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The Fine Structure of the Testicular Interstitial Cells in Men of Normal Androgenic Status

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Summary. The ultrastructural features of the testicular interstitial cells were examined in a group of men of normal androgenic status. In osmium-fixed material three cell types were identified, namely, an immature cell, a light cell, and a dark interstitial cell not previously described. The dark cells possessed increased amounts of tubular agranular endoplasmic reticulum, variable numbers of membrane-bounded bodies, and accumulations of glycogen granules. These differed from the light interstitial cells which possessed both tubular and vesicular forms of agranular endoplasmic reticulum, no demonstrable accumulation of glycogen, and in general the membranous components of the light cells were less electron dense than in the dark cell. Intermediate forms between the cell types were observed. Tubular crystalline inclusions with a regular substructure were described and a hypothesis linking these structures with the formation of the crystals of Reinke is discussed. A new cytoplasmic inclusion consisting of aggregations of short dense rod-shaped structures seen in these cells, was also described.

Introduction

Since the early postulate by BOUIN and ANCEL (1903) that the interstitial cells of the testis are involved in the production of androgenic steroid hormones, much evidence has accumulated to confirm their views. CHRISTENSEN and FAWCETT (1966) have summarized the evidence to date, both cytological and biochemical, which implicates the abundant smooth endoplasmic reticulum of these cells as the biosynthetic site of these hormones in all species studied so far.

The ultrastructural features of the interstitial cells of the testis in infertile men and men undergoing orchidectomy for carcinoma of the prostate, were described by FAWCETT and BURGOS (1956, 1960). These authors recognized, in their study, that material obtained from their patients could not be regarded as normal and no accurate assessment of the androgenic function of the interstitial cells in these patients was made. Apart from a brief report on the foetal and adult testicular interstitial cells by YAMADA (1962) and a study of the gonad in testicular feminization syndrome (GORDON et al., 1964), no report of further studies on these cells in man has come to the notice of the author, despite improvements in methods which have allowed the selection of patients of normal androgenic status. This has been made possible by technical advances in the measurement of the plasma testosterone level, now accepted as the parameter which affords the best indicator of the androgenic function of the interstitial cell (LIPSETT et al., 1966).

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During an ultrastructural study on testicular biopsies from healthy fertile men and from infertile men of normal androgenic status as judged by a normal plasma testosterone level, several noteworthy observations were made. In addition to an immature interstitial cell type similar to that described by FAWCETT and BURGOS (1960), and a light interstitial cell type corresponding in some features to the mature interstitial cell described by FAWCETT and BURGOS (1960), a dark type of cell, as yet unidentified in man, was described. Furthermore, new observations on the cytoplasmic inclusions found in these cells, in particular on the crystals of Reinke, were made. These new findings will form the subject of this paper.

Materials and Methods

Under general anaesthesia testicular biopsies were obtained from two healthy fertile men and from four infertile men whose plasma testosterone was in the normal range. Small pieces of tissue were immediately placed in cold 2.5% osmium tetroxide buffered with a potassium dichromate-calcium chloride mixture (RICHARDSON, 1962) for two hours. After washing in distilled water, the specimens were dehydrated in graded concentrations of acetone and embedded in Araldite.

Thin sections, exhibiting silver to gold interference colours, were stained with aqueous uranyl acetate (WATSON, 1958) followed by lead citrate (REYNOLDS, 1963). The sections were then examined in a Siemens Elmiskop I. B. at 80 K. V.

"Thick" sections of Araldite-embedded material were stained with toluidine blue and examined under the light microscope.

Observations

Light Microscopy. The interstitial cells of the testis were found either scattered singly or in small groups in the intertubular tissue. The study of Araldite-embedded material stained with toluidine blue emphasized the heterogeneity of the population of interstitial cells, and all gradations from intensely staining to poorly staining cells were observed (Fig. 1). This lends support to the observations of SNIFFEN (1950) and other investigators who, using light microscopy, described a variable appearance of the interstitial cells with respect to the granularity of the cytoplasm and the presence or absence of pigment, lipid, and the crystals of Reinke.

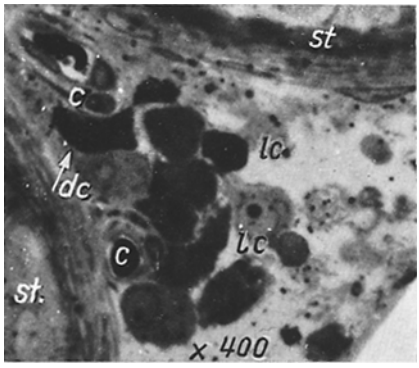


Fig. 1. Photomicrograph showing gradations in the intensity of staining of a group of interstitial cells found between the seminiferous tubules (*st*) in Araldite-embedded material stained with toluidine blue. Dark cells (*dc*), light cells (*lc*), and capillaries (*c*) are easily distinguished. × 400

Electron Microscopy. Ultrastructurally, three basic types of interstitial cells were identified; the fusiform or immature interstitial cell (SNIFFEN, 1950; FAWCETT and BURGOS, 1960), a light interstitial cell corresponding in some features to the mature interstitial cell described by FAWCETT and BURGOS (1960), and a dark type as yet unidentified. Although three definite types of interstitial cells were readily identified, a gradation between the cell types was also seen.

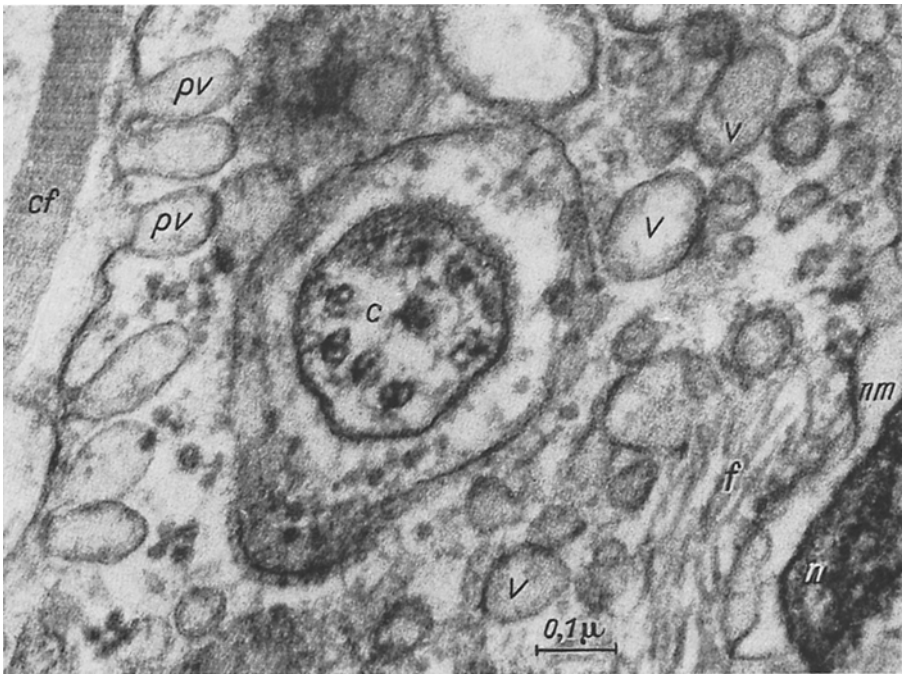


Fig. 2. A small area of an immature interstitial cell which illustrates the nucleus (*n*), nuclear envelope (*nm*), cytoplasmic fibrils (*f*), pinocytotic vesicles (*pv*), and a structure resembling a cilium (*c*). Vesicles (*v*) of the smooth endoplasmic reticulum are present in the cytoplasm and a collagen fibril (*cf*) is extracellularly placed. ×112,000

Fusiform Interstitial Cells

Fusiform interstitial cells, similar to those described by FAWCETT and BURGOS (1960) were seen. The ovoid nucleus, the small variable aggregation of vesicular smooth endoplasmic reticulum, and the distribution of cytoplasmic fibrils corresponded to the description by these investigators. Pinocytotic vesicles were a prominent feature in these cells and, in some, a moderately electron-dense content appeared to be in the process of being extruded or engulfed (Fig. 2). Gradations between this cell type and the contractile cells present in the lamina propria of the seminiferous tubule (ROSS and LONG, 1966) were frequently observed. Rarely, a structure resembling a cilium in cross-section was seen in the fusiform interstitial cell (Fig. 2).

Light Interstitial Cells

The light interstitial cell was characterized by the presence of a well-developed smooth endoplasmic reticulum which varied greatly from cell to cell. In some it appeared as an extremely compact network of vesicles or tubules, whilst in neighbouring cells the density of these components was relatively sparse (Figs. 3, 4, 7).

This system consisted of a number of vesicles whose diameter ranged from 55 mµ to 450 mµ. These vesicles were frequently filled with a homogeneous

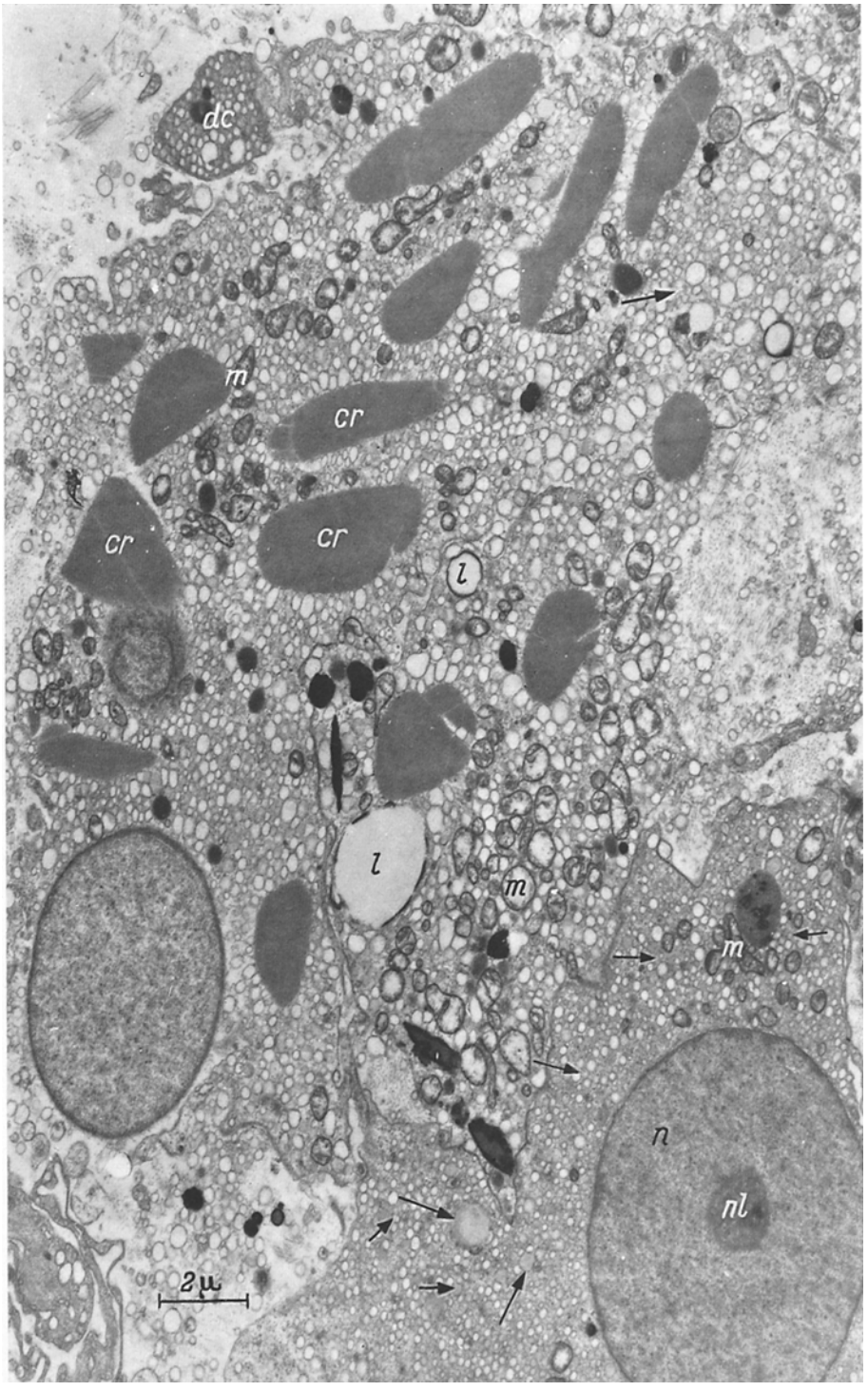


Fig. 3. A group of light interstitial cells shows the gradation in size of the vesicles (arrows) of the smooth endoplasmic reticulum, the nucleus (*n*), nucleolus (*nl*), lipid droplets (*l*), mitochondria (*m*), and the crystalloids of Reinke (*cr*). A dark cell (*dc*) is indicated. ×6,700

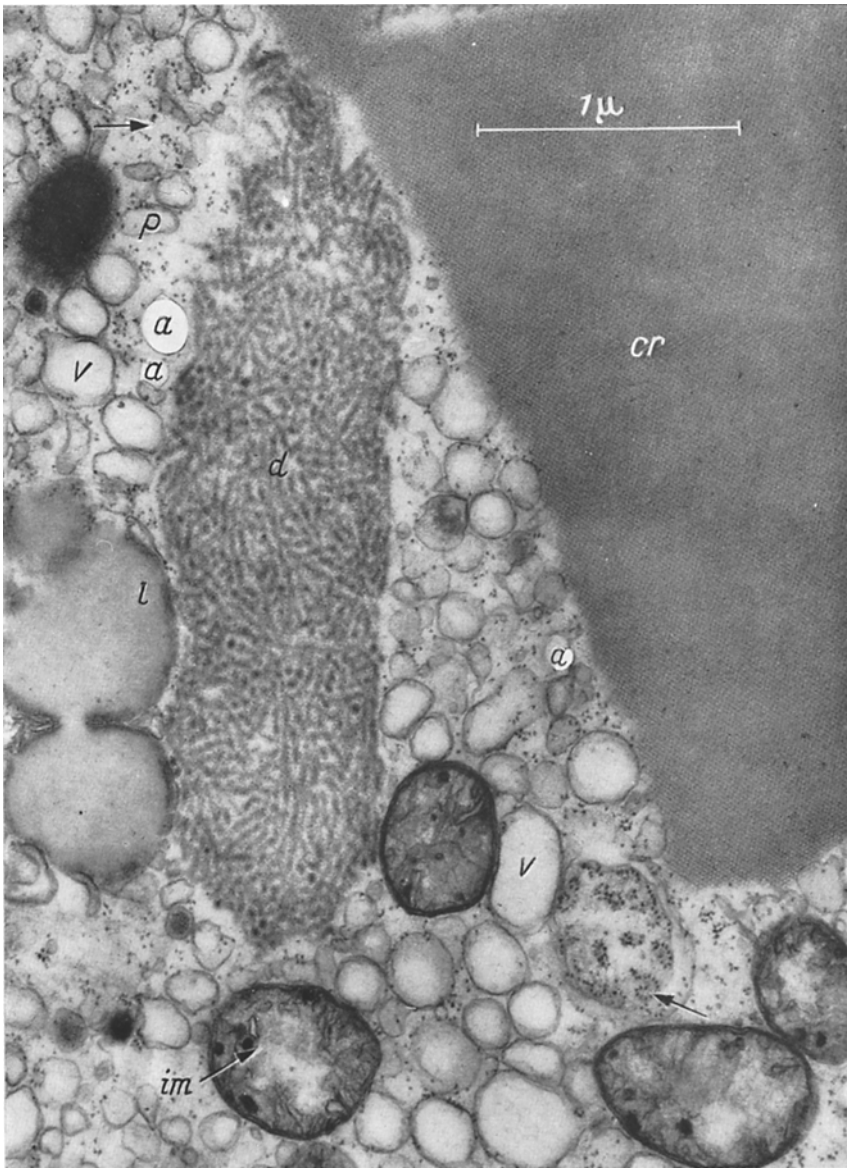


Fig. 4. A cytoplasmic inclusion composed of dense rod-shaped structures (*d*) is seen in the vicinity of the crystal of Reinke (*cr*). The mitochondria reveal many intramitochondrial granules (*im*). Numerous vesicles (*v*) of the smooth endoplasmic reticulum are shown as well as lipid droplets (*l*), pigment accumulation (*p*), and ribosomes (arrows). Areas designated (*a*) are artefacts in section preparation. ×35,000

material of moderate electron density but, in some, only a thin layer of this material was observed around the inner aspect of their walls (Figs. 4, 7).

Many of the interstitial cells possessed a smooth endoplasmic reticulum consisting of interconnected tubules. The diameter of the tubules varied between 25 mμ

and 100 mμ, and the electron density of their content was similar to that found in the vesicular components.

Typical rough endoplasmic reticulum was rarely seen in these cells, but the cytoplasmic matrix contained numerous ribosomes, scattered singly or aggregated to form polysomes (Fig. 4).

The mitochondria of the light interstitial cells were extremely variable in size and shape. The diameter of these organelles ranged from 0.5 μ to 0.80 μ and the appearance of the cristae altered with the mitochondrial dimension. In mitochondria of small diameter the cristae extended transversely across the matrix, whilst, in the larger forms, appeared as blindly-ending tubules identical to the mitochondria of other steroid-producing cells (BELT and PEASE, 1956). Occasionally the outer mitochondrial membrane appeared to be deficient, suggesting a communication between the matrix and the adjacent tubular endoplasmic reticulum. Also occasionally, in some areas of the cell, the mitochondria were incompletely surrounded by one or two membranous layers.

The density of the mitochondrial matrix also varied with the diameter. Smaller mitochondria exhibited a greater overall density, whilst those of larger diameter exhibited a central less dense region. Small dense intra-mitochondrial granules, identical in appearance to those commonly found in other tisssues, were frequently present in the mitochondrial matrix. Larger spheroidal non-membrane-bounded granules, similar to those described by FAWCETT and BURGOS (1960) were also observed (Fig. 4).

A well-defined juxtanuclear Golgi complex consisting of curved parallel arrays of membranes and vesicles whose diameter ranged from 25 mμ to 250 mμ, was observed (Fig. 5). The smaller vesicles contained a material of moderate electron density which, in some areas, appeared to lie freely in the cytoplasm. The larger vesicles consisted of two types. The first, identified as a multivesicular body, was confined solely to the Golgi region. The second type, which contained material morphologically identical to that found in the smaller vesicles, was not only observed in the Golgi region but was distributed randomly throughout the cytoplasm. These structures corresponded to the membrane-bounded bodies described by FAWCETT and BURGOS (1960).

A number of aggregations of moderately osmiophilic material, which were referred to as lipid droplets because of their similarity to lipid inclusions reported in other tissues, were found in the interstitial cells. Many of these lipid droplets were contained in expanded smooth-surfaced vesicles (Fig. 4). The smaller lipid droplets were approximately the same size as the larger vesicles of the smooth endoplasmic reticulum the contents of which, because of the striking similarity in their electron density, were also regarded as lipid. Some of the larger lipid droplets were surrounded by a few concentric membranous layers and the intermembranous space appeared in some views to be continuous with the endoplasmic reticulum.

Deposits of intensely osmiophilic lipochrome pigment, generally membrane-bounded, were found scattered in the cytoplasm. The form of these pigment aggregations was similar to those described by other investigators (FAWCETT and BURGOS, 1960; GORDON et al., 1964). In a few aggregations, however, the limiting membrane was deficient in parts and laminated membranous structures appeared to extend into the cytoplasm (Fig. 6).

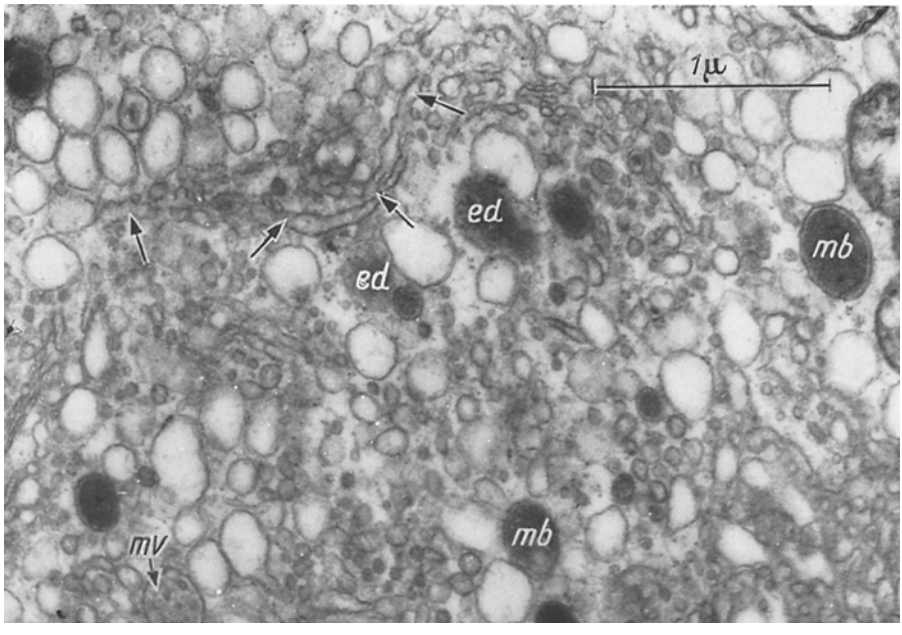


Fig. 5. The Golgi complex of the light interstitial cell consists of parallel arrays of membranes (arrows) and many vesicles. Accumulations of granular electron-dense material (*ed*) are present, some being membrane-bounded (*mb*). A multivesicular body (*mv*) is present. ×33,000

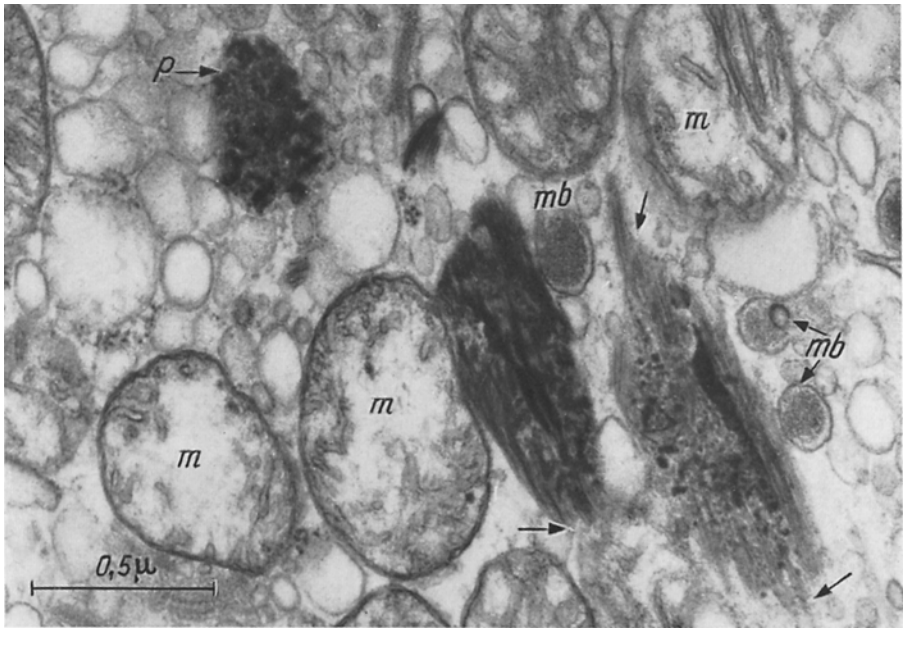


Fig. 6. Lipochrome pigment deposits (*p*) in some areas show laminated membranous extensions (arrows) into the cytoplasm. Membrane-bounded bodies (*mb*) and mitochondria (*m*) are indicated. ×50,600

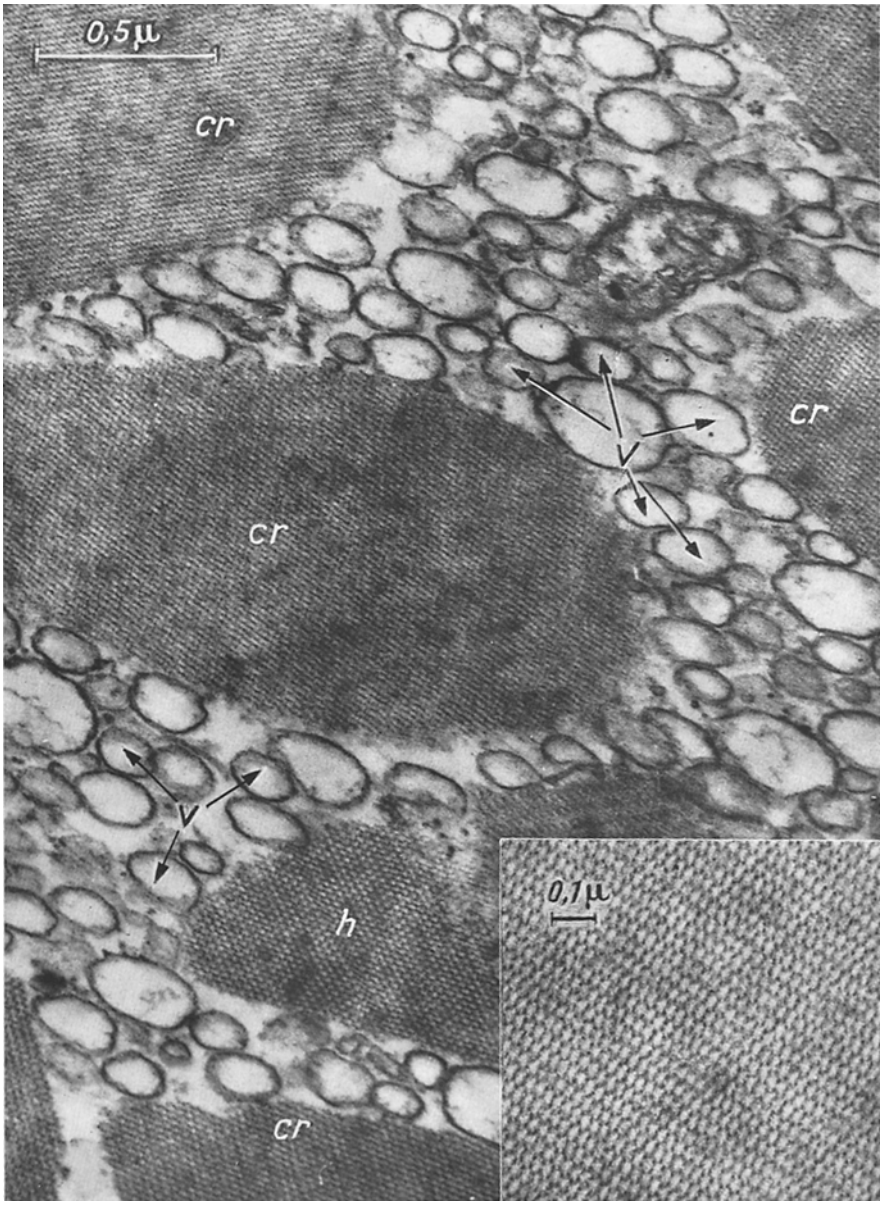


Fig. 7. The patterns of the crystals of Reinke (*cr*) are shown. Note the variable size of vesicles (*v*) of the endoplasmic reticulum. ×49,000. *Insert.* The hexagonal pattern (*h*) at higher magnification, indicates the granules at the interstices of the hexagons. ×66,000

The crystals of Reinke were found irregularly scattered throughout the cytoplasm of the cell. Generally rod-shaped, the internal appearance of these crystals varied with the plane of section as described in the fine study by FAWCETT and BURGOS (1960). The parallel dense-line appearance and the "moiré" effect described

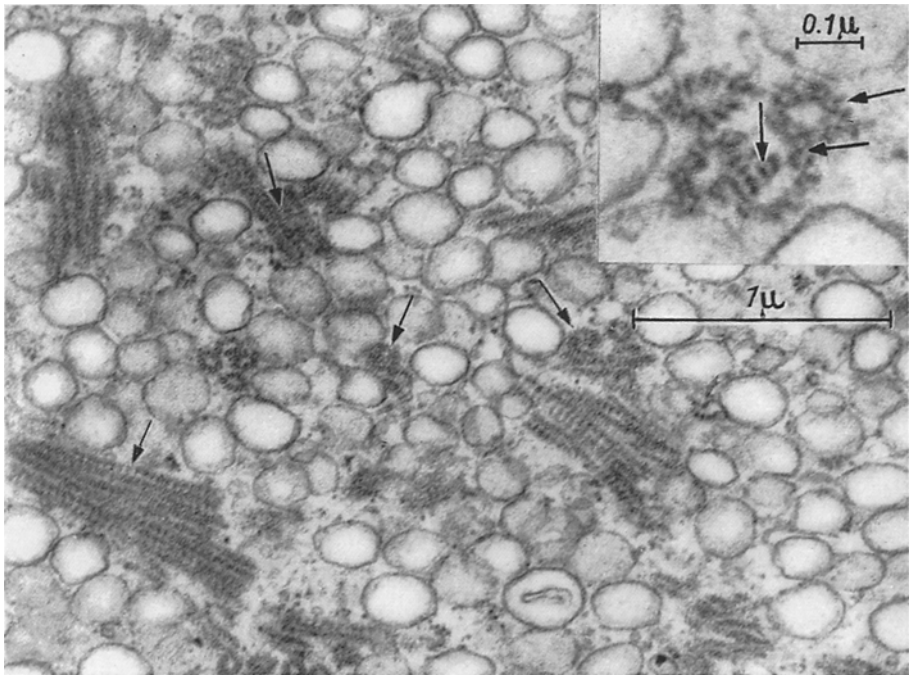
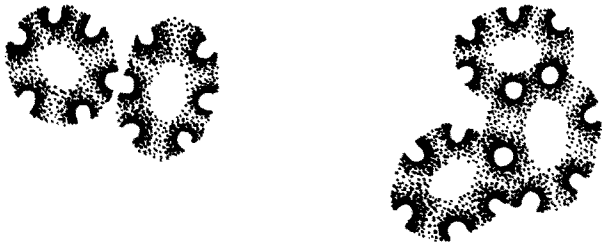


Fig. 8. Crystalline tubular inclusions (arrows) are seen in longitudinal and cross-section. ×35,000. *Insert.* The structure of the horseshoe-shaped inclusions in the walls and deficiencies in these units are indicated (arrows). ×88,000

by these investigators (FAWCETT and BURGOS, 1960), was observed (Fig. 7). In certain planes a strikingly regular hexagonal lattice structure, similar to that briefly described by YAMADA (1962) was seen. The distance between opposite axes was approximately 150—200 Å, and each septum measured 150 Å. At the junction of septa of adjacent hexagons it was possible to distinguish a small granule, approximately 75 Å, which at some intersections appeared as a black dot, whilst at others it appeared circular with a clear centre (Fig. 7 Insert).

A striking feature which had not been comprehensively described before, was the presence of small crystalline tubular inclusions up to 1 μ in length. These structures, arranged singly or aggregated in groups, were frequently seen in the vicinity of the crystals of Reinke or scattered irregularly in the cytoplasm (Fig. 8). Less commonly, relatively large areas of the cell were occupied by these structures lying in a randomly-orientated manner. The tubular inclusions exhibited a regular substructure and in cross-section the shape of the inclusions varied from ovoid to hexagonal with a long diameter which ranged from 600 Å to 700 Å. Their walls contained horseshoe-shaped units approximately 300 Å in diameter, the centre of which was of low electron density. A deficiency was present in the denser wall and it always occupied the outer aspect of each unit (Fig. 8 Insert). The number of horseshoe-shaped units present in the cross-sections of individual tubules was almost invariably six. The walls of centrally-placed tubular inclusions often



a b

Fig. 9. (a) This illustrates the structure of two adjacent tubular crystalline inclusions. (b) A group of these inclusions is shown and illustrates the complete circular profile of the units forming the substructure of these inclusions

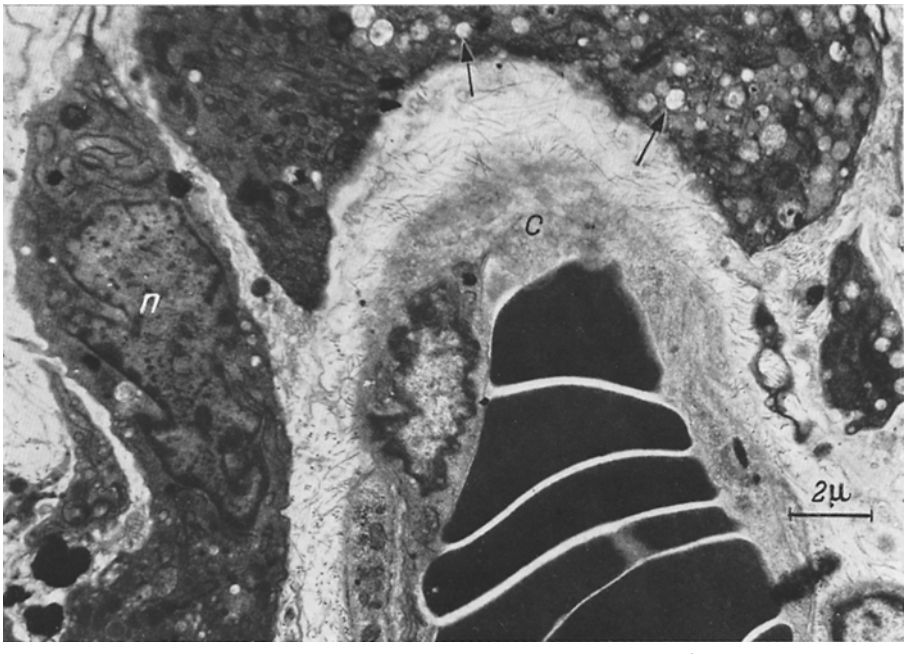


Fig. 10. The dark interstitial cells distributed around a capillary (*c*) filled with erythrocytes. The nucleus (*n*) is of irregular shape and one of the cells contains many membrane-bounded bodies (arrows). ×5,500

contained units which appeared to be complete and this finding tended to suggest a possible method of linkage of adjacent tubular structures (Fig. 9). Groups of units similar in size to those in the walls of the tubular inclusions were observed in the adjacent cytoplasm and probably represent the macromolecules described by FAWCETT and BURGOS (1960).

In longitudinal section the inclusions exhibited a regularly-banded appearance, each band being approximately 150 Å thick. In some tubules which were cut obliquely, the horseshoe-shaped units appeared to be regularly stacked.

Two further types of inclusions, only occasionally present in the interstitial cells, were observed. The first consisted of aggregations of short dense rod-shaped

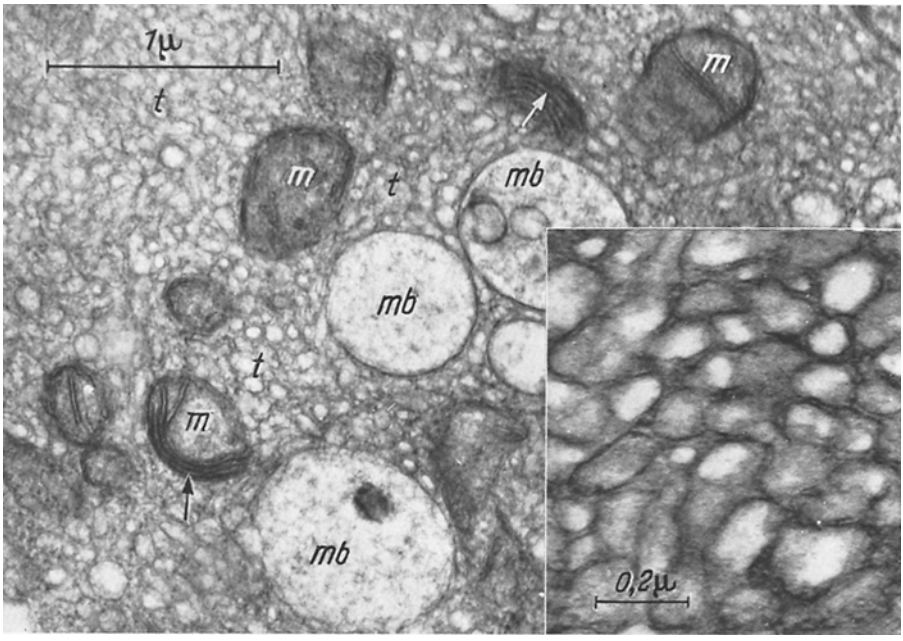


Fig. 11. Cytoplasm of the dark interstitial cell showing tubular agranular endoplasmic reticulum (*t*), membrane-bounded bodies (*mb*), and mitochondria (*m*). The laminated pattern of cristae is indicated (arrows). ×31,200. *Insert.* Shows the relatively high density of the contents and membranes of the endoplasmic reticulum. ×70,000

structures which were observed in the immediate vicinity of the crystals of Reinke or less frequently enclosed within the framework of these crystals (Fig. 4). These short structures which in cross-section appeared solid, were randomly orientated and not bounded by a membrane.

The second type of cytoplasmic inclusion occasionally seen, was comprised of loose aggregations of tubules with a diameter of 30 to 45 mμ, some of which contained a fine granular core. The electron density of the cytoplasmic matrix in the vicinity of these tubules was increased.

Apart from occasional binucleate cells, the nuclear detail presented no unusual feature and was similar to that observed by FAWCETT and BURGOS (1960) (Fig. 3).

Dark Interstitial cells

The dark interstitial cells were seen scattered in the intertubular tissue and their number varied with each biopsy (Fig. 10). The increased electron density of these cells was due to the presence of a densely-packed smooth endoplasmic reticulum which was predominantly tubular, although occasionally cisternae were present (Fig. 11).

The walls and contents of this tubular system exhibited a greater electron density than their counterpart in the light cell (Fig. 11, Insert). In addition, collections of densely staining particles resembling glycogen were scattered throughout the cytoplasm.

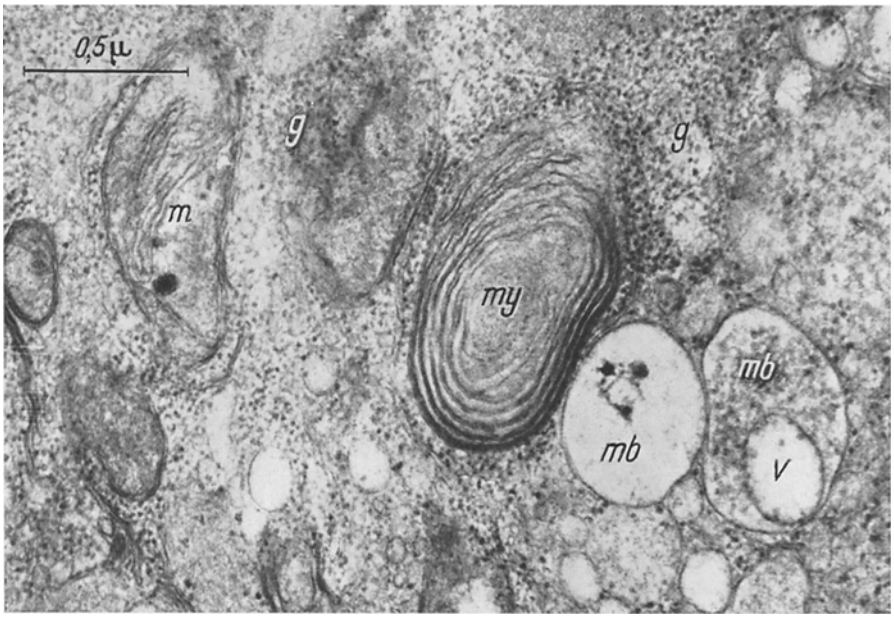


Fig. 12. The cytoplasm of the dark interstitial cell shows glycogen granules (*g*), membrane-bounded bodies (*mb*), and mitochondria (*m*). The mitochondria (*m*) sometimes resemble myelin figures (*my*). ×46,000

The mitochondria of the dark interstitial cells were pleomorphic, irregularly-ovoid forms predominating (Fig. 11). The mitochondrial diameter ranged from 0.18 µ to 0.35 µ, in general being smaller than their counterpart in the light interstitial cell. The mitochondrial matrix was moderately electron-dense and exhibited a fine granularity. Whereas small intra-mitochondrial electron-dense granules were rarely observed, larger electron-dense granules, similar to those in the mitochondria of light interstitial cells, were often seen. Although tubular cristae, similar to those in the light interstitial were noted, the predominant pattern of the cristae was a laminated arrangement. In mitochondria exhibiting this laminated pattern, the cristae were generally aggregated towards one end and appeared as densely staining concentric membranes, the space between cristae often being absent. Occasionally, this type of appearance produced mitochondrial forms with a remarkable similarity to myelin figures (Fig. 12).

The dark cells contained numerous membrane-bounded bodies with a diameter ranging from 0.2 µ to 0.4 µ (Fig. 11), many of which differed in their content from the membrane-bounded bodies found in the light interstitial cells. In these, the contents were less electron dense, more coarsely granular, and occasionally vesicular in appearance. In cells containing large numbers of these membrane-bounded bodies, the tubular endoplasmic reticulum was decreased in amount and poorly defined. The definition and amount of the smooth endoplasmic reticulum seemed to be inversely proportional to the numbers of membrane-bounded bodies.

The Golgi complex of this type of interstitial cell was poorly developed and difficult to distinguish amongst the compact agranular endoplasmic reticulum.

Typical rough endoplasmic reticulum was rarely seen and the content of ribosomes in these cells did not differ from the light interstitial cell. Lipid and pigment aggregations were present in the dark cells in the same form and distribution as the light interstitial cells.

The nuclei of the dark cells differed from those of the light cells in their irregular outline and moderate chromatin clumping. Many of these chromatin clumps were continuous with a peripheral nuclear chromatin rim (Fig. 10).

Discussion

This study confirms the pleomorphic appearance of the interstitial cells of the testis in men of normal androgenic status. The presence of dark interstitial cells has been clearly demonstrated and the observations indicate that the dark cells differ significantly from the light cells in the distribution, quantity, and electron density of their organelles.

Many of the features of the dark interstitial cells described in this study, such as the increase in tubular endoplasmic reticulum, the increase in glycogen content, and the presence of numbers of membrane-bounded vesicles, were similar to those of the dark cells described in other species (CRABO, 1963; LEESON, 1963; MURAKAMI, 1966).

The description of the dark interstitial cells in man differs from the description of these cells in the opossum (FAWCETT and CHRISTENSEN, 1961) and the guinea pig (CHRISTENSEN, 1965). In both the guinea pig and the opossum, the only major difference between the light and dark cells was an increase in the electron density of the dark cell and no marked difference in the distribution and quantity of their organelles was noted. The demonstration in man, of a difference, not only of electron density, but also in the quantity and distribution of the organelles in the dark and light cells, supports the consideration that two distinct cell types are present.

It is worth noting that CHRISTENSEN (1965) in a study on the guinea pig, in which he used osmium tetroxide, and glutaraldehyde by perfusion and immersion, failed to demonstrate the dark cells in the glutaraldehyde-fixed material. He suggested that the dark cells found in osmium-fixed material maybe an artefact of fixation. However, in the present study, and in the study of rat testicular interstitial tissue by MURAKAMI (1966), who fixed his material in glutaraldehyde by immersion, dark cells were clearly demonstrated. These opposing findings may indicate that a species difference exists. Further studies using glutaraldehyde and osmium tetroxide by perfusion in a number of species are necessary to clarify these issues, but the difficulties in carrying out such perfusion studies in the human are considerable.

The gradation in appearance from the light cells, through cells containing relatively large amounts of densely staining endoplasmic reticulum and glycogen, to dark cells containing large numbers of membrane-bounded bodies, may indicate a changing physiological role. The presence of increased amounts of tubular smooth endoplasmic reticulum in the dark cell, the organelle implicated in steroid biosynthesis (CHRISTENSEN, 1965), led to the consideration that it is a more active form than the light interstitial cell. This is substantiated by the findings of an increase in the smooth endoplasmic reticulum of the interstitial cells of the

testis in men treated with human pituitary gonadotropin (DE KRETSER, 1967). The loss of definition and a decrease of tubular endoplasmic reticulum, associated with an increase in membrane-bounded bodies, observed in some dark cells, may indicate a degenerating or exhausted form.

The biosynthetic importance of the smooth endoplasmic reticulum of the interstitial cell has been reviewed by CHRISTENSEN (1965). The difficulty in deciding whether the vesicular form of the endoplasmic reticulum is a fixation artefact, as suggested by the investigations of CHRISTENSEN (1965), is again raised by the observations reported in this study. The demonstration of both tubular and vesicular forms of smooth endoplasmic reticulum, often in adjacent cells, clearly makes it difficult to understand why fixation damage affects only certain cells. The observations of both vesicular and tubular types in this study and in the glutaraldehyde-fixed human material used by GORDON et al., (1964) may indicate that factors other than fixation are involved. CRABO (1963), in the rabbit, also described both forms of endoplasmic reticulum in the interstitial cells.

The change from vesicular to tubular endoplasmic reticulum in rats treated with Amphenone B, an inhibitor of certain steps of steroid biosynthesis, demonstrated by SCHWARZ and MERKER (1965), leads to the consideration that physiological changes in the cells may be the cause of the differing appearances of this organelle.

It may well be that the described gradation in the size of the vesicles of the smooth endoplasmic reticulum, the similarity between their content and the aggregations of lipid, indicates that the contained material is either utilized or produced by these vesicles. However, it is difficult to comment on the dynamic aspects of these cells from purely morphological studies.

The close proximity of mitochondria to the tubular endoplasmic reticulum which, in some cases, appears to surround those structures has been noted in the mouse (CHRISTENSEN and FAWCETT, 1966). The demonstration in man, in this study and that of GORDON et al. (1964), of regions which suggest a communication between these structures, may indicate a method of transfer of substances between these organelles. The close relationship of these organelles lends support to the postulate of CHRISTENSEN and FAWCETT (1966) who suggest a transfer of cholesterol to, and pregnenolone from, the mitochondria.

The description of the mitochondria of the light interstitial cells in this study, indicates again the similarity of these structures to their counterpart in other steroid hormone-producing cells (BELT and PEASE, 1956). In Methacrylate-embedded material, FAWCETT and BURGOS (1960) demonstrated large mitochondria in the human interstitial cell and suggested that this appearance was due to swelling during fixation. However, the demonstration, in Araldite-embedded material used in this study, of large and small mitochondrial forms in adjacent cells and often in the same cell, suggests that the larger mitochondria are a normal finding in this tissue. This suggestion is supported by the findings of similar mitochondrial sizes in the glutaraldehyde-fixed material used by GORDON et al., (1964) in their study of the gonad in testicular feminization syndrome.

In contrast to the findings of FAWCETT and BURGOS (1960), small intramitochondrial granules were observed mainly in the light interstitial cells. The signi-

ficance of these granules in this and other tissues (FAWCETT, 1966) remains obscure. The large dense intramitochondrial bodies found in both dark and light cells were similar to those described by FAWCETT and BURGOS (1960) and may represent the electron-dense bodies interpreted by GORDON et al., (1964) as intramitochondrial lipid. These dense bodies differ from the "Corpus intra cristam" or intracristal dense bodies found in other normal tissues (HONJIN et al., 1965; SILVA, 1966) by the lack of a membranous boundary. The exact nature of these large intramitochondrial inclusions is not known.

The nature and function of the membrane-bounded bodies found in both types of interstitial cell remains obscure. These structures, described in other species (FAWCETT and BURGOS, 1960; CHRISTENSEN and FAWCETT, 1961; LEESON, 1963; CHRISTENSEN and FAWCETT, 1966) have been interpreted as being collections of cell metabolites, as precursors of mitochondria, or lysosomes. The findings in this study suggest that the membrane-bounded bodies containing finely granular electron-dense material are probably produced in the Golgi complex. This supports the view of FAWCETT and BURGOS (1960) that these structures are lysosomes, as the Golgi complex is a site which has been implicated in the production of hydrolytic enzymes (WEISSMAN, 1965). However, histochemical evidence of the presence of hydrolytic enzymes in these structures is needed before they can conclusively be labelled as lysosomes. Suggestions that these structures are intermediates in pigment formation was refuted by FAWCETT and CHRISTENSEN (1961).

The tubular crystalline inclusions described in this study were briefly referred to by FAWCETT and BURGOS (1960) who indicated that the walls of these inclusions were granular in structure. Similarly YAMADA (1962) described "fibrous structures with a periodic banded appearance". The present study indicates that the two inclusions briefly referred to by these investigators were probably the tubular crystalline inclusions.

The postulate, that the method of linkage of adjacent inclusions is by the union of contiguous horseshoe-shaped units, is emphasized. It is considered that linkage and condensation of these tubular structures represent steps in the formation of the crystals of Reinke. This hypothesis is supported by the demonstration that the crystals of Reinke in some planes exhibit a hexagonal lattice structure and it is supplemented by the presence of circular granules or macromolecules at the interstices of the axes of adjacent hexagons of the crystal lattice. The nature and function of these crystals is still obscure. FAWCETT and BURGOS (1960), after reviewing suggestions that these crystals may represent stores of specific cell product or crystalline virus particles, considered that these crystals were probably a protein related to the normal metabolic activities of the human interstitial cell. The absence of these crystals before puberty supports their view. However, the failure to detect these crystals in the gonad in testicular feminization syndrome (GORDON et al., 1964) is against the hypothesis, as androgen production by the interstitial cells in this condition reaches levels found in the normal male (SOUTHREN et al., 1965).

The significance of these crystals must await consideration of their biochemical characteristics together with further studies of the distribution of these structures in the normal and the abnormal testis.

References

BELT, W. D., and D. C. PEASE: Mitochondrial structure in sites of steroid secretion. J. biophys. biochem. Cytol. **2**, Suppl. 369—372 (1956).

BOUIN, P., et P. ANCEL: Recherches sur les cellules interstitielles du testicule des mammifères. Arch. Zool. (Stockh.) **1**, Ser. IV, 437 (1903).

CHRISTENSEN, A. K.: The fine structure of testicular interstitial cells in guinea pigs. J. Cell Biol. **26**, 911—935 (1965).

—, and D. W. FAWCETT: The normal fine structure of opossum testicular interstitial cells. J. biophys. biochem. Cytol. **9**, 653—670 (1961).

— The fine structure of testicular interstitial cells in mice. Amer. J. Anat. **118**, 551—572 (1966).

CRABO, B.: Fine structure of the interstitial cells of the rabbit testes. Z. Zellforsch. **61**, 587—604 (1963).

DE KRETSER, D. M.: Changes in the fine structure of the interstitial cells of the human testis after treatment with human pituitary gonadotrophin. (In Preparation.)

FAWCETT, D. W.: The cell, its organelles and inclusions. Philadelphia: W. B. Saunders Co. 1966.

—, and M. H. BURGOS: Observations on the cytomorphosis of the germinal and interstitial tissue of the human testes. Ciba Foundation Colloquia in Ageing **2**, 86—99 (1956).

— — Studies on the fine structure of the mammalian testes. II. The human interstitial tissue. Amer. J. Anat. **107**, 245—269 (1960).

GORDON, G. B., L. R. MILLER, and K. G. BENSCH: Electron microscopic observations of the gonad in the testicular feminization syndrome. Lab. Invest. **13**, 152—160 (1964).

HONJIN, R., A. TAKAHASHI, T. HANYO, and S. MURDNO: Electron microscopy of intracristal dense body within mitochondria of epithelial cells of mouse stomach and rectum. Okajimas Fol. anat. jap. **41**, 267—275 (1965).

LEESON, C. R.: Observations on the fine structure of rat interstitial tissue. Acta anat. (Basel) **52**, 34—48 (1963).

LIPSETT, M. B., H. WILSON, M. A. KIRSCHNER, S. G. KORENMAN, L. M. FISHMAN, G. A. SARFATY, and C. W. BARDIN: Studies on Leydig cell physiology and pathology; secretion and metabolism of testosterone. Recent Progr. Hormone Res. **22**, 245—281 (1966).

MURAKAMI, M.: Elektronenmikroskopische Untersuchungen am interstitiellen Gewebe des Rattenhodens, unter besonderer Berücksichtigung der Leydigschen Zwischenzellen. Z. Zellforsch. **72**, 139—156 (1966).

REYNOLDS, E. S.: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17**, 208—212 (1963).

RICHARDSON, K. C.: The fine structure of autonomic nerve endings in smooth muscle of the rat vas deferens. J. Anat. (Lond.) **96**, 427—442 (1962).

ROSS, M. H., and I. R. LONG: Contractile cells in human seminiferous tubules. Science **153**, 1271—1273 (1966).

SCHWARZ, W., and H. J. MERKER: Die Hodenzwischenzellen der Ratte nach Hypophysectomie und nach Behandlung mit Chorion Gonadotropin und Amphenon B. Z. Zellforsch. **65**, 272—284 (1965).

SILVA, D. G.: The fine structure of multivesicular cells with large microvilli in the epithelium of the mouse colon. J. Ultrastruct. Res. **16**, 693—705 (1966).

SNIFFEN, R. C.: The human testis. Arch. Path. **50**, 259—268 (1950).

SOUTHREN, L. A., S. TOCHIMOTO, N. C. CARMODY, and K. ISURUGI: Plasma production rates of testosterone in normal adult men and women and in patients with the syndrome of feminizing testes. J. clin. Endocr. **25**, 1441—1450 (1965).

WATSON, M. L.: Staining of tissue sections for electron microscopy with heavy metals. J. biophys. biochem. Cytol. **4**, 475—478 (1958).

WEISSMAN, G.: Lysosomes (Review). New Engl. J. Med. **273**, 1084—1090, 1143—1149 (1965).

YAMADA, E.: Some observations on the fine structure of the interstitial cell in the human testis. 5th Internat. Congr. for Electron Microscopy, vol. 2, LL 1. New York: Academic Press, 1962.

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