Immature interstitial cells. Intermediate stages between indifferent mesenchymal cells and light interstitial cells are often observed. They have been called immature interstitial cells. Lipid droplets are often seen in these cells (Figur 12). The endoplasmic reticulum is more or less expanded and of the same type as in the light interstitial cells. The diameters of the mitochondria are rather constant within a single cell, but vary considerably between cells (0.2 to 0.6 μ). At a diametre of about 0.3—0.4 μ, the internal membranes of the mitochondria begin to change from cristae to tubules. On the whole, cells with small mitochondria also show the least developed structures. The transition between

immature interstitial cells and light interstitial cells is gradual, and those cells which appear to be most mature contain the largest mitochondria. The diameter of the mitochondria thus seems to be a good measure of the developmental stage of those cells.

"Pigment cells". Sometimes cells are observed, the size and shape of which varies from those of fibroblasts or indifferent mesenchymal cells to those of mature interstitial cells. Their mitochondria are of about the same size and structure as those seen in the immature interstitial cells. The endoplasmic reticulum shows, as a rule, a relatively simple structure and is most often tubular. The main difference between these cells and the immature interstitial cells is the occurrence of osmiophilic inclusions of varying structure in the former ones (Figs. 16, 17). Sometimes they look like myelin figures and other times they consist of more or less dense clumps containing granules with a stronger osmiophilia. Occasionally mitochondria have been observed in intimate contact with such inclusions.

Discussion

The common feature of the interstitial cells of those species which have hitherto been examined in the electron microscope is a well developed agranular endoplasmic reticulum (FAWCETT and BURGOS 1956, 1960, FOLLENIUS and PORTE 1960, CHRISTENSEN and FAWCETT 1961). In most cases, it has the shape of small vesicles. However, CHRISTENSEN and FAWCETT (1961) showed that the endoplasmic reticulum sometimes has the shape of flattened, fenestrated cisternae similar to those, occuring in pancreatic acinar cells. Most often the endoplasmic reticulum occurred as a tubular system with a tubular diameter of 30—40 mμ. In the matetial embedded in Epon, the endoplasmic reticulum of the Leydig cells always appeared in the shape of small vesicles. This was considered to depend on the embedding medium. In the present material, no obvious differences in the structure of the endoplasmic reticulum which could be due to the type of embedding medium used have been observed. The interstitial tissue of the testes seems to be more suspectible to polymerisation damage during methacrylate embedding than most other tissues. Therefore, the pictures obtained after embedding in Epon are considered to be more reliable.

After fixation in osmium tetroxide (according to CAULFIELD or DALTON), the endoplasmic reticulum most often appeared vesicular. The vesicles measured 0.07—0.2 μ in diameter. However, after fixation in permanganate, profiles were observed which probably represented a tubular system with a tubular diameter of 0.03—0.1 μ. Thus, permanganate fixation gives a picture which is strongly reminiscent of that observed in the opossum by CHRISTENSEN and FAWCETT (1961) after fixation according to DALTON and methacrylate embedding. FAWCETT and BURGOS (1960) did not succeed in demonstrating a tubular or cisternal type of endoplasmic reticulum in human interstitial cells, in spite of attempts under conditions identical to those used for the opossum tissue. It is therefore not easy to decide whether some of these pictures may be an artifact. It is wellknown that material fixed in dichromate or permanganate solutions shrinks. After such a treatment the vesicles may be compressed and appear as tubules. Osmium tetroxide is considered to give a better preservation of fine structure than permanganate. If that is true, the large, sometimes communicating vesicles may

represent the true picture. However, it is also known that the endoplasmic reticulum when treated roughly is easily transformed into vesicles (Bernhard et al. 1954). After osmium fixation, the endoplasmic reticulum has a somewhat different appearance in different types of rabbit interstitial cells. This may indicate different functional stages of the cells. The endoplasmic reticulum is possibly more easily transformed into vesicles in certain cells types; i.e., in the dark cells, it appears as elongated, irregular profiles. Those cells which contain osmiophilic inclusions, have a simple, tubular endoplasmic reticulum, whereas the reticulum is widely spread and vesicular in the light cells.

The large number of vesicles communicating with the intercellular spaces in some (probably young) dark cells is difficult to explain. Formation of new endoplasmic reticulum vesicles from the cell membrane, pinocytosis, or liberation of hormones are possible explanations. The steroid hormones are, to a great extent, synthesized by the endoplasmic reticulum (cf. Christensen and Fawcett 1961). At the same time as an increased surface activity is observed, strongly increased amounts of the *fine calibre* tubular system are seen in the dark cells. The fine calibre system may be a modification of the ordinary endoplasmic reticulum and perhaps consists of cisternae which have emptied themselves.

There are many ribosomes in the immature and light interstitial cells. They are mainly arranged in rosettes in the cytoplasm. This arrangement is common in actively growing cells and the immature and light interstitial cells belong to this category.

The mitochondria of the interstitial cells in previously examined species have shown a somewhat variable picture. In human interstitial cells, they are often swollen and bizarre in shape (Fawcett and Burgos 1956, 1960). This probably depends on the great sensitivity of the mitochondria to fixation and polymerisation damage. In the rat and the fish, *Lebistes reticulatus*, mitochondria with an obviously tubular inner structure have been observed (Belt and Pease 1956, Wilke and Scheuhardt 1958, Follenius and Porte 1960). The mitochondria in the interstitial cells of the opossum are somewhat irregular, scanty, and of such a simple structure, that Christensen and Fawcett (1961) judged them to be of little or no importance for the specialized function of the cell.

In the rabbit, the case is different. As a rule, especially in the light interstitial cells, there are a large number of regularly ovoid mitochondria with tubular inner structures. They often have a cross section exceeding $2\,\mu$ and are obviously similar to the giant mitochondria observed by Belt and Pease (1956) in the adrenals of the rat.

After hypophysectomy, the mitochondria of the interstitial cells in the rat diminished in size (Christensen 1959) and the inner tubules were replaced by ordinary cristae. When hypophysectomized animals were treated with gonadotrophins, the opposite occurred (Wilke and Scheuhardt 1958).

The same changes in structure, as Wilke and Scheuhardt (1958) observed after treatment with gonadotrophins can be seen in normal rabbits. During the development from indifferent mesenchymal cells to mature interstitial cells the mitochondria grow continuously and their cristae are transformed into tubules. The mitochondrial fine structure may therefore be used as a criterion of the developmental stage of the interstitial cell.

The Golgi apparatus shows no special features and seems, in the rabbit, to have the same position in the cell as in the opossum (DUESBERG 1918, cit. by RASMUSSEN 1932). However, those Golgi vesicles which have dense contents are interesting. They are of various sizes and the largest ones approach the dense bodies in size. These dense bodies are similar, both in size and structure to the microbodies observed by RHODIN (1954) in kidney tubules. They occur most abundantly around the Golgi apparatus. It is thus possible that microbodies originate from the Golgi apparatus. Similar lines of thought have earlier been followed by OBERLING and ROUILLER (1957) and RHODIN (1958).

The microbodies of the rabbit interstitial cells are probably identical to the "secretion granules", which were described by RASMUSSEN (1932) as being present in the interstitial cells of many species. These "secretion granules" had many of the staining properties of mitochondria and were difficult to distinguish from these organellae. Many authors consider them to be derived from mitochondria. When the interstitial cells hypertrophy, the "secretion granules" are described as increasing in number and size (cf. RASMUSSEN 1932). In the present material, mitochondria with a very dense matrix and generally looking very similar to microbodies were observed, particularly in the light interstitial cells. Nowadays, a connection between mitochondria and microbodies is considered credible. Under experimental conditions, ROUILLER and BERNHARD (1958) observed how, during cell regeneration, the microbodies increased first in number before increasing numbers of mitochondria were seen. From this observation they concluded that the microbodies develop to mitochondria.

Mitochondria with osmiophilic inclusions and membranes can be seen particularly in the dark interstitial cells. These mitochondria are sometimes swollen and probably degenerating. They are possibly precursors of the lipopigments which are seen in certain cells. Mitochondria have been considered to be the origin of lipopigments in the adrenal cortex by LEVER (1955) and GADRAT et al. (1960). These authors based their conclusions upon pictures which were similar to those observed in the interstitial cells of the rabbit. In the liver, however, lysosomes are thought to be the origin of the lipopigments (cf. DE DUVE 1959).

The interstitial cells of most species are considered to develop from fibroblast-like cells. Such a development has been reported in man by MONTAGNA and HAMILTON (1951) and MANCINI et al. (1952), in the bull by HOOKER (1944) and in several other species (cf. RASMUSSEN 1932). A similar development was also observed by WILLIAMS (1950) in autogenous grafts of testicular tissue in rabbits.

Fibroblast-like cells developing into interstitial cells contain lipids. In man and opossum, they have been identified with the electron microscope as indifferent mesenchymal cells (FAWCETT and BURGOS 1956, 1960; CHRISTIANSEN and FAWCETT 1961). In man they contained fibrils (FAWCETT and BURGOS 1956, 1960). Such fibrils are not seen in the rabbit, but large fibrillar whorls have been observed both in immature and mature interstitial cells of the dog (CRABO, unpublished observations).

Dark interstitial cells have previously been demonstrated in the opossum by CHRISTENSEN and FAWCETT (1961), but they also occur in dogs, bulls, and boars (CRABO 1963). LEVER (1955) considered light and dark interstitial cells in the adrenal cortex to represent different functional stages of the cells. CHRISTENSEN

and Fawcett (1961) give a similar opinion about the dark interstitial cells of the opossum.

A comparison with Williams' (1950) experiments gives a better understanding of the different interstitial cells types of the rabbit testes. He transplanted testicular tissue to a transparent chamber in the ear of the rabbit and observed the cells for an extended period of time. Cell groups emerged from the connective tissue and grew progressively larger. Finally they reached a stage of maximal granulation. This stage lasted for about one week. Then the cells lost their transparent appearance and, within 6 to 12 weeks, disappeared into the surrounding connective tissue.

The maximally granulated cells observed by Williams (1950), probably correspond to the light interstitial cells in the electron micrographs and the non-transparent, dense cells to the dark cells, which seem to degenerate gradually. In the light microscope, the cytoplasm grows more and more acidophilic during this process and the nucleus shrinks. However, it is, at present, impossible to exclude the possibility that transformations between dark and light cells may occur before the final degeneration. As a rule, only one or two cell types have been observed within each cell group. When two types are present, they represent adjacent stages in the chain of development discussed here. This observation is also in agreement with those of Williams (1950).

Those cells, which contain pigments and which show a low activity as judged from their ultrastructure, were seen very sporadically. They are probably interstitial cells, which, for some reason, have not regressed in the usual way. Most human interstitial cells contain structural osmiophilic inclusions, and, therefor, Fawcett and Burgos (1956, 1960) felt that these inclusions might have some physiological function. Follenius and Porte (1960) found many interstitial cells containing great amounts of lipopigments in the fish, *Lebistes reticulatus*. The general structure of these cells made them think that the cells were expended interstitial cells. In the wood-chuck, the amounts of lipopigments increase after the sexual season at the same time as the cells diminish in size and number (Rasmussen 1917). Therefore, the occurrence of pigments is probably a sign of low or decreasing activity in the interstitial cells. Those interstitial cells of the rabbit which contain pigments may possibly be transformed into active interstitial cells when under the influence of gonadotrophins. Such cell transformation is described in the wood-chuck (Rasmussen 1917).

Summary

Interstitial tissue from 14 normal, sexually mature rabbits was examined in the electron microscope. A short description is given of the basement membrane of the seminiferous tubules and the contractile cells, which are connected with it.

Four different interstitial cell types are described: immature interstitial cells, light interstitial cells, dark interstitial cells, and interstitial cells containing lipopigments. Indifferent mesenchymal cells are the probable origin of immature interstitial cells which transform to light interstitial cells. The latter ones seem to be the most active cells judging from their ultrastructure. The dark interstitial cells are degenerating light cells. The sporadically occuring cells containing pigments are thought to be expended interstitial cells which have not degenerated and which are perhaps returning to a lower stage of differentiation.

In the mature interstitial cells, the agranular endoplasmic reticulum is the most prominent structure with the exception of the mitochondria. It is vesicular after osmium fixation and tubular after permanganate fixation and occurs in two modifications. The influence of different fixation fluids and embedding media on its structure is discussed.

The mitochondria of mature interstitial cells reach a considerable size and have a tubular inner structure similar to the mitochondria of many steroid hormone producing cells. During the development from indifferent mesenchymal cells to mature cell, the mitochondria increase in size and the cristae are replaced by tubules. A possible chain of development: Golgi vesicle — microbody — mitochondrion is also discussed.

The dark interstitial cells differ from the light ones in that they show a higher degree of osmiophilia and more irregular surfaces. The former very often show degenerating mitochondria which contain structures reminiscent of lipopigments. The pigment cell type is very similar to the immature interstitial cell except for its content of lipopigments.

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