HISTOLOGICAL AND ULTRASTRUCTURAL STUDY OF THE MALE REPRODUCTIVE SYSTEM OF NON-BREEDING NAKED MOLE RATS (*Heterocephalus glaber,* Ruppell) AND *IN VITRO* INTERSTITIAL (LEYDIG) CELL RESPONSE TO LUTEINIZING HORMONE (LH)

by

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DECLARATION

I, Daniel W. Onyango, hereby declare that the work contained in this thesis is my original work and has not been presented for a degree in any other University.

Signed:

Date: . . Z l / l Z j . . ! !

This thesis has been submitted for examination with our approval as University supervisors

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SUMMARY

The morphology of the male reproductive system of the naked mole rats was studied. The testes of these animals are located intra-abdominally and on histological examination, they reveal the presence of a continuous mass of Leydig cells with dark staining granules within the interstitial tissue among which are isolated seminiferous tubules. Connective tissue, blood and lymphatic capillaries are also few and scattered. Ultrastructurally, the Leydig cells show a conspicuous accumulation of lipid droplets, elongated mitochondria and abundant smooth endoplasmic reticulum that is largely vesicular. Endocrinological investigations, however, revealed that the Leydig cells are unresponsive to an *in vitro* challenge by LH and that these animals have low plasma LH but high plasma testosterone levels. The low levels of LH in plasma suggests depressed release of gonadotrophin releasing hormone (GnRH) from the hypothalamus. The seminiferous epithelium comprises of Sertoli (sustentacular) and spermatogenic cells. The Sertoli cells exhibit irregularly outlined nuclei with a single nucleolus, predominant network of smooth endoplasmic reticulum, mitochondria oriented perpendicularly and parallel to the long axis of the cell, lipid droplets and lysosomes. These cells form recesses into which fit the spermatogenic cells. All stages of spermatogenesis occur although secondary spermatocytes, spermatids and spermatozoa are few compared to spermatogonia and primary spermatocytes. This suggests that spermatogenesis progresses to

completion even though the rate appears to be reduced considering the number of mature spermatogenic cells seen. This may possibly be due to an arrest in further development occurring at primary to secondary spermatocyte stage.

The excurrent duct system comprises of the ductuli efferentes, ductus epididymis and ductus deferens. Ductuli efferentes occur as small and numerous ducts whose histology and ultraslructure reveal the presence of simple cuboidal epithelium with two main cell types namely the principal (non-ciliated) and ciliated cells. The ductus epididymis comprises of the initial, middle and terminal segments. The entire duct has a pseudostratified columnar epithelium that is high in the initial segment and a slightly reduced height in the middle and terminal segments. The epithelial cells are mainly the principal and basal cells although apical cells and lymphocytes are also present in the initial segment. The cytoplasm of principal cells in both initial and middle segments show no conspicuous cytoplasmic modification but in the terminal segment, apical vacuoles and basal granules are evident. In all segments, microvilli (stereocilia) occur and the lumina of the tubules contain some cellular debris. Ductus deferens has two segments; the proximal and the distal segments. The proximal segment has a markedly reduced epithelial heights with the microvilli forming the brush border. The point of transition between the two segments is recognised by a dramatic increase in the epithelial height and presence of clear cells. The principal cells from the transition point

distally show an apocrine mode of secretion. The distal segment has a thickened wall and the two opposite parts of the ducts are bundled together by a fold of connective tissue. A third duct, utriculus musculinus (remnant of Mullerian duct) is also evident at this level. The epithelium of the ductus deferens in this segment is thrown into folds resulting from the thick muscular coat.

Seemingly, the prostate gland is the only accessory sex gland in these animals. The pelvic urethra has the ejaculalory ducts (continuations of the ductus deferens) in addition to utriculus musculinus dorsally and urethral epithelium is mainly the transitional type. The pelvic urethra gives rise to the penile urethra that is within the penis. The penis is of the vascular type and is covered at its tip by a prepuce.

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

The naked mole rat *Heterocephalus glaber*, belongs to the rodent family Bathyergidae which is exclusively an African group. They are small rodents found in hot arid parts of Africa mainly Somalia, Eastern Ethiopia and Kenya where they are confined to the Eastern and North Eastern parts of the country. They have often been regarded as "reliable indicators" of arid environment due to their exclusive adaptation to arid conditions (Kingdon, 1974). The adult body weight ranges between 30-80 gms. and a nose to base of tail measurement of between 8-13 cms. (Hill *et ai*, 1957; Jarvis, 1969, 1984; Brett, 1985). Their life-span under natural conditions has not been determined but under captivity, they have been reported to live for over ten years (Jarvis, 1984; Brett, 1985) which is quite long for rodents of comparable size.

The naked mole rats are completely fossorial mammals, living in burrows up to 40 cm. deep. They have very small eyes which are thought to be sightless and show no obvious external sexual dimorphism (Jarvis, 1984; Jarvis & Sale, 1971; Brett, 1985). It has also been shown by Quay (1981) that the pineal gland of the naked mole rat is atrophic. It is therefore unlikely that photoperiod plays any significant role in their reproductive rhythms. These animals are practically hairless with a low body temperature of 32° Celsius and the lowest capacity for thermoregulation of any known

mammals (McNab, 1966). They have poorly pigmented integument and exhibit wriggling movements with their comparatively feeble limbs resembling the naked new-bom stage of many other rodents (Hill *et al.,* 1957; Kingdon, 1974). The rats live in colonies consisting of approximately 70-80 members and have a communal toilet within the nest. The naked mole rats are the only mammals known to have a colony structure similar to that of the social insects (eusociality). The colony is divided into castes comprising of the working caste made up of numerous small sized rats; the non-working caste made up of fewer large sized rats which spend most of their time in the nest with the breeding female and may be involved in defence of the colony and the breeding caste which consists of one breeding female and one or two breeding male(s) (Jarvis, 1981; Brett, 1985).

The gestation period of the naked mole rat is about 70 days with an average litter size of 12 although a litter size of 27 has been reported in captivity and the "queen" gives birth four times a year (Jarvis, 1969, 1984). Like other hystricognath rodents a gestation length of 70 days is normal for the naked mole rat but relatively long for most of the other rodents of comparable size. Although experiments have shown that the non-breeding members of the colony are not sterile (because they can found new colonies and also replace the breeding pair if the latter dies) it appears the "queen" mole rat suppresses fertility in the non-breeders through a combination of chemicals (Pheromones) she produces and behavioural dominance. It has been observed that just before a litter

is born, all the colony members develop teats and look like females; male hormone level drops and some females come close to breeding condition strongly suggesting that the colony is responding to chemical stimuli produced by the breeding female (Jarvis, 1984).

1-2 Morphology of the reproductive system

1.2.1 Testes

Testes are found located in various positions in different animal species. In most species the testes are scrotal, in others inguinal and in yet some animals, they are intra-abdominal (true testicondas). In other animals, however, the testes are either scrotal or inguinal depending on the season of the year. The testes generally consist of the interstitial tissue and seminiferous tubules both encapsulated by a tough fibrous connective tissue band, the tunica albuginea.

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Morphological studies on the testes of various animals have been widely covered including those of rodents. Studies involving the interstitial tissue of rodents include those on rats (Niemi & Ikonen, 1962; 1963; Leeson, 1963; Ross, 1963; Clegg, 1966), guinea pigs (Christensen, 1965), mice (Baillie, 1961; Christensen & Fawcett, 1966; Russo, 1970; Russo & De Rosas, 1971), European moles, *Talpa europaea* (Suzuki & Racey, 1978), spring hares, *Pedetes surdaster larvalis* (Mugisha-Girasi *et ai,* 1979) and Australian rodents, *Notomys* and *Pscudomys* (Breed, 1982; Tierce & Breed, 1984) among others.

Studies on the interstitial cells of mature and active rodents have shown that the cells l^ave an abundance of smooth endoplasmic reticulum (sER), spherical or ovoid mitochondria, juxtanuclear Golgi apparatus (GA) and few lipid droplets (Leeson, 1963; Christensen, 1965; Christensen & Fawcett, 1966; Russo, 1970; Breed, 1982; Fierce & Breed, 1984). Occasionally, strands of rough endoplasmic reticulum (rER) have been seen to be continuous with sER although these are rare (Christensen & Fawcelt, 1966; Russo, 1970). It has been frequently observed that mitochondria in steroid producing cells are ovoid or spherical in form with well developed cristae (Belt & Pease, 1956). It has therefore been suggested that enzymes for androgen biosynthesis are located on the surfaces of the sER membranes and the mitochondrial cristae (Christensen & Gillim, 1969; Burgos *et al,* 1970) such that during active synthesis these organelles proliferate. Although GA is well developed in steroid secreting cells, their role in steroid secretion is unclear even though it has been suggested that it may be the site of steroid conjugation in preparation for synthesis or secretion (Christensen & Gillim, 1969). The quantity of lipid droplets in these cells has been taken to be indicative of the state of activity to the extent that in an inactive state as in prepubertal males (Baillie, 1961; Niemi &Ikonen, 1963; Russo, 1970; Russo & De Rosas, 1971; Mugisha-Girasi *et al.,* 1979; Zirkin & Fwing, 1987), seasonal influence (Neaves, 1973; Lam & Earner, 1976; Leceta *et al.,* 1982; Gustafson, 1987) or inadequate levels of pituitary gonadotropins (Lynch & Scott, 1951; de Krester, 1967) there are numerous lipid droplets accompanied by immature sER. Conversely in an active state, there is a dramatic depletion of

lipid droplets and a conspicuous proliferation of sER. This general phenomenon is not without exceptions. It has been observed in some animals that rather than a reduction in the amount of lipid droplets during the active state, the inclusions persist as in European moles (Suzuki & Racey, 1978), ground squirrel, *Cilellus lateralis* (Pudney, 1986), horseshoe bat, *Rhinolopus capensis* (Bernard, 1986). Lipid droplets are thought to contain cholesterol which is an important intermediate for testosterone synthesis such that cells that are more active have fewer or smaller droplets and the less active ones have larger or more droplets (Christensen & Gillim, 1969; Christensen, 1975). Various unique cytoplasmic inclusions have also been described in a number of animals. Reinke's crystals have been observed in the cytoplasm of Leydig cells of humans (Fawcett & Burgos, 1960; Christensen, 1975). In the hopping mouse, *Notomys alexis,* Pierce & Breed (1984) observed the presence of lysosomal end products with paracrystalline lattice. In the rock hyrax (Neaves, 1973) and rats (Russel *el al.,* 1987) myelin figures and various filaments (actin and vimentin) were observed in the cytoplasm respectively.

The existence of testicular interstitial lymphatics is no longer in doubt as this has now been demonstrated by various workers on several animal species such as ram (Lindner, 1963), goat (Oduor-Okelo, 1974), guinea pig and chinchilla (Fawcett *el al., 1969,* 1970). Fawcett et al., (1973) reported various forms of lymphatics in 14 laboratory and domestic animal species. These latter workers observed that in some animals, the interstitial lymphatics were

mainly sinusoids or lymph spaces, in others there were centrally or eccentrically located lymphatic vessels and in yet some other animals the lymphatics were few and small sized. The significance of interstitial lymphatics in androgen transportation has not been clearly defined although there is incriminating evidence that they may take part in androgen transportation to the remote target organs. Also, because of the slow rate of lymph flow, testicular lymphatics may be important in ensuring an adequate concentration of testosterone around the seminiferous tubules to maintain spermatogenesis. Lindner (1963) observed that the amount of androgen carried to the general circulation through lymph was lower than what remained in the tissue fluid which diffuses into the seminiferous tubule. In goats it was found that the Leydig cells were located at points equidistant between the lymphatics and blood capillaries (Oduor-Okelo, 1974) suggesting a lymphatic role in transporting androgens as do capillaries. Fawcett *et al,* (1973) have speculated the possible role of interstitial lymphatics in androgen transportation in the groups of animals studied. Transportation of androgens is not only a function of lymphatics but also of the blood vessels although a higher proportion of the androgens diffuse into the seminiferous tubules than that transported through the general circulation. The significance of these escape routes (capillaries and tubules) has been underscoted by studies on human testis (Halley, 1960; Kormano & Suaranto, 1971). The morphology of the lymphatics shows a thin endothelial layer with no basal lamina (Leak & Burke, 1966, 1968; Oduor-Okelo, 1974) but instead connected to the underlying connective tissue by numerous filaments (Leak &

Burke, 1966,1968). Micropinocytotie vesicles and open gaps have been implicated in transportation across the lymphatic endothelial wall (Leak, 1971; Fawcett *et al,* 1969, 1970) in addition to free particles in the cytoplasm observed in goats (Oduor-Okelo, 1974).

The seminiferous tubules comprise of a population of static tall columnar (Sertoli or sustentacular) cells which support the actively dividing and mobile spermatogenic cells. The tubules are surrounded by external limiting membranes also called boundary tissue. Morphology of the boundary tissue reveals differing components in various animal species. Among the rodents it has been reported that newborn rats have four poorly defined layers that become clearly demarcated with progressive development to form an inner non-cellular layer, inner cellular layer, outer non-cellular layer and outer cellular layer (Leeson & Leeson, 1963). A similar number of layers has also been reported in Swiss mouse (Gardner & Holyoke, 1964). In newborn mice, however, the tubule epithelium is surrounded by 3-4 layers of fibroblasts which reduce in number with growth and development to become well defined and specialised (Ross, 1967). Similar investigations in domestic animals such as camels and the West African dwarf goats revealed presence of three layers namely inner non-cellular layer, middle (inner) cellular layer and peripheral (outer) cellular layer of fibrocytes, fibroblasts and collagen (Moniem *et al.,* 1980; Ezeasor, 1988). In humans, 3-4 layers were recognized as being separated by varying amounts of collagen (Ross & Long, 1966). Based on the observed variations of the boundary tissue components attempts have been

made to group them into three categories. Type "a" membranes consisting of two lamellae between which a cell layer is sandwiched found in rats, mouse and hamster; type "b" where the components of the internal lamellae are fused together and interlamellar cells are present in 2-4 layers found in guinea pigs and type "c" with inner lamellae having two components (an inner layer containing minute fibrils and an outer layer having collagen fibres) and plicatures or infoldings on those parts occupied by the basal portion of Sertoli cells and this type occurs in man, cats (Burgos *et al,* 1970) and goats (Ezeasor, 1988).

Considering all the layers of the boundary tissue, one that has aroused a lot of interest is the middle (inner) cellular layer. Most studies on this layer have revealed the presence of highly flattened cells also referred to as myoid cells with smooth muscle-like properties such as the presence of intracytoplasmic filaments and micropinocytotic vesicles (Leeson & Leeson, 1963; Gardner & Holyoke, 1964; Ross & Long, 1966; Ross, 1967). It is for this reason that Ross & Long (1966) suggested that these cells may have a role in the movement of tubular content due to their contractile property. This cell layer, apart from propelling the tubular contents, has also been incriminated in regulation of passage of nutrient materials and secretory products between the tubules and the surrounding interstitium and is thus considered as part of the blood-testis barrier. This role was recognized through physiological and morphological studies. Setchel (1967) observed that plasma and testicular lymph were richer in some constituents, notably proteins, than the

seminiferous/rete testis fluid in sheep. This was further confirmed when it was found that whereas some compounds in rete testis fluid equilibriated readily with blood plasma, some did so slowly and others never even appeared in the rete testis fluid at all (Setchel *et ai,* 1969). Setchel *et al.* (1969), therefore, suggested that this permeability barrier regulates access to the seminiferous epithelium of some constituents of blood plasma, isolating the germinal cells immunologically and helping to maintain the concentration differences between rete testis fluid and lymph or blood plasma. Morphological investigations into this cell layer concurred with the previous suggestion of a possible permeability barrier in this region. It was demonstrated using *en bloc* uranyl acetate staining and lanthanum nitrate that the cells forming this layer exhibited both occluding junctions which prevented passage of lanthanum nitrate into the germinal epithelium and a continuous interspace of 200 A where lanthanum nitrate penetrated the myoid layer into the tubules hence, forming partial permeability barrier to the penetration of substances into the seminiferous epithelium (Dym & Fawcett, 1970). In monkeys, however, no occluding junction was observed in this cell layer (Dym, 1973).

Sertoli (1865) described the presence in human testis of cells with irregularly shaped bodies and indistinct margins showing two extremities: an upper one which is larger with a well marked margin and a contracted lower extremity resting onto the basement membrane. These cells had extensions that surrounded the germinal cells, hence, he referred to them as branched cells'. He

observed that these cells had fat droplets in their cytoplasm which extended even to the cell branches and the nuclei were always containing nucleoli. These cells later came to be known as the Sertoli cells. Several workers to-date have confirmed Sertoli's observations in addition to new findings resulting from species variations and more detailed morphological analysis, Among the presently known cytoplasmic organelles of these cells are, better developed sER than rER (the latter being mainly basal in location), mitochondria parallel to the long axis of the cell in the supranuclear column, lysosomes, lipid droplets, lipofuchsin granules, muccopolysaccharides (Bawa, 1963; Gardner & Holyoke, 1963; Nicander, 1963; Burgos *et al,* 1970; Dym, 1973; Fawcett, 1975) together with crystals of Charcot - Bottchner (or crystalloids) in humans (Bawa, 1963; Burgos *et al,* 1970; Fawcett, 1975) and residual bodies of Regaud in monkeys (Dym, 1973). The nuclei of Sertoli cells are large, ovoid with infoldings (Burgos *et al,* 1970; Dym, 1973; Fawcett, 1975) and lobulated in some species (Fawcett, 1975) with prominent nucleoli that are reticulated in most species (Nicander, 1963; Fawcett, 1975).

At histological level identification of the limits of Sertoli cells are complicated by superimposition coupled with their elaborate intercrescence with each other and with the germinal cells (Fawcett, 1975). Ultrastructurally, however, the Sertoli - Sertoli cell junctions in various animal species have been found to comprise of tight (occluding) junctions that are apparently effective barriers than those of the myoid cells (Bawa, 1963, Flickinger & Fawcett, 1967;

Burgos *et al,* 1970; Dym & Fawcelt, 1970; Dym, 1973; Felletier & Friend, 1983; Pelletier, 1986, 1988). The formation of these tight junctions results in the compartmentalisalion of the seminiferous tubules into two compartments namely the basal compartment containing the spermatogonia and preleptotene spermatocytes and the adluminal compartment above Sertoli cell junctions containing the more advanced forms of germ cells (Dym & Fawcelt, 1970). These special junctions between Sertoli cells have been thought to favour the cell-to-cell communication necessary for the regional synchronization of developmental events characteristic of spermatogenic wave (Flickinger & Fawcett, 1967). Three years later, Dym & Fawcett (1970) added that when processes from neighbouring Sertoli cells meet below a germ cell, the formation of such junctions and their extensions from base towards lumen would zipper up the junction and tend to move the germ cell towards lumen. These blood-testis barriers (myoid-myoid and Sertoli-Sertoli) selectively allow passage of some materials while blocking others (Dym & Fawcett, 1970; Fawcett *ct al,* 1970; Dym, 1973) leading to the observed differences between seminiferous or rele testis fluid on the one hand and testicular lymph and plasma components on the other (Voglmayr *ct al,* 1966; Setchel *ct al.,* 1969). Furthermore, the Sertoli-Sertoli barrier prevents foreign proteins from the advanced forms of germ cells (since they differ genetically from their precursors in the basal compartment) from reaching the blood and inducing formation of antibodies which could lead to autoimmune infertility (Fawcett, 1986).

The role of Sertoli cells in androgen biosythesis is an intriguing one since there are some observations pointing out that even though Leydig cells are the principal androgen producers, Sertoli cells are also capable of doing so although at a lower level (Christensen & Masson, 1965). Furthermore, it has been suggested that there is a local feedback from the seminiferous tubules exerting an influence on Leydig cells such that atrophy of the tubules is accompanied by stimulation and increase in abundance of the nearby Leydig cells while those cells far from atrophic tubules show no evidence of stimulation (Aoki & Fawcett, 1978). Tabone *et al.* (1984) when studying the interactions between immature porcine Leydig and Sertoli cells *in vitro* concluded that Sertoli cells exert a trophic effect on Leydig cells and it was thought that the stimulatory effect of follicle stimulating hormone (FSI I) on Leydig function was probably via Sertoli cells. It is known that FSII stimulation results into production of androgen-binding-protein (ABP) which binds testosterone thereby increasing its concentration around the tubule (Fawcett, 1986). Furthermore, cases of feminisation of organisms have been associated with Sertoli cell tumours due to over-production of estrogens (Dellmann & Wrobel, 1976) and the presence of both sFR and rFR in mature and active Sertoli cells of ground squirrel further stresses this role of steroidogenesis (estrogens production in particular) and protein synthesis and secretion respectively (Pudney *et al.,* 1985; Pudney, 1986). On the other hand, Burgos *ct al.* (1970) stated that the presence of tubules, cisternae, vesicles and rich numbers of inclusions in these cells may render them (become) obligatory anatomical pathway for metabolic

interchange between germinal cells and blood stream. Other functions of Sertoli cells include phagocytosis of regressive spermatozoa and attached residual bodies of spermatids and production of anti-mullerian factor responsible for the regression of Mullerian ducts in male embryos. Sertoli cells also produce inhibin which suppresses the production of FSII by the hypophysis in addition to elaborating fluids that serve as vehicle for transporting spermatozoa (see Fawcett, 1986).

Spermatogenic cells form the mobile (non-fixed) population of the seminiferous epithelium. Frequently encountered cells here include spermatogonia (A and B), spermatocytes (primary and secondary), spermatids and spermatozoa. Type A spermatogonia are less numerous and rest on the basement membrane. They are characterized by presence of dome-shaped or oval cell bodies, round or oval mitochondria, scattered tubular or vesicular forms of sFR, dense granules and small GA. The nuclei are spherical or ellipsoidal with smooth or regular surface, have pale and homogeneous nucleoplasm with fine filamentous texture. Type B spermatogonia are found in groups with similar morphology. In their last mitotic division, the cell bodies become incompletely divided forming intercellular bridges. From a single spermatogonium, several germ cells which maintain protoplasmic continuity through intercellular bridges arise. The nuclei are round and central with flakes of chromatin. Nucleoli, usually one or two per nucleus, are irregular and heterogeneous. The type B spermatogonia may be found completely surrounded by lateral extensions of Sertoli cells, in

partial contact with other type B spermatogonia or the basal lamina. Primary spermatocytes arise from the last mitotic division of spermatogonia B. These cells develop progressively through premeiotic interphase, prophase (leptotene, zygotene, pachytene, diplotene, diakinesis), metaphase, anaphase and telophase stages of meiosis. The resulting haploid secondary spermatocytes undergo mitosis to eventually give rise to spermatids whose further maturation lead to formation of spermatozoa (Gardner & Holyoke, 1964; Burgos *et al.,* 1970; Dyrn & Fawcett, 1971; Fawcett, 1986). During spermatogenesis, the spermatogenic cells are held up at the edge of Sertoli cells. Late spermatids, however, shift their position to occupy apical recesses within the Sertoli cells (Fawcett, 1986) and are held in these positions by half junctional complexes (Flickinger & Fawcett, 1967). The appearance of various spermatogenic cellular associations within the seminiferous epithelium have been used to determine the number of stages of the cycle of the seminiferous epithelium in various animals. Among laboratory animals, rats have been shown to have a cycle consisting of 14 stages (Leblond & Clermont, 1952; Perey *et al.,* 1961; Clermont, 1962; Clermont & Bustos-Obregon, 1968), guinea pig 12 (Clermont, 1960; Fawcett, 1986), domesticated giant rats, *Cricetomys gambianus,* Waterhouse, 14 (Aire, 1980), Opossum, *Didephis azarae,* Temminck, 10 (Orsi & Ferreira, 1978), mink 12 (Pelletier, 1986). Larger animals, on the other hand, show a reduced number of stages as exemplified by humans 6 (Clermont, 1963) and camels 8 (Osman *et al.,* 1979).

1.2.2 Excurrent duct system

The components of the excurrent duct system include the tubuli recti, rete testis, ductuli efferentes, ductus epididymis (caput, corpus and cauda) and the ductus deferens.

1.2.2.1 Tubuli recti and rete testes

Towards the end of the seminiferous tubule, there is an abrupt narrowing of the tubule accompanied by disappearance of spermatogenic cells leaving only the Sertoli cells. These narrow continuations of the seminiferous tubules are connected lo some irregularly shaped cavities, intratesticular rete testis, by tubuli recti or straight tubules (Roosen-Runge, 1961; Dym, 1974; Fawcett, 1986). At histological level, the tubuli recti and the intratesticular rele in most mammalian species are lined by simple cuboidal to columnar epithelium resting upon a thick basal lamina. This in turn is surrounded by dense collagen bundles among which are scattered smooth muscle cells (Roosen-Runge, 1961; Bustos-Obregon & Holstein, 1976; Dym, 1974, 1976; Johnson, 1978). Ultrastructurally either one epithelial cell type in most mammalian species (Dym, 1976) or two in man (Bustos-Obregon & Holstein, 1976) have been recognized. In these mammalian species other than man, the epithelial cells have deeply indented nuclei, spherical or rod-shaped mitochondria, supranuclear Golgi profiles, cisterns of rFR, free ribosomes, pinocytotic vesicles and microvilli in addition to a single, long flagellum (Dym, 1974, 1976). In man, however, flat dark cells with slender microvilli and microvesicles were seen besides

prismatic light cells with more organelles (Bustos-Obregon & Holstein, 1976). It has been suggested that these epithelial cells could be secreting substances (Bustos-Obregon & Holstein, 1976) as evidenced by the cytoplasmic organelles. Further, it has been advanced that there may be a blood-testis barrier between the epithelial cells of the intratesticular rete (Dym, 1976).

1.2.2.2 Ductuli efferentes

The intratesticular rete testis is connected by extra testicular rete testis to the ductuli efferentes (Roosen-Runge, 1961; Dym, 1974). Initially these ducts are many but later converge to form the caput epididymis. Morphology of the ductuli efferentes has been studied in a number of mammalian species such as the rat (Hoffer, 1972; Hamilton, 1975; Hamilton *et al,* 1977), guinea pig (Hoffer & Greenberg, 1978), mouse (Hoffer, 1972), hamster (Flickinger *et al,* 1978; Vicentini *et al,* 1990), rabbit (Jones *et al,* 1979), giant rat, *Cricetomys gambianus,* Waterhouse (Oke *et al,* 1988), Australian rodents, *Pseudomys australis* and *Notomys alexis* (Pierce & Breed, 1989) and opossum (Ladman, 1967) where the epithelium mainly consisted of principal (non-ciliated) cells with oblong and indented nuclei, numerous dense granules, microvilli on the luminal surface, vesicles and vacuoles in the supranuclear cytoplasm. Another dominant cell type in these ducts are ciliated cells with long cilia projecting into the lumen of the ducts and lobated nuclei (Hamilton, 1975). Similar epithelial cells have also been recognized in the ductuli efferentes of other larger mammalian species such as the boar, ram and bull (Hemeida *et al,* 1978), goats

(Hemeida *et al.,* 1978; Goyal & Williams, 1988), stallion (Hemeida *et al.,* 1978; Johnson, 1978), monkey (Ramos & Dym, 1977) and elephant, *Loxodonta africana* (Jones & Holt, 1981). In some animals studied, these ducts showed the presence of additional epithelial cell types apart from the two dominant ones. It has been shown that the lumen of ductus efferentes in some animals is stellate in appearance and the epithelium is surrounded by one to four layers of elongate smooth muscle cells (Hamilton, 1975; Ramos & Dym, 1977; Hoffer & Greenberg, 1978).

1.2.2.3. Ductus epididymis

The ductuli efferentes gives rise to the ductus epididymis which is morphologically divided into caput, corpus and cauda segments. Nicander & Glover (1973), however, divided this duct into initial, middle (arising from caput flexure to the corpus-cauda junction) and terminal segments. The epithelium of ductus epididymis, mainly pseudostratified columnar, rests onto a basement membrane which is further surrounded by varying amounts of smooth muscle in the entire duct (Hamilton 1975; Oke *et al.,* 1988; Tierce & Breed, 1989). These segments were further found to consist of several zones. Most laboratory rodents have 3-7 zones with the Australian rodent *(Notomys alexia)* having the least number of zones (three) (Pierce & Breed, 1989) and guinea pig the highest (seven) (I loffer & Greenberg, 1978).

The initial segment (zone 1) of the caput epididymis marks the transition from ductuli efferentes to caput epididymis

characterized histologically by an abrupt cessation of ciliated cells, acquisition of basal cells, a dramatic increase in the height of principal cells and a wide lumen as in the golden hamster (Nicander & Glover, 1973) or a narrow lumen as reported in the laboratory rat, giant rat and Australian rodents (Hamilton, 1975; Hamilton *et ai,* 1977; Oke *et ai,* 1988; Pierce & Breed, 1989). Other cell types have also been encountered within the segment among which are the apical cells (Hamilton, 1975; Pierce & Breed, 1989), macrophages (Oke *cl ai,* 1988) and 'hallo' or intraepithelial lymphocytes (Hoffer *rf ai,* 1973; Hamilton, 1975; Oke *et ai,* 1988). Under the light microscope, the principal cells show small basal nuclei with varying number of nucleoli, mitochondria, GA, small granules, several vesicles and vacuoles (Hoffer *et ai,* 1973; Hamilton, 1975; Flickinger *et ai,* 1978; Pierce & Breed, 1989). Rare vesicles and vacuoles have been observed in the apical cytoplasm and long clumped microvilli on the apical surface (Oke *et ai,* 1988). Basal cells are small, and have pale cytoplasm, ovoid nuclei with the long axis oriented parallel to the basement membrane (Hamilton, 1975; Oke *et ai,* 1988; Pierce & Breed, 1989). Apical cells, on the other hand, have their nnclei towards the apical cytoplasm and their cytoplasm seems not to extend to the basement membrane. These cells have few, less developed microvilli (Hamilton, 1975; Pierce & Breed, 1989). 'Halo' cells occur mostly towards the basal lamina and they appear as cells with a space around the nuclei (Hoffer *et ai,* 1973; Hamilton, 1975; Oke *et ai,* 1988).

Ultraslructurally, the principal cells have nuclei that are variable in size, exhibit membranous endocytotic invaginations on the apical surface of plasmalemma and compartmentalisation of cytoplasmic organelles where the apical cytoplasm has numerous coated vesicles, multivesicular bodies and frothy areas of GA. Strands of rER and sER, mitochondria, free ribosome and polysomes occur in the basal cytoplasm (Hoffer *et al.,* 1973; Hamilton, 1975; Flickinger *et al.,* 1978; Oke *et al.,* 1989; Nicander & Malmquist, 1977).

The epididymal duct serves as a transporting conduit, maturation and storage site for spermatozoa (Bedford, 1975) the achievement of which is gradual from segment to segment. The presence of coated vesicles, multivesicular bodies, vacuoles in the cytoplasm and membranous endocytotic invaginations on the apical plasmalemma of the principal cells in the initial segment suggests absorptive function (Friend & Farquhar, 1967; Hoffer *et al.,* 1973; Hamilton, 1975; Flickinger *et al.,* 1978). Using mercuric sulfide, Burgos (1964) confirmed that the cells of caput epididymis participate in the uptake of colloidal particles assisted by the microvilli which increase the surface area exposed to the material in the lumen and facilitate uptake and transport of substances by pinocytosis. Absorption of sperm-specific protein also occurs within this segment (Barker & Amman, 1971). These latter workers further suggested a secretory role for the principal cells in this area when they observed, using immunofluorescence, some non-ciliated antigen-secreting cells. In line with the foregoing, it has been argued that the presence of sER and/or rER and GA which are associated with protein

synthesis and secretion (Bennett *et al.,* 1974) may confer a secretory role in these cells (Hoffer *et al.,* 1973; Flickinger *ct al.,* 1978).

The middle segment begins from caput flexure to the proximal cauda (Nicander & Glover, 1973) and consists of various zones depending on the animal species. Each zone is identified by the overall duct histology which includes a progressive increase in luminal diameter, reduction in epithelial cells and microvilli height from caput flexure distally (Hamilton, 1975; Hamilton *et al.,* 1977; Vicentini & Orsi, 1987; Oke *et al.,* 1988; Pierce & Breed, 1989). The epithelium in this region mainly consists of principal and basal cells (Hamilton, 1975; Oke *et al.,* 1988; Pierce & Breed, 1989) and in some species as in the giant rat, 'halo' cells and macrophages occur (Oke *et al.,* 1988). The main histological and ultrastructural leaturcs ot principal cells in this segment include basal nuclei with one or two nucleoli, vacuoles and vesicles that become fewer and smaller distally, multivesicular bodies (which were not observed in the corpus), well developed GA, mitochondria that are dispersed throughout the cytoplasm and well developed ER. Lipid droplets and dense bodies have also been observed (Hamilton, 1975; Hamilton *et al.,* \977-, Flickinger *el al.,* 1978; Jones *et al.,* 1979; Oke *et al.,* 1988,1989; Pierce & Breed, 1989). Flickinger *et al.,* (1978) reported that within this region, sodium and potassium are absorbed but not fluids. The same authors also found that it is within this region that the cytoplasmic droplet of the luminal sperm begin to move caudally.

The terminal segment begins from the proximal cauda to the distal cauda embracing the whole cauda epididymis (Nicander & Glover, 1973; Oke *et al,* 1988; Pierce & Breed, 1989). In this segment, the luminal diameter is wider than in the middle segment, the epithelium is low and the microvilli form a "brush border" (Nicander & Glover, 1973; Hamilton, 1975; Hamilton *et al.,* 1977; Vicentini & Orsi, 1987; *Okeet al.,* 1988; Pierce & Breed, 1989). The epithelium in this region consists of principal, basal (numerous in this region) (Hamilton, 1975; Flickinger *et al.,* 1978; Hoffer & Greenberg, 1978; Jones *et al.,* 1979; Oke *et al.,* 1988; Pierce & Breed, 1989) and in some

species clear (light) (Hamilton, 1975; Hamilton *et al.,* 1977; Flickinger *et al.,* 1978; Oke *et al.,* 1988; Pierce & Breed, 1989; Vicentini & Orsi, 1989) and 'halo' cells (Hamilton, 1975; Oke *et al.,* 1988). Principal cells in the terminal segment morphologically show fewer vacuoles and vesicles than the middle segment formed from GA, mitochondria scattered within the cytoplasm, concentric array of rER basally located, small basal nuclei with one or two nucleoli (Hamilton, 1975; Jones *et al.,* 1979; Oke *et al.,* 1988; Vicentini & Orsi, 1989). Vicentini & Orsi (1989) also reported presence of multivesicular bodies, micropinocylotic vesicles and membrane-bounded vesicles in apical cytoplasm of principal cells in the hamster. Clear cells however, show presence of numerous vesicles, vacuoles and dense bodies, centrally located pale staining nuclei and no microvilli on the apical surface of the cells (Flickinger *et al,* 1978; Oke *et al,* 1988; Pierce & Breed, 1989; Vicentini & Orsi, 1989). This segment serves as a storage and maturation site for spermatozoa. It

as motile as ejaculated ones (Hamilton, 1975; Johnson, 1978). The cytoplasmic organelles found in principal cells and clear cells in this region suggest a secretory function (Hamilton, 1975; Flickinger *et al.*, 1978; Jones *et al.*, 1979; Vicentini & Orsi, 1989) which is further corroborated by the isolation of cellular androgen binding proteins (ABP) from cauda epididymis of dogs (Wango, 1983; Oduma, 1988) although evidence of absorption has also been reported in principal cells of this segment in laboratory rats and hamsters (Hamilton, 1975; Vicentini & Orsi, 1989).

1.2.2.4 Ductus deferens (vas deferens)

This is the caudal continuation of the epididymis and together with accessory sex gland ducts, the ductus deferens open into the pelvic urethra, a short distance caudally from the neck of the urinary bladder (Nickel et al., 1973). For descriptive purposes, ductus deferens has been conveniently divided into two segments: proximal or epididymal and distal segments (Flickinger *et al.*, 1978; Hamilton & Cooper, 1978; Kennedy & Heidger, 1979; Pierce & Breed, 1989; Leong & Singh, 1990) but a terminal segment has also been described (Hamilton & Cooper, 1978). These workers reportedly observed a low epithelial height comparable to that of the cauda epididymis and distended lumen in the former segment while the distal segment exhibited a convoluted mucosa, tall epithelial cells with long microvilli and surrounded by a thick muscular coat. In laboratory rats, the epithelium of the ductus deferens mainly consists of principal and basal cells (Hamilton *et al.*, 1977; Flickinger *et al.*, 1978; Kennedy & Heidger, 1979) but may (Kennedy & Heidger,

1979) or may not (Hamilton, 1975) have clear cells. A further cell type whose identity is obscure but which has been referred to as pencil cell (Niemi, 1965; Hamilton, 1975; Hamilton *et al.,* 1977) has also been observed. Kennedy & Heidger (1979), on the other hand, reported the presence of a "Mitochondrion-rich" cell and intraepithelial macrophages in the distal segment and the entire duct epithelium respectively. Principal and basal cells have also been found as predominant cell types in the epithelium on this duct in guinea pigs and a few clear cells in Australian rodents have also been reported (Hoffer & Greenberg, 1978; Pierce & Breed, 1989).

1.2.3 Accessory sex glands and external genitalia

1.2.3.1 Accessory sex glands

Commonly encountered accessory sex glands are seminal (vesicular), prostate and bulbourethral glands whose occurrence in animals is species dependent. Many mammals including the stallion, bull, boar, rats and rabbits have all the three major glands and in addition some rodents have coagulating gland while the rabbit and stallion have the ampulla (Greene, 1963; Weisbroth *et al.*, 1974). Similar observations were seen in the rock hyrax where all the three glands were present but with no coagulating gland nor ampulla (Glover & Sale, 1968). In European mole rats, however, only the prostate and bulbourethral alongside the preputial gland are present (Racey, 1978). Some mammals like the spotted skunk (Mead, 1970) and the dog (Nickel *et al.,* 1973) have the prostate gland only.

Growth and differentiation of the accessory sex glands are influenced by sex hormones such that removal of androgenic stimulus by orchiectomy results into marked structural alteration and depression of functional activities while administration of exogenous androgenic compounds reverses the effects (Brandes, 1974a; Narbaitz, 1974). Bernard (1986) further showed in the cape horseshoe bat that during the period of sexual activity, the increase in plasma testosterone concentration is accompanied by increased secretory activity of the accessory sex glands. On the other hand, Neaves & Bramley (1972) in their study of the relationship between blood levels of testosterone and accessory sex gland weights in impalas, which are animals that breed all the year round, found that there was no correlation between plasma testosterone and accessory sex gland weights.

1.2.3.2 External genitalia

The external genitalia comprises of the penis and the urethra. The mammalian penis is made up of three cylindrical masses of erectile tissue namely two corpora cavernosa penis, unpaired corpus cavernosum urethrae (surrounding urethra) and glans penis (Nickel *et al.,* 1973; Kelly *et al.,* 1984). Each corpus cavernosum is surrounded by a capsule, tunica albuginea, underneath which are venous plexuses. Within the corpus cavernosum are large spaces or lacunae lined by endothelium and are separated by fibrous trabeculae rich in smooth muscle fibres (Dellmann & Wrobel, 1976; Kelly *et al.,* 1984). The tunica albuginea of corpus cavernosum

urethrae is thin and contains many elastic fibres and the lacunae are uniform in size. In some mammals such as dogs and cats (Nickel *et al*, 1973), walruses, otters, guinea pigs (Owen, 1868) and bushbabies (Harrison & Lewis, 1986) an os penis or bacculus is present in the penis. In the glans penis, the erectile tissue has dense venous plexus but no tunica albuginea (Kelly *et al.*, 1984). Rabbits, however, do not have a glans penis but instead have a free extremity called pars libera (Weisbroth *et al.*, 1974). The prepuce is a fold of skin that overlies the glans and consists of connective tissue with bundles of muscle fibre covered by a thin epidermis (Kelly *et al.*, 1984). A number of modified sweat glands are found on the inner surface of the prepuce.

The urethra can be divided into the pelvic (prostatic), bulbar (membranous) and penile (cavernous) portions (Dellmann & Wrobel, 1976; Kelly *et al*, 1984). The urethral mucosa is thrown into longitudinal folds and the ureteric crest of urinary bladder continues into the urethra with a prominent and permanent fold called urethral crest (Dellmann & Wrobel, 1976; Fawcett, 1986) which terminates at the colliculus seminalis. The ejaculatory ducts and utriculus masculinus (vestiges of Mullerian ducts) similarly terminate at the colliculus seminalis. In most animal species, the pelvic and penile urethra are lined by transitional and stratified epithelium respectively but dog, cat and buck have a bulbar portion lined by stratified columnar epithelium (Dellmann & Wrobel, 1976). The propria submucosa of urethra contain erectile tissue rich in elastic fibres and plexus of capillaries and thin-walled veins in its deeper portion.

13 M?le reproduction in the naked mole rat

1.3.1 Morphology

Morphological studies on the reproductive organs of the naked mole rat are scanty. Hill *et al.* (1957) in their field and laboratory studies on the naked mole rat presented an in-depth macroscopic description of the various components of both male and female reproductive systems. Kayanja and Jarvis (1971) examined the ovaries of the naked mole rat and reported presence of rich interstitial gland tissue. Similarly, the only reported microscopic account of the male reproductive organs of the naked mole rat is contained in a comparative study of the organisation of the interstitial tissue of the mammalian testis by Fawcett *et al.* (1973). These workers showed that in the naked mole rat testis, the seminiferous tubules are sparsely distributed in a mass of lipid-rich interstitial cells of Leydig which form about 60% of the volume of testis. A similar observation was reported in the boar testis by the same authors where the Leydig cells constitute about 60% of the testicular volume. In addition, Sink (1967) showed that the testis of the boar is the source of the odorous steroid 16-androsterone which is thought to serve as a pheromone. Fawcett *et al.* (1973) therefore speculated that the abundance of Leydig cells in such animals as the naked mole rat may indicate the synthesis of other steroids besides testosterone.

1.3.2 Endocrinology

The breeding habits of the naked mole rats in a colony are indeed puzzling, for example, why only one female and one or two males should be capable of breeding is not clearly understood. Moreover, when the non-breeding members of the colony are removed from the influence of the colony, they start breeding. It has been suggested that the pheromone from the female or its behavioural dominance could be the cause (Jarvis, 1984). It has been determined, at least in female primates and naked mole rats, that the sexual suppression is as a result of suppressed secretion of gonadotrophin releasing hormone (GnRH) from the hypothalamus (Abbot, 1987; Faulkes *et ai,* 1990b).

Selective breeding has also been observed in the common marmoset monkeys (Hearn, 1983; Abbott, 1987; Abbott *et ai,* 1989) where the breeding female is thought to produce pheromones that cause sexual suppression in other females. In wild mice, male sexual maturity is suppressed by the mature wild male mice (Pandey & Pandey, 1986). On the other hand, exposure to pheromonal cues does not always lead to sexual suppression. In prairie voles *(Microtus ochrogastcr)* exposure of females to male urine leads to an increased secretion of luteinizing hormone releasing hormone (LHRH) and norepinephrine from the olfactory bulb. This causes increased LH levels in blood (Dluzen et al., 1981).

There have been attempts to determine the sex hormone levels in the naked mole rats to form a basis for explaining this

observed lack of breeding. In recent studies Faulkes *et al.* (1989a, 1989b, 1990a) found plasma LH and urinary progesterone and testosterone levels in non-breeding females and males to be low respectively. However, neither plasma testosterone nor progesterone levels were measured to give an overview of what the level of these two hormones are in blood. But as stated earlier, the volume of Leydig cells in the testes of these animals is large, forming 60% of testicular volume and may also be a source of estrogens as in the boar (Fawcett *et al.* 1973). However, there has been no attempt to stimulate this massive volume of Leydig cells *in vitro* or *vivo* with gonadotropin to determine their response and if so to quantify the amount of testosterone they produce.

i

1.4 Aims and objectives

The present study aims at achieving the following:

1. To shed light into the hitherto little known histological and ultrastructural features of the male reproductive system of the naked mole rat.
2. To observe the effect of *in vitro* stimulation of the massive volume of the testicular interstitial cells using pituitary gonadotropin (Ovine LH). Hopefully this will provide a fair assessment of the steroidogenic capacity of this mass of cells.
3. To determine the plasma testosterone and LH levels and compare this with what had previously been observed by other workers.

CHAPTER 2

MATERIALS AND METHODS

2.1 Experimental animals

A total of 50 naked mole rats were caught from Mtito Andei area of Eastern Province, Kenya. This is a semi-arid area with a total annual rainfall of about 2,000 mm and located about 240 km from Nairobi on the main Nairobi-Mombasa road. Trapping of the animals was done with the help of the local people of Mtito Andei area. The animals live in an extensive burrow system with branching and anastomosing tunnels. Trapping therefore involved surprise excavating of soil using a hoe immediately ahead of the tunnel entrance where the mole rats were busy throwing out soil. The rats are active in the mornings and evenings, hence, these are the best times for trapping. Trapping is even easier during the rainy season because the soil is soft. These animals were caught in different months (table I) and transported back to the laboratory. In the laboratory, each monthly batch was kept in a cage measuring 60 cm x 30 cm x 30 cm with soil for burrowing and infra-red lamp as a heat source provided. The cage was kept in a room with 60% humidity for a period of 48 hours. The animals were fed on carrots and cassava tubers. In addition, six 6-weeks old BALBC mice were obtained for the purpose of comparing the testicular interstitial cell response to LH stimulation to that of the naked mole rat cells and also for naked mole rat plasma LH level determinations.

2.2 Animal handling and sample collection

Just before killing, the animals were weighed and their sizes in terms of head to base of the tail measured. The animals were then anaesthetized using ether and the abdominal cavity opened via the ventral mid-line to expose the reproductive tract. The females (identified by lack of testis close to the fundus of the urinary bladder) were overdosed with Pentobarbitone sodium (200 mg/ml) and discarded. The male animals were divided into two groups. Group one animals (see table I, Nos. 2-15) had blood samples withdrawn from the heart using needle gauge 25 and syringe and testes removed for use in the assays. The animals were then overdosed with pentobarbitone sodium as before and the remaining reproductive tracts collected and the animals discarded. Group two animals (table I, Nos. 1, 16-34) were perfusion fixed using 2.5% gluteraldehyde after blood clearing using physiological saline and the entire reproductive tract removed for morphological studies. Out of 50 animals, 34 were males with an average body weight of 34.03 ± 1.26 g (n=34) and a head to base of the tail length of 9.80 ± 0.16 cm (n=34). The testes on the average measured 3 x 4 mm.

2.3 Fixation, processing and examination

2.3.1 Morphological samples

I

Basically two methods of fixation were employed to preserve the morphological samples. The animals for direct morphological studies were first cleared of blood using physiological saline and perfusion fixed through the abdominal aorta with 2.5%

gluteraldehyde delivered at a constant pressure. Perfusion was necessary to fix the tissues in situ to avoid the possibility of autolysis. The harvested tissues were further fixed by immersion in 2.5% gluteraldehyde and kept in the fridge for a period of 24 hours. Tissues from animals of group one were only fixed by immersion.

The penile urethra and penis obtained for morphological studies because of their large sizes were processed for routine histological studies and stained with hematoxylin and eosin (H & E). In brief, the tissues were dehydrated in ascending concentrations of ethyl alcohol (50%, 70%, 90% and 100%), cleared using methyl benzoate, infiltrated in liquid paraffin and embedded in wax. The embedded tissues were mounted on wooden blocks and sections of 7jim thick cut from the block using a sliding microtome. These sections were then stained using H & E and observed under light microscope.

The remaining organs of the tract due to their small sizes were processed for electron microscopy (EM) using the routine method. In brief, the tissues were rinsed in phosphate buffer, post-fixed in osmium tetroxide (OSO_4) buffered with Phosphate buffer, dehydrated in ascending concentrations of ethyl alcohol (50%, 70%, 95% and 100%) followed by further dehydration in propylene oxide. The tissues were infiltrated with propylene oxide-resin mixture and embedded in resin mixture. The resin blocks were cut with glass knives using Porter Blum ultramicrotome (Sorval microtome^{R}).

The obtained 1 nm thick sections were stained with toluidine blue and observed under light microscope while 60 nm thick sections were mounted on 200 mesh grids, stained with uranyl acetate and counter-stained with lead citrate and observed under Phillips CM12 or Zeiss 9 electron microscope.

2.3.2 Endocrinological samples

Blood samples were obtained from 10 (Table I, Nos. 2-11) out of the initial 14 animals. Blood sample from each animal was transferred into a separate and independent tube containing EDTA. About 300-400 p1 was found to be adequate for the hormone assay. The collected blood samples were immediately centrifuged at 1200 g and 4°C for 10 minutes. The plasma was then separated from the remaining cellular residues by decanting and kept at -20°C until the time of assay {Radioimmunoassay (RIA)}. The residues were discarded.

The testes harvested for *in vitro* endocrinological studies were trimmed off excess fatty tissue and rinsed in a culture medium containing 12 ml Basal Medium Eagles (Flow lab. reagents), 2.1 ml of 7.5% sodium bicarbonate, 1 ml calf serum (prepared from 3-4 months old fetuses), 3 drops of antibiotic (streptopen), 100 ml double distilled water and aerated slowly for 10 minutes using medical gas containing 95% O2 and 5% CO_2 (East African Oxygen Company, Kenya) maintained in an ice bath. Mice testes were also removed and similarly treated. For each assay six testes were used as had been previously found to be adequate in mice (Van Damme *et al.*, 1974).

The Leydig cells were dispersed by finely mincing the testes in an ice bath using a pair of scissors. The minced tissues were then transferred to a plastic flask containing the same medium maintained in an ice bath and stirred using a magnetic stirrer for 15 minutes. The medium and the minced tissues were filtered through a fine nylon mesh into a plastic beaker. The filtrate (cells) were then pre-incubated and maintained in an atmosphere of 95% O2 and 5% CO_2 in a water bath at 24°C for 1 hour. Simultaneously the gonadotrophin tubes were appropriately labelled according to the various concentrations of LH to be put in. After incubation, the tissues were centrifuged at 4°C and 500 g for 10 minutes. The supernatant was discarded and the cells rinsed in tap water and fresh medium added for further centrifugation. The cells were then re-suspended in a total of 10 mis fresh medium in a plastic beaker and stirred. They were stained with trypan blue (0.1% Fluka AG, Switzerland) and counted in a haemocytometer to determine the viability of the cells. Viability of mice and mole rat cells were as in table II and fig. 46.

2.3.3 LH Bioassays

About 250,000 cells of either the naked mole rat or mice prepared as before were dispensed separately into assay tubes containing varying concentrations (0 to 25 m IU/ml) of standard ovine LH supplied by World Health Organization (WHO). The cells and the stimulating hormone (LH) were then incubated in a shaking water bath for 3 hours at 24°C in an atmosphere of 95% O2 and 5%C02). The incubation was stopped by immersing the tubes in an

ice bath and subsequent addition of 1 ml cold steroid buffer (WHO method manual, 1990). The tubes were centrifuged at 4°C for 10 minutes at 1200 g to sediment the Leydig cells.

Plasma LH assays were done using an indirect method of measuring plasma LH levels where this was estimated from the **amount** of testosterone produced by dispersed mice interstitial cells (Van Damme *et al.* 1974). 100 ^il samples and LH standards (WHO Matched Reagent Programme) were incubated for 3 hours at 24°C in an atmosphere of 95% O2 and 5% CO2 in a shaking water bath with 6 weeks old mice interstitial cells. The standard LH dose ranged from 0.375-6 mIU/ml. The incubation was stopped as already outlined and the cells centrifuged at 4°C for 10 minutes and 1200 g to sediment the cells. The supernatant from the *in vitro* study and the plasma LH bioassays together with the plasma for testosterone determination were analysed using RIA.

2.3.4 Radioimmunoassay (RIA)

This was done according to WHO protocol (1990). The initially frozen samples (plasma for both LH and testosterone determination and supernatant from Leydig cells) at -20°C were thawed and treated with reconstituted WHO reagents. These WHO reagents included antitestosterone serum, testosterone standard, testosterone tracer, charcoal reagent, gelatin and dextran reagents. Anti-testosterone serum was provided in lyophilized form and stored at 4°C until needed. When needed, one bottle of the anti-serum (enough for one assay batch of 100 tubes) was reconstituted

with 10 ml of steroid assay buffer and left to stand for 5-10 minutes before use. Testosterone standard was provided in ethanolic solution at a concentration of 220 nmol/L and this was cooled to 4°C. When required 10 mis of assay buffer was added and the solution heated to 40°C. This solution contained 2.2 nmol/L. 2 mis of this solution was dispensed into a tube containing 2 ml of assay buffer, mixed and 2 ml withdrawn. This withdrawn 2 ml was transferred to a second tube containing 2 ml buffer and mixed again. Further 2 mis was withdrawn from the second tube to a third tube containing 2 mis buffer and mixed. This procedure was carried out through tubes 4 to 5 each containing 2 ml buffer and in each case the solutions mixed and 2 ml withdrawn except in tube 5. 500 [il of testosterone standard was dispensed directly into the assay tubes.

Testosterone tracer was provided in sealed ampoules containing 9.25 MBq (250 ^Ci). The contents of the ampoule was transferred to a 25 ml volumetric flask, the ampoule rinsed with toluene:ethanol (9:1 by volume) and the "washing" added to the flask contents. The volume of this stock solution was made up to 25 ml with toluene:ethanol (9:1) solution. This was stored in the dark at -20°C till needed. When needed, 150 ^il of the stock solution was transferred into a tube, the solvent evaporated and the residue redissolved in 15 ml assay buffer for 30 minutes. This solution contained 3.7 KBq/ml (100 nCi/mL) and was enough for one assay.

10 nl of thawed plasma was transferred into two tubes and to this was added 1.5 ml freshly purified diethyl ether. Into two other tubes only 1.5 ml diethyl ether was added to form "ether blanks".

Into another tube containing 10 of radioactive standard and 10 ^il of thawed sample was added 1.5 ml diethyl ether. This last tube was the recovery tube. All these tubes were vortex mixed, shaken in an agitator for 3 minutes and frozen using solid carbon dioxide ("Dry Ice") and acetone. The ether fraction in each tube was transferred into clean, dry assay tubes, evaporated and 500 of assay buffer added. Into the previously prepared assay lubes, various reagents and samples (both plasma and Leydig cell samples) were aliquoted as in table III.

Recovery tube was taken directly for counting after extraction. The assay tubes were incubated overnight at 4°C, 200 |il dextran-charcoal reagent added and centrifuged at 1500 for 15 minutes. The supernatants were then decanted into fresh, clean scintillation vials and the charcoal residue discarded. To the scintillation vials, about 4 mis of the scintillation cocktail was added and the tubes left for 1 hour to equilibriate in a water bath. After this, the tubes were counted in a gamma counter. A dose-response curve was plotted from these counts by using computer and testosterone levels read-off from this standard curve. LH levels, however, were obtained from the LH-graph plotted using testosterone levels obtained by stimulating mice Leydig cells with standard LH against stimulating LH. Sample LH levels were then directly read-off from this graph. To monitor sample lost during extraction of the plasma testosterone, counts from tubes containing recovery correction samples were expressed as per cent recovered. This gave an average of 90% recovery.

Table 1. : Naked mole rats caught during the year (1990), their weights and body lengths

Month	Total No.	Sex		Serial Nos.	Weights (gms)		Head-tail base Length (cm)	
		Males	Females		Males	Females	Males	Females
Feb.				1	33.15	40.37	10.00	10.10
						35.95		10.50
marcn	14	10	4	2	26.58	26.05	9.00	9.00
				3	34.08	35.66	10.00	9.00
				4	38.42	26.44	10.05	9.00
				5	41.45	40.46	10.00	10.50
				6	36.67		10.00	
				7	33.34		10.00	
				8	24.25		9.00	
				9	53.11		10.20	
				10	28.87		9.50	
				11	40.45		10.50	
April	12	8	4	12	25.46	30.93	9.80	9.00
				13	37.07	34.70	10.00	10.00
				14	46.92	48.80	11.00	10.90
				15	44.18	27.60	11.20	11.00
				16	34.12		10.50	
				17	38.93		11.00	
				18	30.00		10.00	
				19	28.02		9.83	
May	5	5		20	37.07	-	10.01	-
				21	36.63		11.00	

Table 1 cont.

				22	34.35		10.52	
				23	37.72		10.80	
				24	33.21		9.00	
July	16	10	6	25	33.50	34.40	7.60	7.50
				26	16.50	30.30	10.00	9.50
				27	20.40	19.90	7.40	10.00
				28	25.05	20.07	8.00	10.10
				29	33.80	16.82	10.50	9.20
				30	34.73	22.25	8.00	9.80
				31	37.64	-	10.00	
				32	29.03		9.80	
				33	40.01		10.00	
				34	32.30		9.00	
	n = 50	n = 34	n = 16		(xs.34.03tl.26)		(x=9.80+0.16)	

N B i) No mole rats were caught in the months of January, June, August, September, October, November and December due to dry periods from October to January. I did not go for trapping in June, August and September.

ii) The serial numbers mainly apply to the male rats.

iii) The average weight and body length of males was 34.03+1.26 gms and 9.80±0.16 cms respectively.

CHAPTER 3

OBSERVATIONS

3.1 General remarks

The naked mole rats were practically hairless except in a few circumscribed areas notably around the muzzle and the anal areas including tail base where there were a few vibrissae (fig. 1). The skin of the animals were lightly pigmented and they had short stubby limbs with large palms. The eyes of the animals were small and covered by a fold of skin as if not in use, the ears were short and small and there were a pair of prominent incisors on both the upper and lower jaws. Externally, the naked mole rats showed no obvious sexual dimorphism such that sexing the animals on external features was not possible. However, on opening the ventral abdominal wall, the testes were found to be intra-abdominal occupying spaces slightly proximal and on either side of the fundus of the urinary bladder. The testes appeared as ovoid structures measuring approximately 3 x 4 mm on average. On the medial border of each testes were masses of convoluted tubules held together by a peritoneal fold and these represented the epididymides. Three segments of the ducts were recognized grossly and these were designated as caput (head) epididymis lying onto the proximal aspect of the testis and in close contact with it, corpus (body) epididymis which appeared as a narrow continuation from caput distally and the cauda (tail) which occurred as a convoluted mass at the distal end of the testis and continued as a long straight tube, the vas deferens, running towards the neck of the urinary bladder criss-

crossing the ureters as they approached the bladder. Close to the neck of the bladder and prior to the penetration of the urethral wall, the two ducts (vasa defferentia) came close to each other, enlarged and became united by a fold of tissue to continue into the urethral wall as one unit but with two openings of the ducts (fig. 2a, b,e). On either side of this unit comprising two ducts were small grain-sized nodules (about 0.5 mm diameter) of the prostate gland not readily visible to the naked eye. The wall of the pelvic urethra was made up of skeletal muscles enclosing the urethral lumen in the centre. This was consistently seen all the way from the neck of the bladder to the glans penis. The glans penis was enclosed in a mass of connective tissue, muscle fibres and skin which had vibrissae to the outside. At the junction of the bladder neck and the penile urethra ventrally was a mass of grey, fatty tissue-like structure lying transversely and held in position by fascia (see figs. 2a, b, c, d and e).

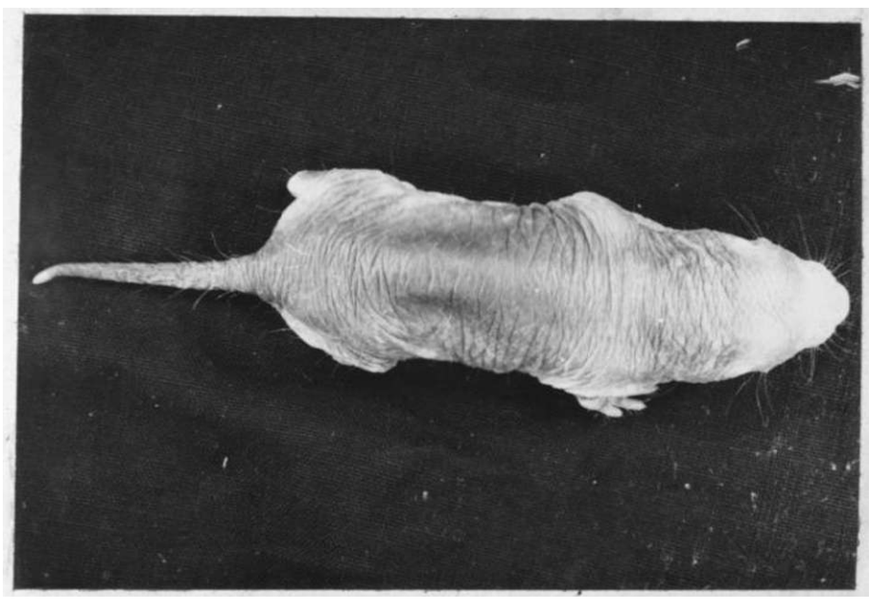


Fig. 1. Naked mole rat. Note the complete absence of hair on the skin except vibrissae in the head and tail regions.

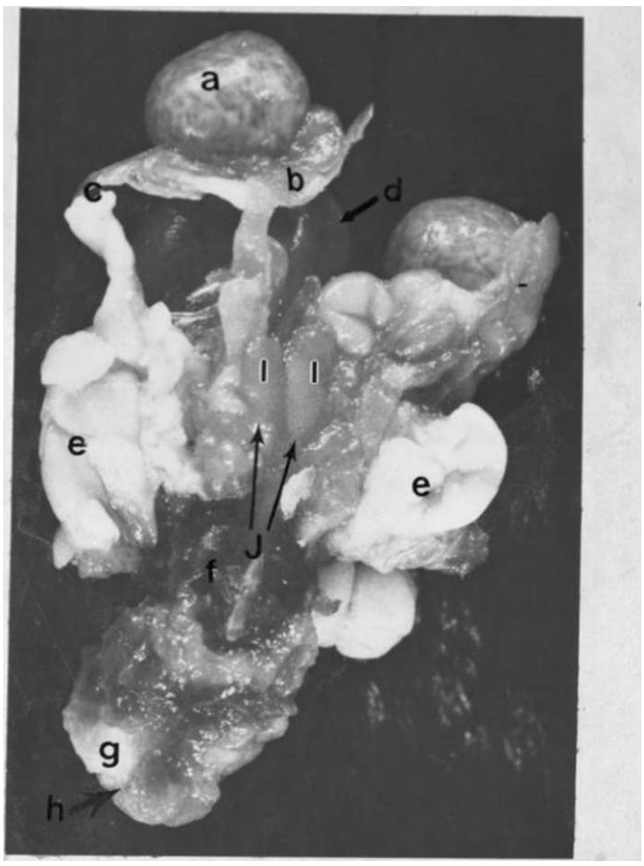


Fig. 2a. A photograph of the dorsal view of the male reproductive organs of the naked mole rat.

a = testis

b = epididymis

c = proximal ductus (vas) deferens

d = urinary bladder

e = fatty tissue

f = pelvic urethra

g = prepuce

h = urethral orifice

I = distal ductus deferens

J = prostate gland positions

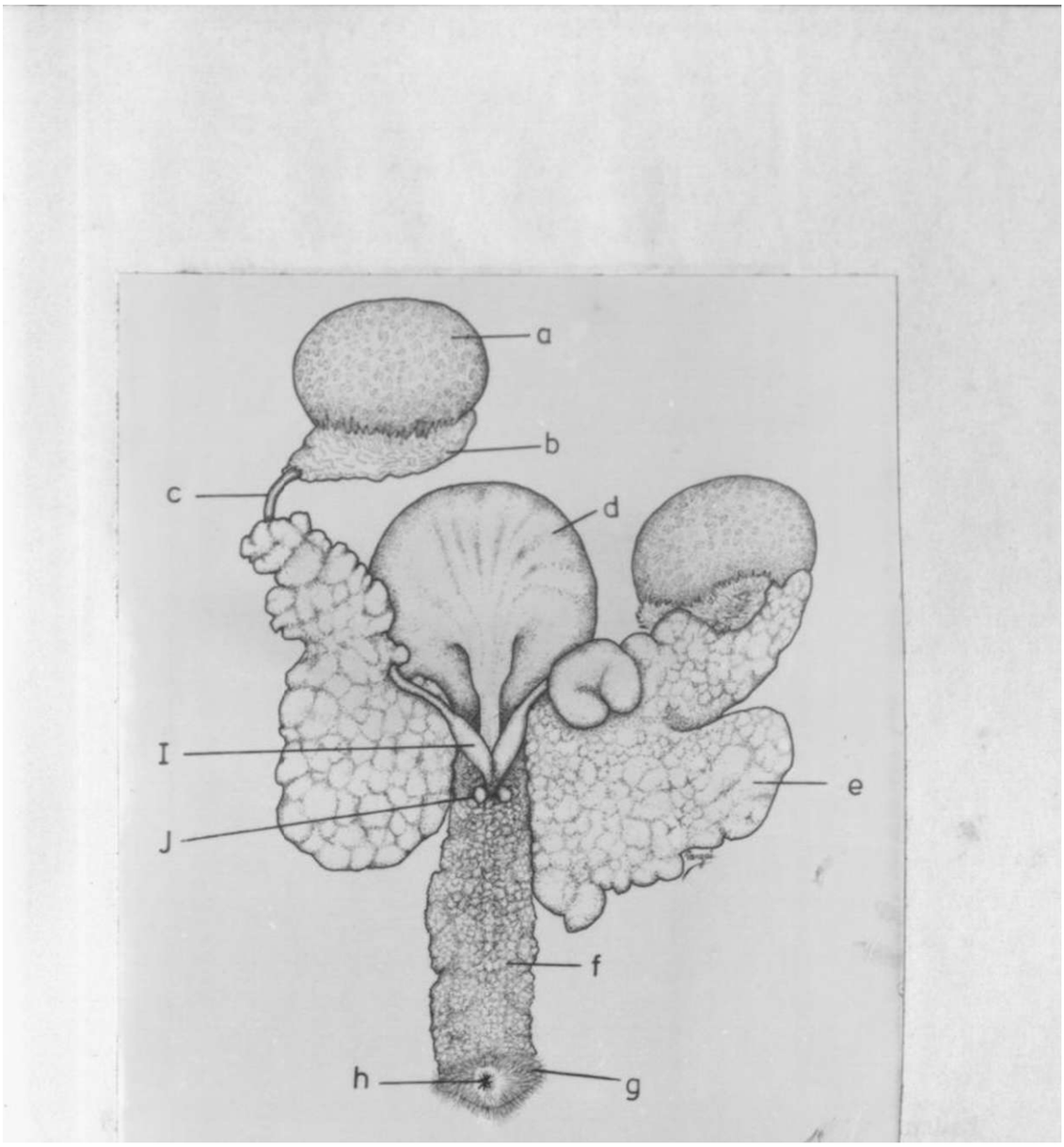


Fig. 2b. A schematic diagram of the male reproductive organs of the naked mole rat (dorsal view).

a = testis

b = epididymis

c = proximal ductus (vas) deferens

d = urinary bladder

e = fatty tissue

f = pelvic urethra

g = prepuce (covers the penis)

h = urethral orifice

I = distal ductus (vas) deferens

J = prostate glands

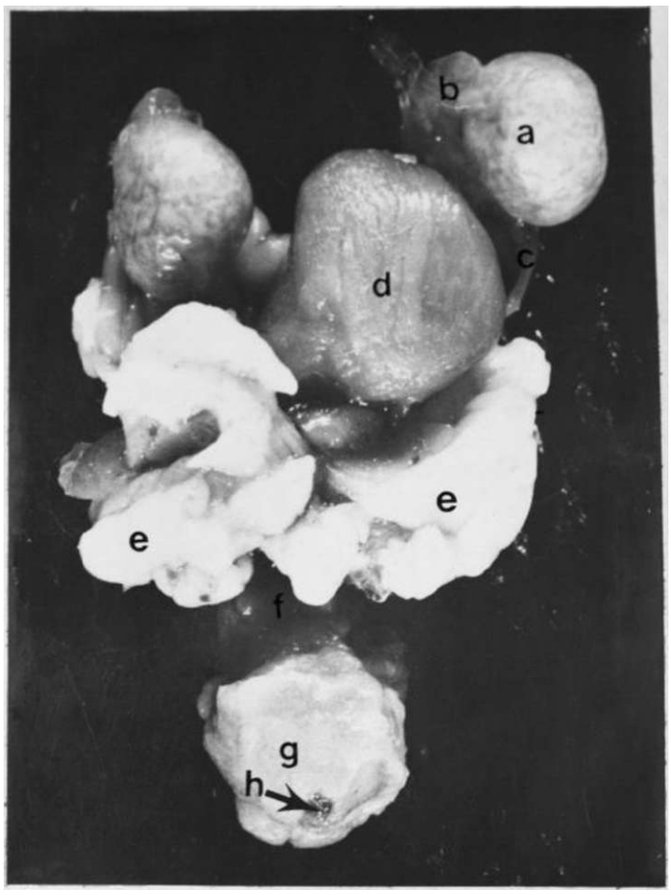


Fig. 2c. A photograph of the male reproductive organs *of* the naked mole rat (ventral view).

a = testis

b = epididymis

c = proximal ductus (vas) deferens

d = urinary bladder

e = fatty tissue

f = pelvic urethra

g = prepuce (covers the penis)

h = urethral orifice

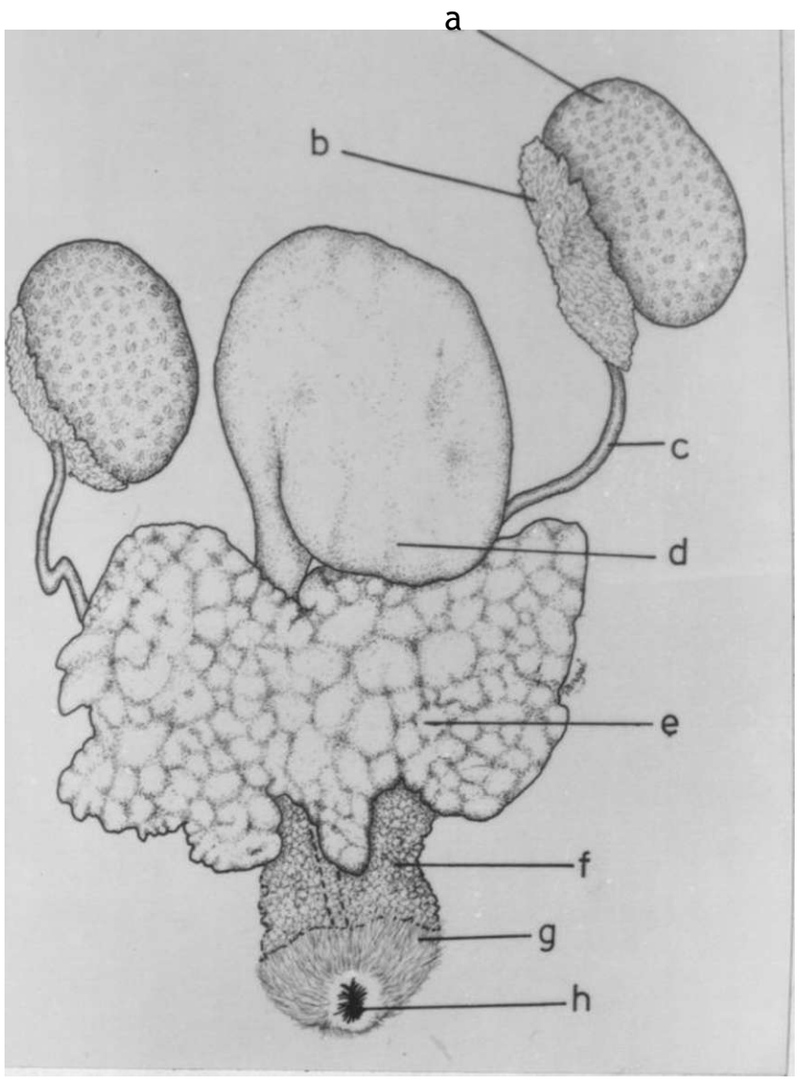


Fig. 2d. A schematic diagram showing the male reproductive organs of the naked mole rat (ventral view).

a = testis

b = epididymis

c = proximal ductus (vas) deferens

d = urinary bladder

e = fatty tissue

f = penile urethra

g = prepuce (covers the penis)

h = urethral orifice

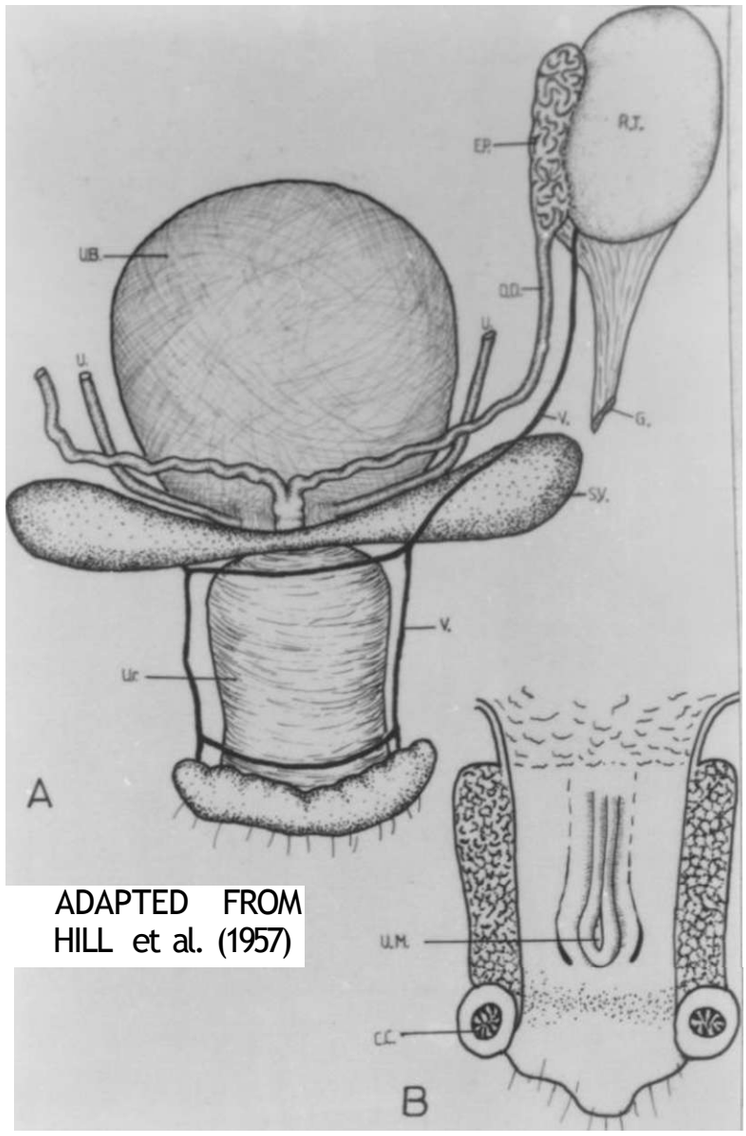


Fig. 2e. A schematic diagram of the male reproductive system of the naked mole rat (Adopted from Hill *et al.,* 1957). Note the presence of seminal vesicle (S.V.).

A (Dorsal view)

RT = Right testis

EP = Epididymis

DD = Ductus deferens

UB = Urinary bladder

V = Venous channels

SV = Seminal vesicle

Ur = urethra

G = Gubernaculum

U = Ureter

B (Interior of urethra)

UM = Utriculus masculinus

CC = Corpus cavernosum

3.2 Microscopic observations

3.2.1 Testes

At light microscopy, the testes were surrounded by a dense collagen fibrous layer, tunica albuginea. This connective tissue layer contained blood vessels and fibroblasts. The testicular parenchyma consisted of a continuous sheet of interstitial cells of Leydig among which were scattered seminiferous tubules (fig. 3), little connective tissue fibres, few capillaries and small lymphatics (fig. 4). The interstitial cells were polyhedral or polyangular in outline with round or oval nuclei displaced towards one pole and in the centre of the nuclei were round densely staining nucleoli. The cytoplasm contained a preponderance of round osmiophillic or pale staining granules (figs. 4 &5) which were at the pole opposite that of the nucleus. At electron microscopy the nucleus appeared irregular in outline surrounded by a double unit membrane. The chromatin material was mainly the euchromatic type which was scattered within the nucleoplasm although areas of marginated condensation were seen along the nuclear membrane (fig. 6). Within the nucleus was a single nucleolus eccentric in position. The cytoplasm around the nucleus and within the nuclear pole had a relatively sparse distribution of organelles except for the abundance of sER but at the opposite pole, the cytoplasm showed presence of numerous round pale staining lipid droplets of varying sizes. Intermingled among the lipid droplets were numerous mitochondria predominantly elongated in form although ovoid and round forms were occasionally seen (figs. 6 & 7). The mitochondria were surrounded by

a double unit membrane with the inner membrane transversely folded to form cristae. The rest of the cytoplasm in this pole as in the nuclear pole was invested by sER (fig. 8). Scattered within the cytoplasm were numerous free ribosomes and microfilaments. The blood capillaries in the interstitial tissue were few and of the continuous type. Their walls possessed more than one endothelial cell joined together by endothelial cell junctions (fig. 9). In addition, few lymphatic capillaries with a single layer of endothelial cells were also evident.

The seminiferous tubules were surrounded by a basement membrane comprising a layer of myoid cells with their nuclei oriented towards the circumference of the tubule and the lumina of the tubules were devoid of spermatozoa (figs. 4 & 10). Resting onto the basement membrane were two populations of cells namely, the spermatogenic cells and the Sertoli (sustentacular) cells. The spermatogenic cells were mainly the spermatogonia and primary spermatocytes even though few secondary spermatocytes, very few spermatids and spermatozoa were occasionally encountered. Two types of spermatogonia were recognized; spermatogonia type A whose position was basal with its long axis parallel to the boundary tissue and had an oval or round nucleus and prominent nucleolus (fig. 10 & 11a) and a type B whose nuclei were perpendicularly oriented to the boundary tissue (figs. 10 & lib). Primary spermatocytes occurred as round cells with round nuclei occupying a large part of the cytoplasm (fig. 12). The chromatin materials in these cells were dispersed with no nucleoli. Occasionally, the

primary spermatocytes showed presence of chromaloin bodies. The cytoplasm was pale staining and restricted to a narrow band around the nucleus with sparse distribution of organelles (fig. 12). Secondary spermatocytes, few in number, together with early forms of spermatids showing presence of acrosome and occasional spermatozoa were seen towards the apical surface of some Sertoli cells (figs. 13 & 14). All these spermatogenic cells were enclosed in recesses delineated with branches of the Sertoli cells (see figs. 11a, 11b, 11c & 12). The Sertoli cells appeared as tall, irregular columnar cells arising from the basement membrane to the lumen of the tubule. Ultrastructurally, these cells had irregular nuclei with a prominent nucleolus (figs, 11b & 15). The cytoplasm showed presence of elongated mitochondria oriented perpendicular to the basement membrane. Strands of ER were also visible within the basal cytoplasm. There were also round osmiophillic structures resembling lipid droplets (fig. 15). In some cases, well developed sER profiles were found in close association with secondary spermatocytes (fig. 16).

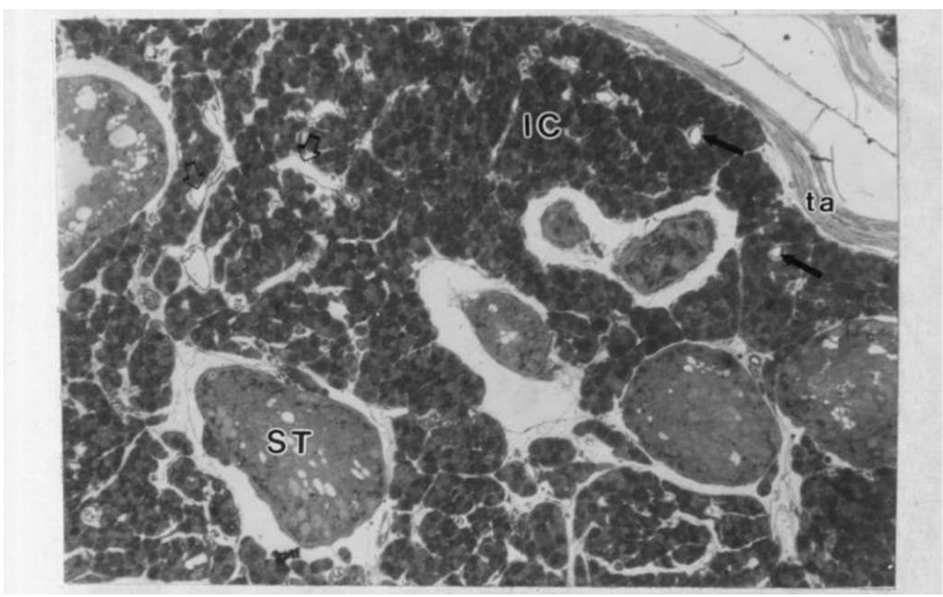


Fig. 3. A photomicrograph of testis showing a tough tunica albuginea (ta) surrounding an expansive volume of interstitial cells of Leydig (IC) among which are few scattered seminiferous tubules (ST). Note the presence of few blood capillaries (dark arrows) and lymphatics (open arrows) within the interstitial tissue.

x 250 (Toluidine blue).

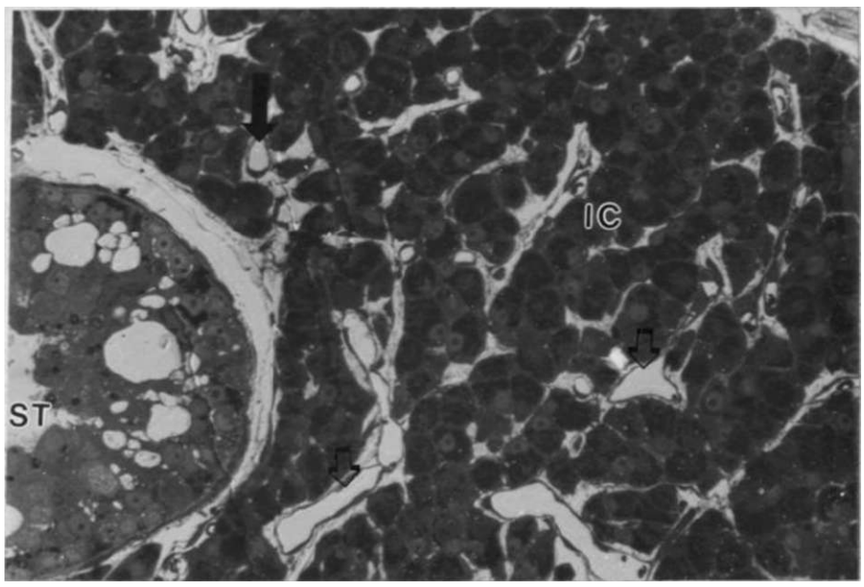


Fig. 4. A photomicrograph showing a massive volume of interstitial cells of Leydig (IC) containing lipid droplets. Blood capillaries (dark arrow) and lymphatics (open arrows) are also present. Note that the seminiferous tubule (ST) is devoid of spermatozoa.

x 400 (Toluidine blue)

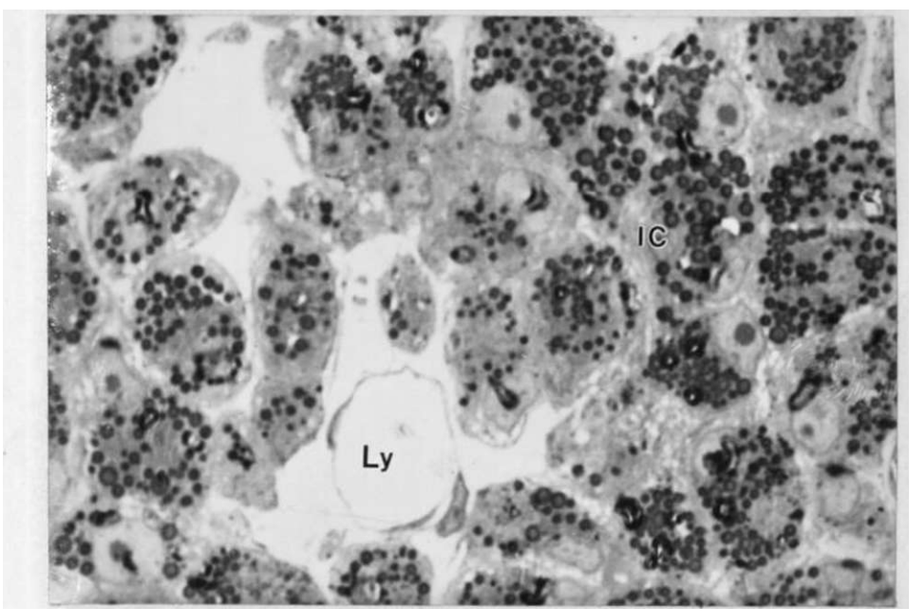


Fig. 5. A higher magnification of interstitial cells of Leydig (IC) displaying the presence of numerous osmiophilic granules (lipid droplets) within the cytoplasm. Note the presence of a lymphatic capillary (Ly.) within the tissue.

x 1,000 (Toluidine blue)

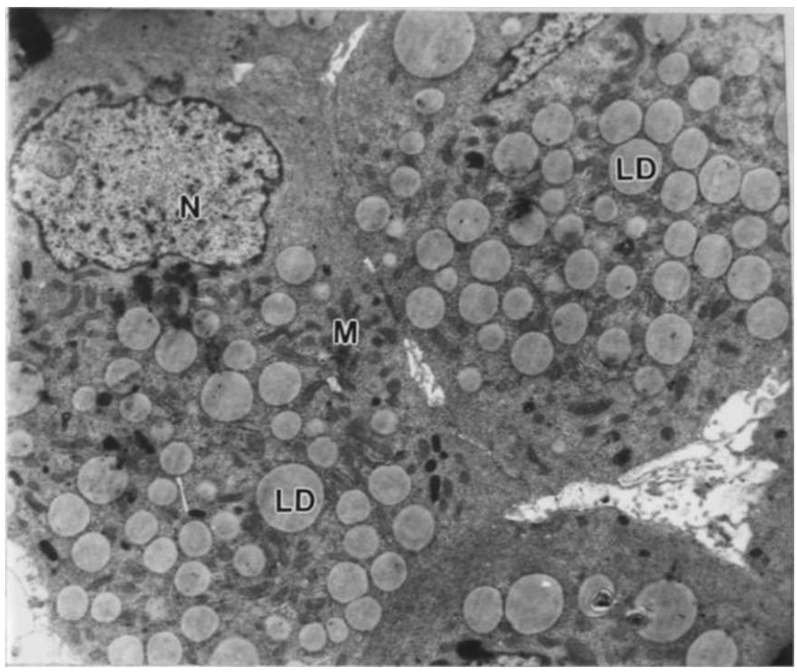


Fig. 6. An electron micrograph of interstitial cells of Leydig with irregularly outlined nucleus (N) having mainly euchromatin. Numerous lipid droplets (LD) and mainly elongate mitochondria (M) can also be seen in the cytoplasm.

x 2,800

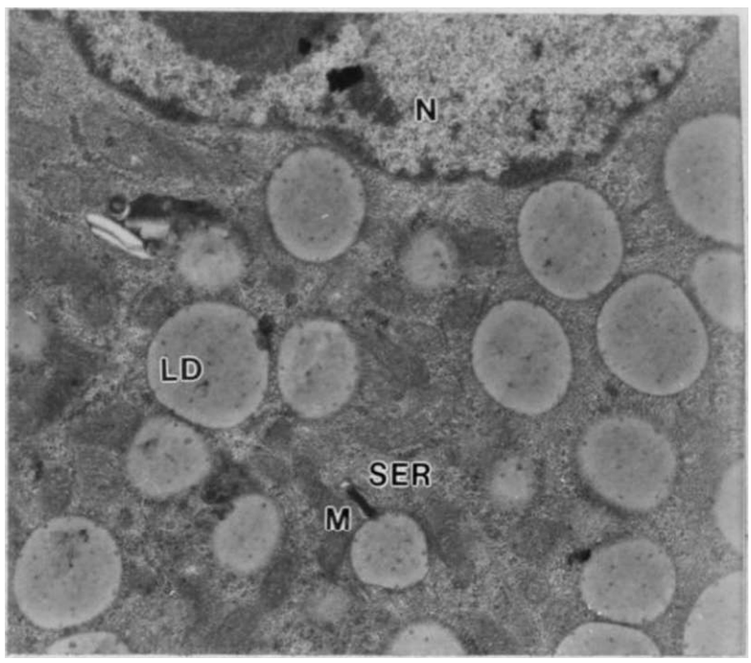


Fig. 7. An electron micrograph showing part of the nucleus (N) and cytoplasm of an interstitial cell. Note the presence of marginated heterochromatin in the nucleus. Lipid droplets (LD), elongate mitochondria (M) and smooth endoplasmic reticulum (SER) are abundant in the cytoplasm.

x 3,900

Fig. 8. Cytoplasm of an interstitial cell of Leydig showing a vast network of smooth endoplasmic reticulum (SER). Various sizes of lipid droplets (LD) occur within the cytoplasm.

x 4,200

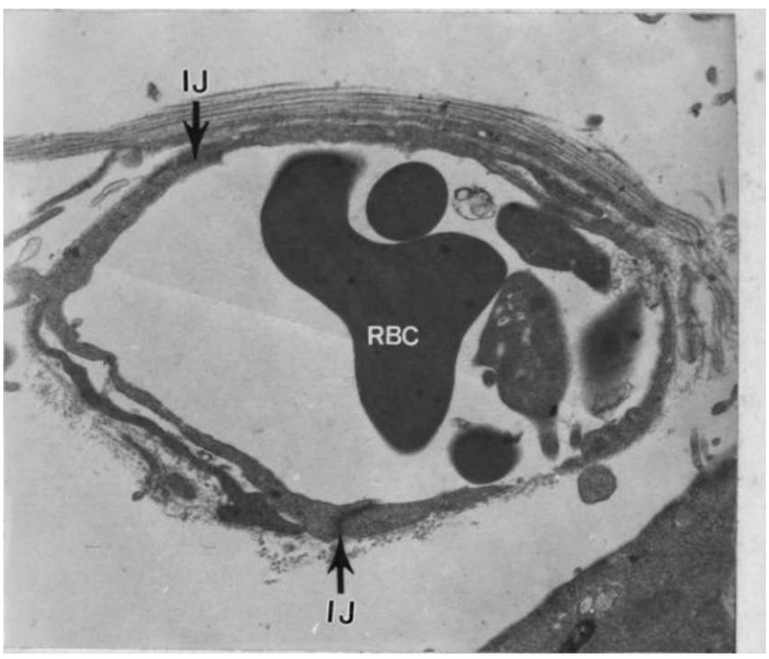


Fig. 9. An electron micrograph of testicular blood capillary. Note the presence of red blood cells (RBC) in the lumen and tight interstitial junctions (IJ) on the capillary wall characteristic of testicular capillaries.

x 2,950

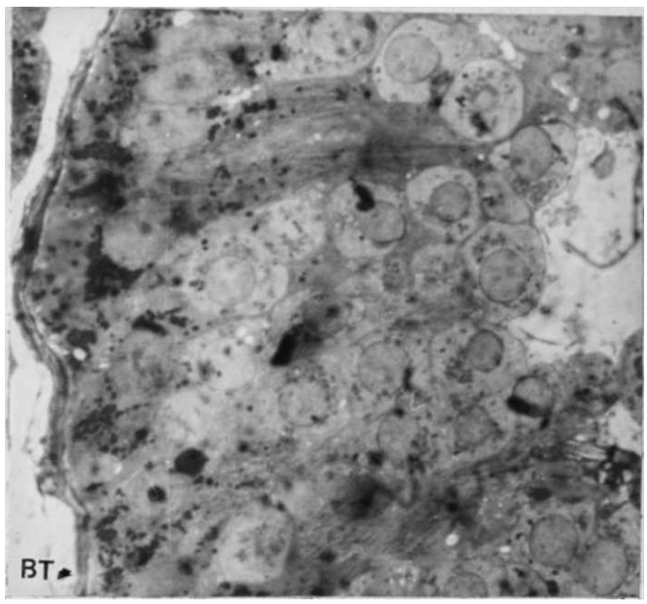


Fig. 10. A photomicrograph of the seminiferous tubule with its epithelial cells resting onto the boundary tissue (BT). Note the absence of spermatozoa in the lumen.

x 1,000 (Toluidine blue).

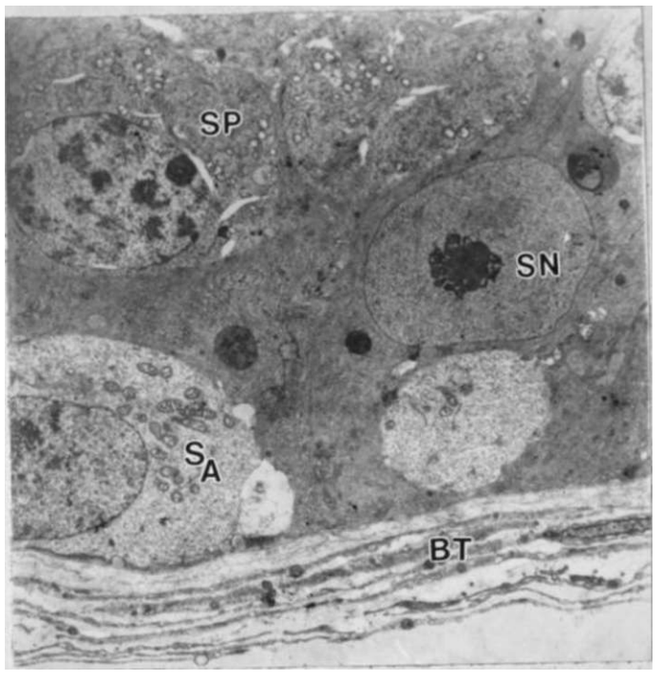


Fig. 11a. An electron micrograph of a part of seminiferous tubule showing spermatogonium type A (**SA**) with its long axis parallel to the boundary tissue (BT), Sertoli cell nucleus (SN) and primary spermatocyte **(SP).**

x 1,800

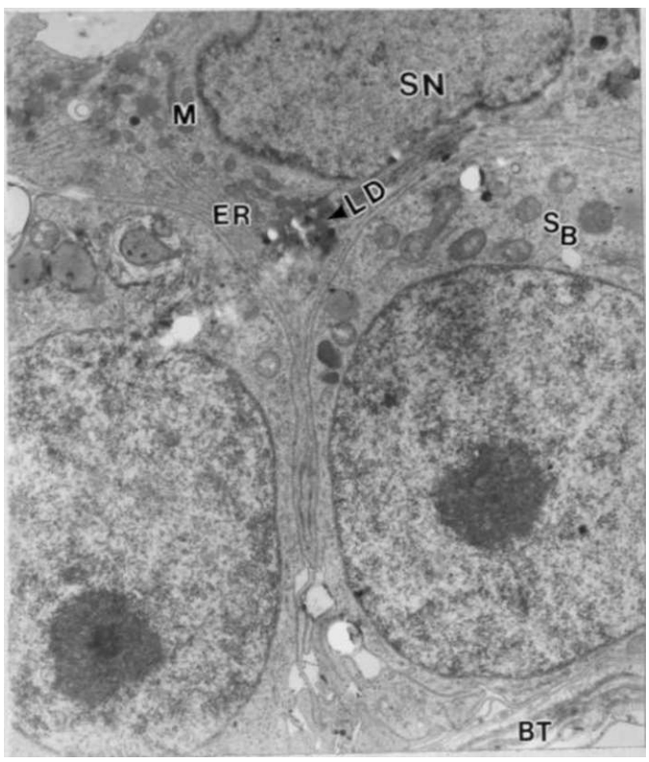


Fig. 11b. An electron micrograph showing spermatogonia type B **(SB)** with their long axis perpendicular to the boundary tissue (BT), Sertoli cell with its irregularly outlined nucleus (SN). Mitochondria (M), endoplasmic reticulum (ER) and lipid droplets (LD) can also be seen in the basal cytoplasm.

x 1,850

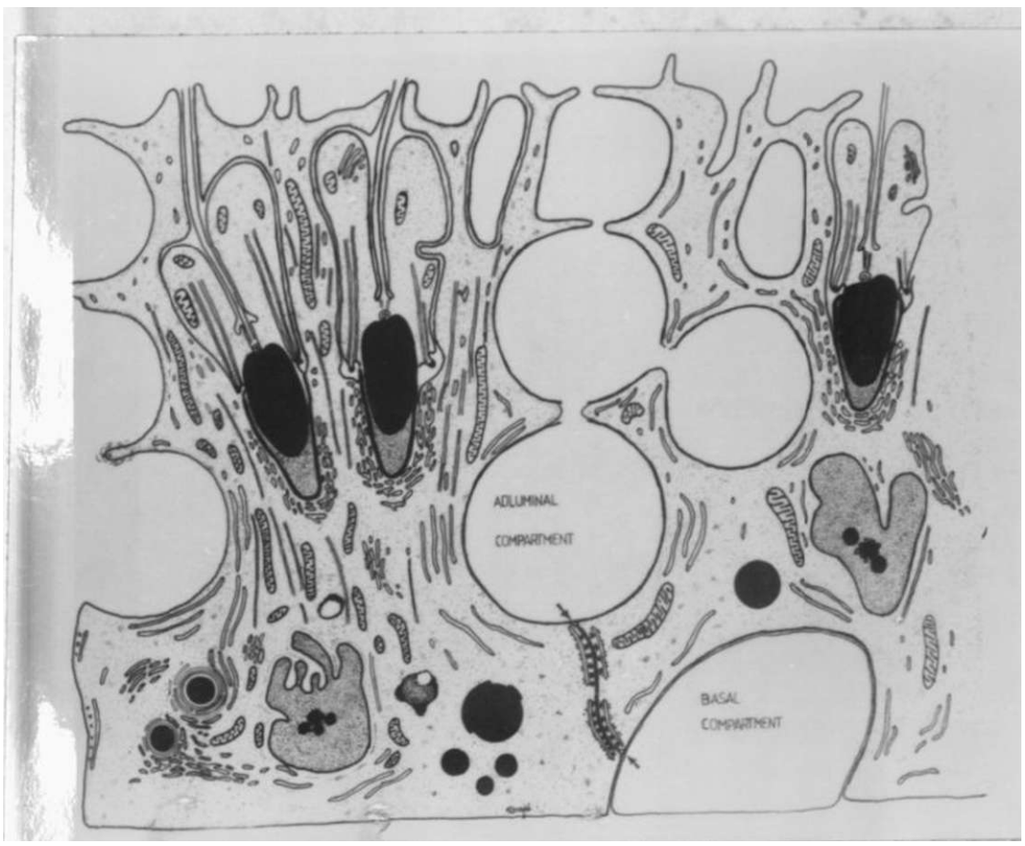


Fig. 11c. A schematic illustration of the supportive function of Sertoli cells and the presence of tight Sertoli-Sertoli cell junctions (arrows) resulting into the compartmentalization of the tubule into adluminal and basal compartments (Adopted from Fawcett, 1986).

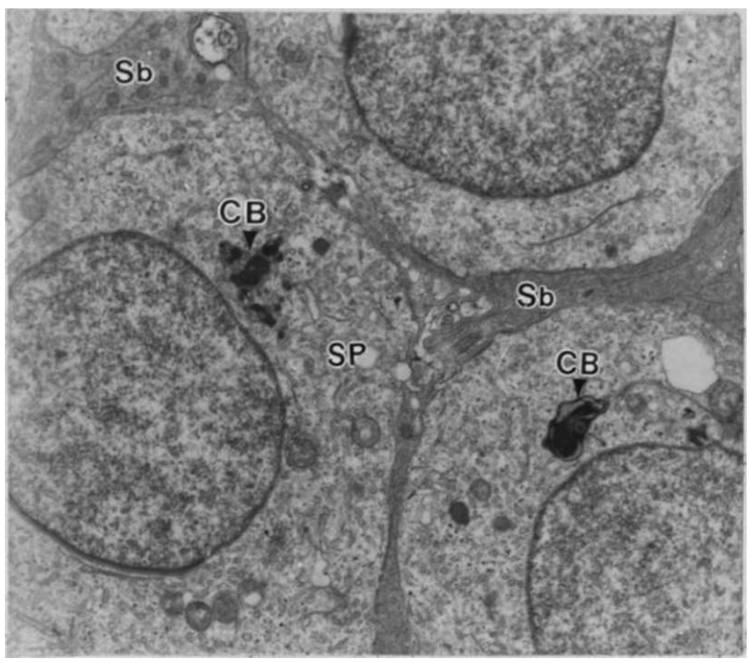


Fig. 12. An electron micrograph showing primary spermatocytes (SP) held in recesses formed by Sertoli cell branches (Sb). Note the presence of chromatoin bodies (CB) within the primary spermatocytes.

x 1,850

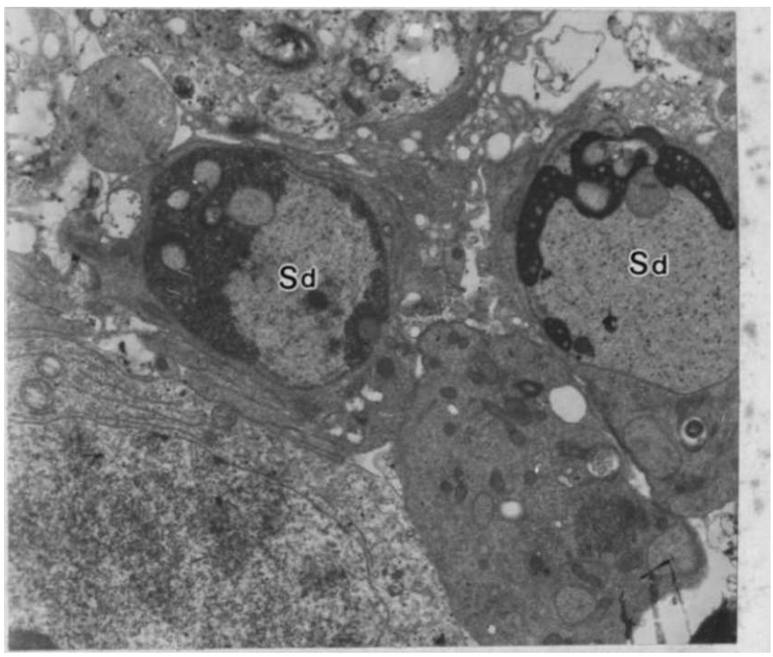


Fig. 13. An electron micrograph showing two spermatids (Sd) in the acrosomal phase of development within the seminiferous epithelium.

x 1,850

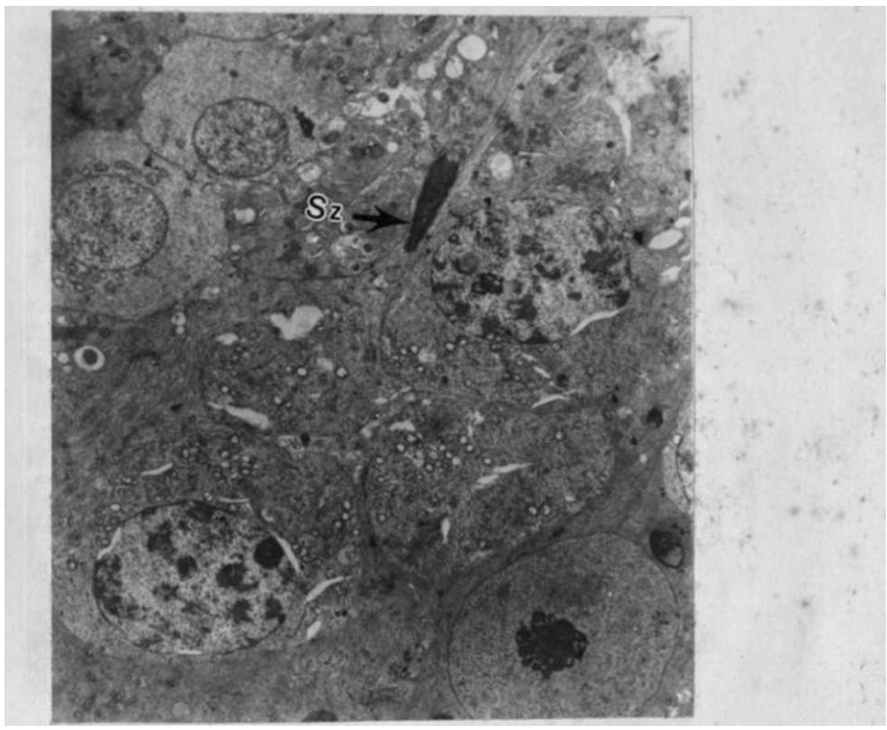


Fig. 14. An electron micrograph showing one spermatozoon (Sz) within the seminiferous epithelium.

x 4,200

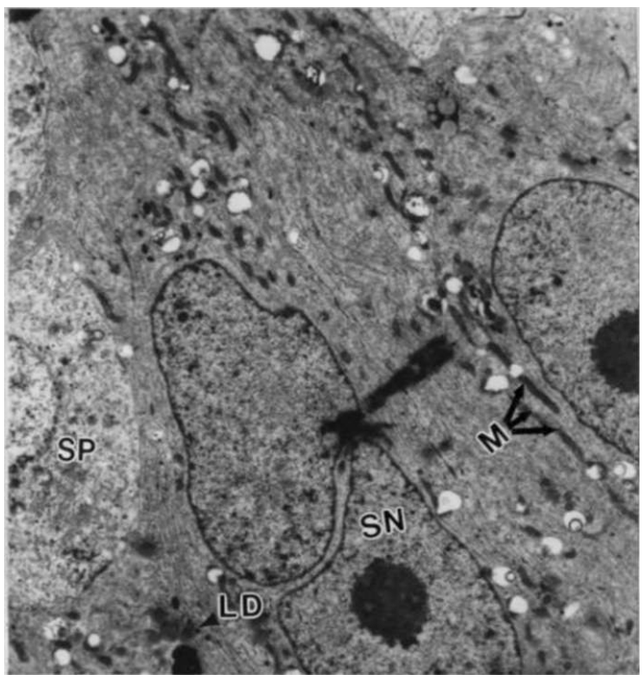


Fig. 15. An electron micrograph of a Sertoli cell with an irregular nucleus (SN) showing perpendicularly oriented mitochondria (M) and lipid droplets (LD). Note the presence of primary spermatocytes (SP) within the lateral recesses.

x 2,900

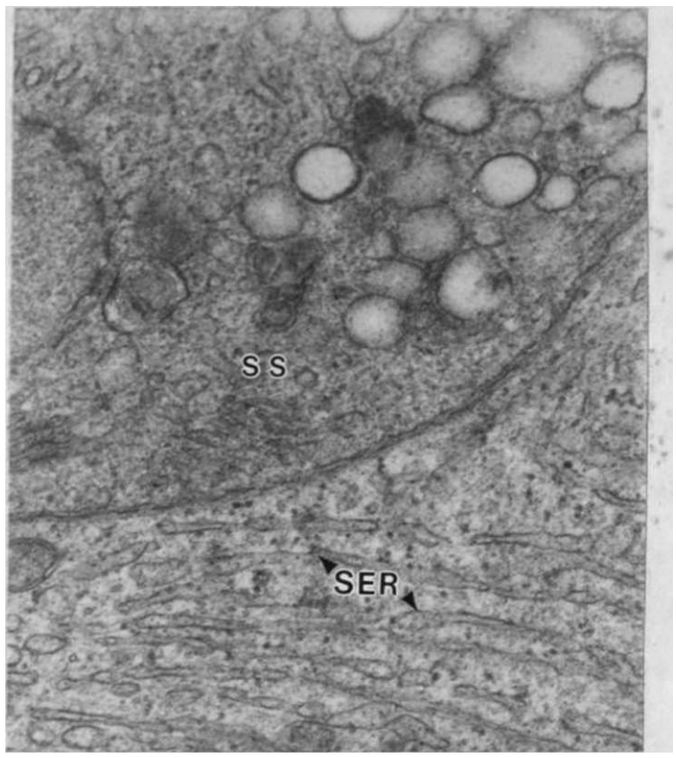


Fig. 16. An electron micrograph showing a secondary spermatocyte (SS) surrounded by profiles of smooth endoplasmic reticulum (SER) from Sertoli cell cytoplasm.

x 10,500

3.2.2 Excurrent durfs

3.2.2.1 Ductuli effprpntps

i

Anteriorly and on the medial surface of the testis was a part of the excurrent duct system whose histology revealed the presence of tubular cross-sections held in position by a fold of connective tissue representing a detachment from the peritoneum (fig. 17). These tubules represented the **ductuli efferentes.** The epithelia of these tubules were mainly made up of two groups of cells: the principal cells which were the predominant cells with large and irregular nuclei containing one nucleolus which was either central or eccentric in location and had microvilli on the luminal surface (figs. 18 & 19) and the ciliated cells easily identified by the presence of long cilia projecting into the lumen (figs. 18 & 21). Principal cells were seen to arise from a narrow basement membrane surrounded by a layer of smooth muscle fibres into which blood vessels penetrate to supply the organ. The apical cytoplasm of these principal cells showed an occasional occurrence of apical granules and unusually long mitochondria (fig. 20). Adjacent principal cells were held together by junctional complexes (see fig. 20). The lumina of the tubules were narrow and devoid of spermatozoa.

3.2.2.2 Ductus epididymis

Further posteriorly, the **initial, middle** and **terminal segments** of the epididymis were recognized by the height of the principal cells, diameter of the lumen and the height of microvilli. In the **initial segment,** the height of the principal cells increased,

there was cessation of the ciliated cells and instead apical cells and in some cases halo cells or lymphocytes became evident (figs. 22, 23 & 24). The microvilli also increased in height becoming more prominent than in the ductuli efferentes. No vacuoles and vesicles were observed in the cytoplasm of principal cells and the lumen of the tubule was devoid of spermatozoa except for a few cellular debris (figs. 22 & 23). The lumen became wider with continued sectioning accompanied by a slight reduction in the epithelial cell height. The predominant cells were still the principal cells although basal cells were also seen (figs. 25 & 26). This marked the anterior end of the **middle segment.** Further posteriorly, the duct lumen became enlarged, principal cell heights reduced slightly and the lumen contained numerous cellular debris (figs. 27 & 28). The epithelial cells in this segment showed no cytoplasmic modifications. In the terminal **segment,** there was no appreciable reduction in epithelial cell height but the diameter of the lumen increased and the microvilli formed short brush-like projections in the lumen (figs. 29 & 30). The epithelium consisted mainly of principal cells although basal cells were also seen. The principal cell cytoplasm was characterized by presence of vacuoles, prominent GA and strands of rER in the apical cytoplasm (fig. 31). Basally, the cytoplasm showed presence of numerous basal granules (fig. 32). Blood vessels were seen penetrating the intertubular connective tissue. The lumina of the tubules contained secretory material and cellular debris (see fig. 29).

3.2.2.3 Ductus deferens

Continuing from the terminal portion of the cauda epididymis was the ductus deferens characterized by a straight proximal part having a greatly reduced epithelial height and short microvilli (figs. 33 & 34). Apical vacuoles and basal granules persisted as in the terminal segment. Distally, there was a dramatic increase in the height of epithelium, emergence of clear cells and an increase in the thickness of muscular coat around the epithelium (figs. 35, 36 & 37). The lumen was devoid of spermatozoa as in other parts of excurrent ducts. The epithelial cells were mainly the principal cells with elongate or round nuclei containing one or two nucleoli. The cytoplasm of these cells showed presence of numerous vacuoles within the cytoplasm and a bleb-like apical evagination on the apical surface (figs. 35 & 38). The two ductuli deferentes became joined together by connective tissue and acquired a third duct called the utriculus masculinus. On either side of these ducts were the prostate gland follicles (fig. 39).

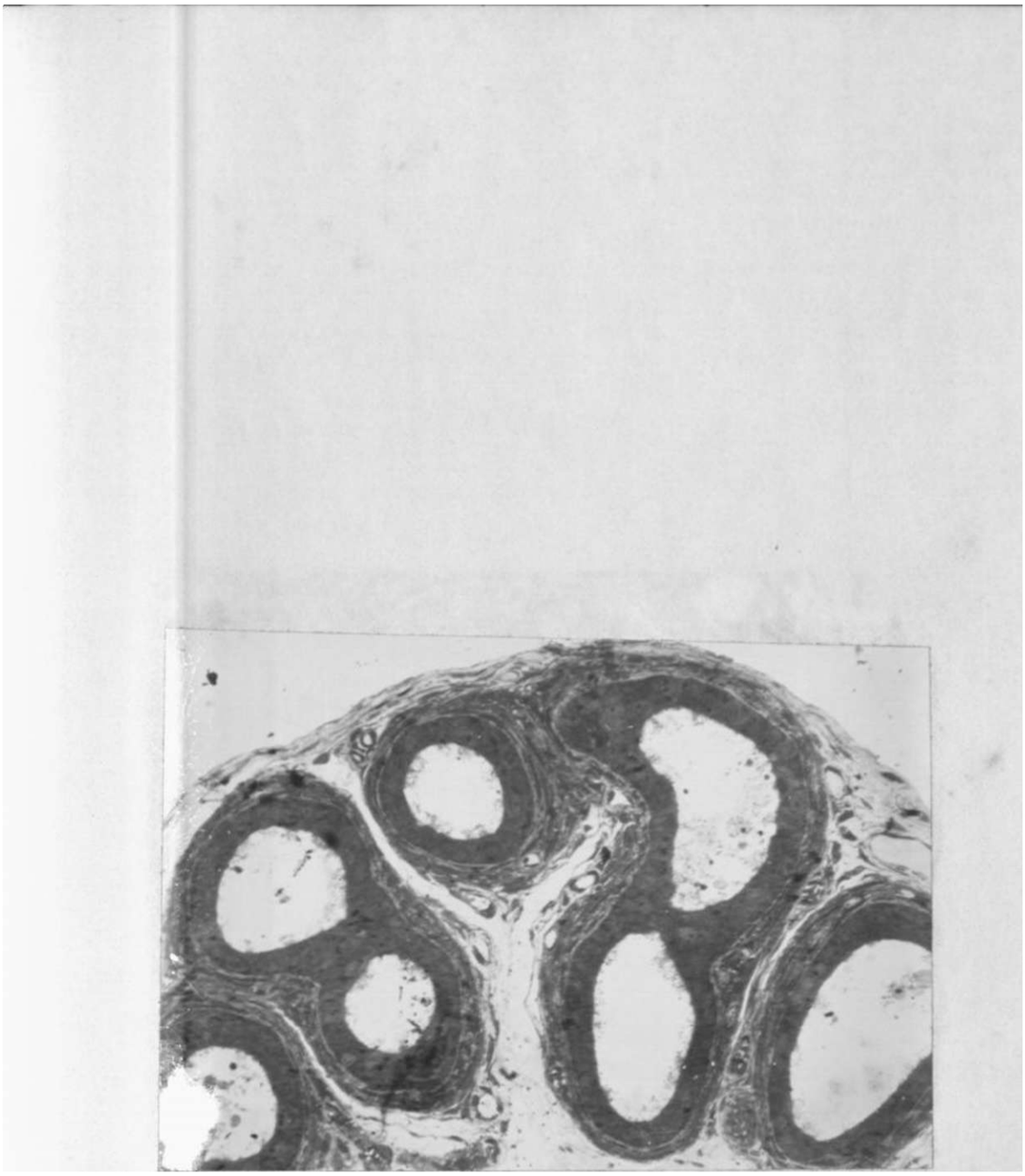


Fig. 17. A photomicrograph of ductuli efferentes showing several tubular cross-sectional profiles.

x 250 (Toluidine blue)

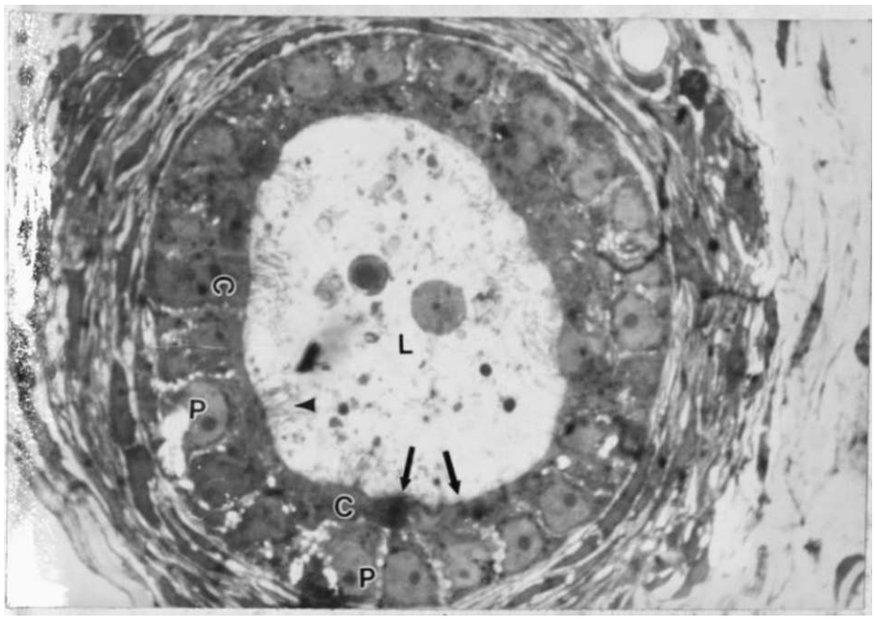


Fig. 18. A high power magnification of ductus efferentes. Two cell types are evident namely the ciliated cells (C) with their long cilia (arrow head) and principal cells (P) having short microvilli (long arrows). The lumen (L) of the duct contain only a few cellular debri.

x 1,000 (Toluidine blue).

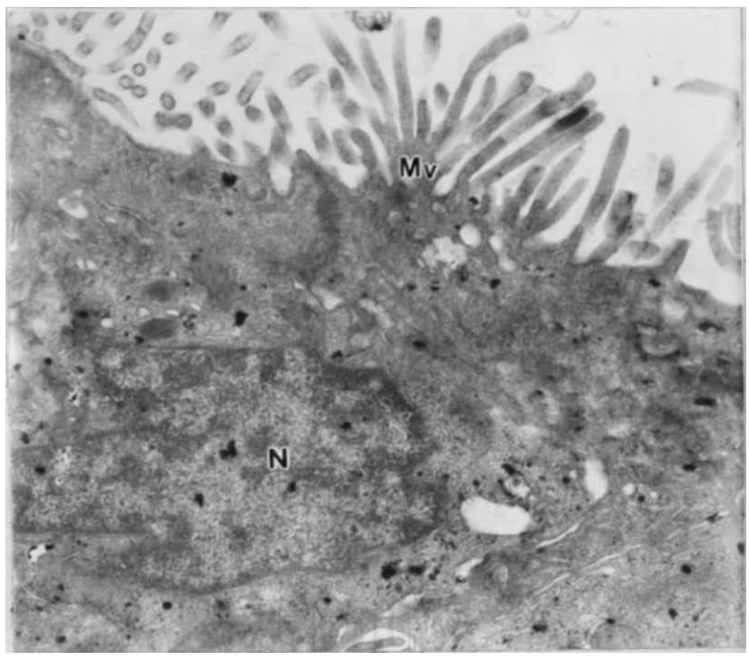


Fig. 19. An electron micrograph of a part of the principal cell of the ductuli efferentes. Note the presence of irregularly outlined nucleus (N) and branched microvilli (Mv) - also referred to as stereocilia.

x 5,000

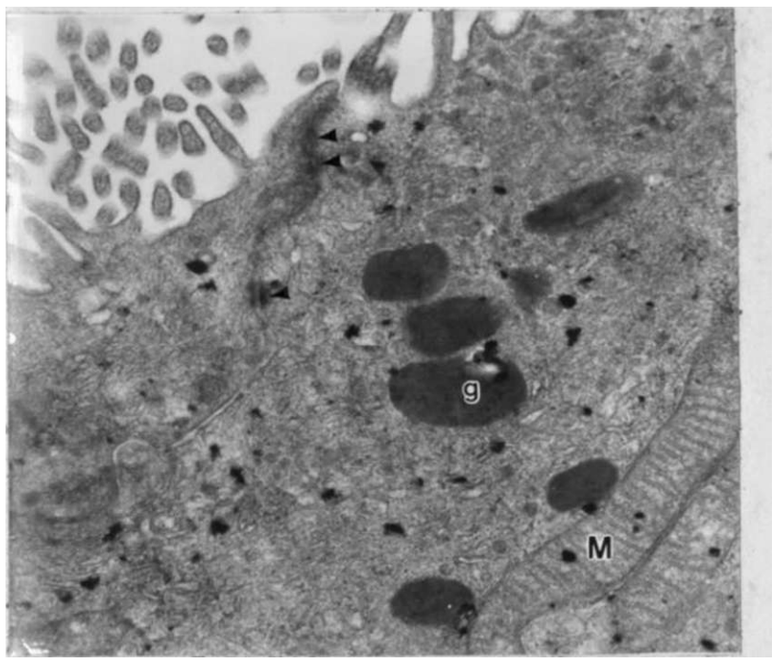


Fig. 20. Part of principal cell of the ductuli efferentes showing an unusually long mitochondrion (M), apical granules (g) and junctional complexes between adjacent principal cells (arrow heads).

x 6,000

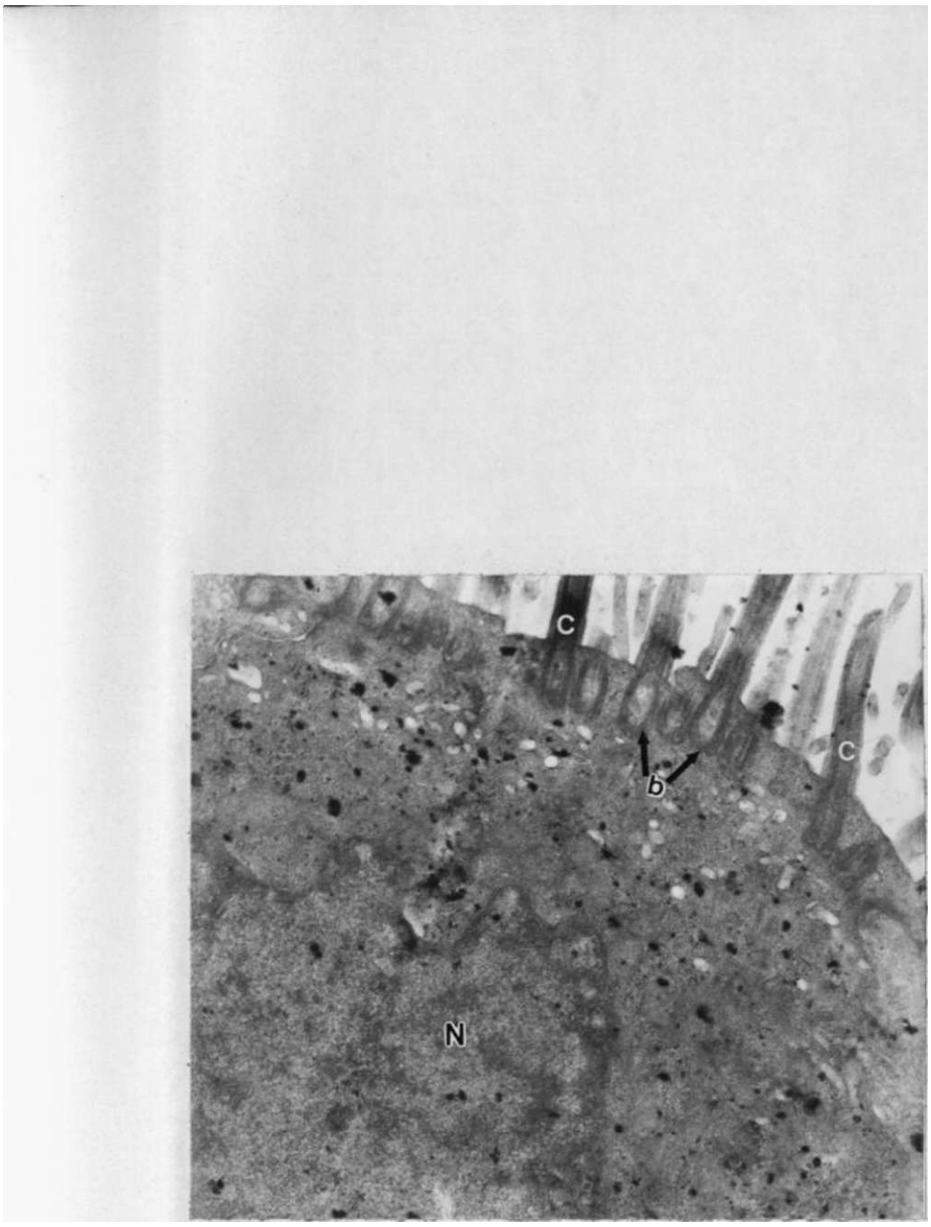


Fig. 21. An electron micrograph of the apical part of ciliated cell showing nucleus (N) and cilia (C) with their basal bodies (b).

x 5,000

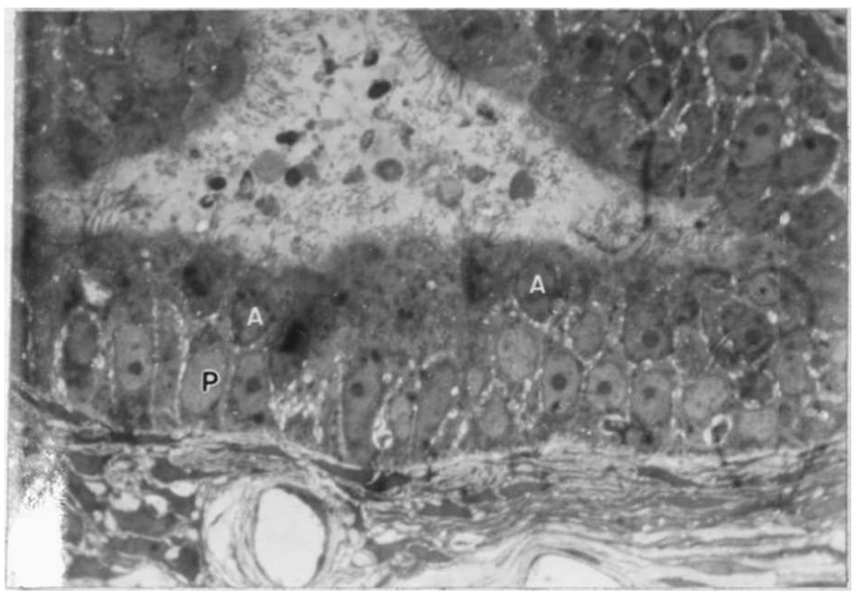


Fig. 22. An initial segment. Notice the obvious increase in the height of principal cells (P) compared to those of fig. 18, presence of apical cells (A) and the long microvilli.

x 1,000 (Toluidine blue).

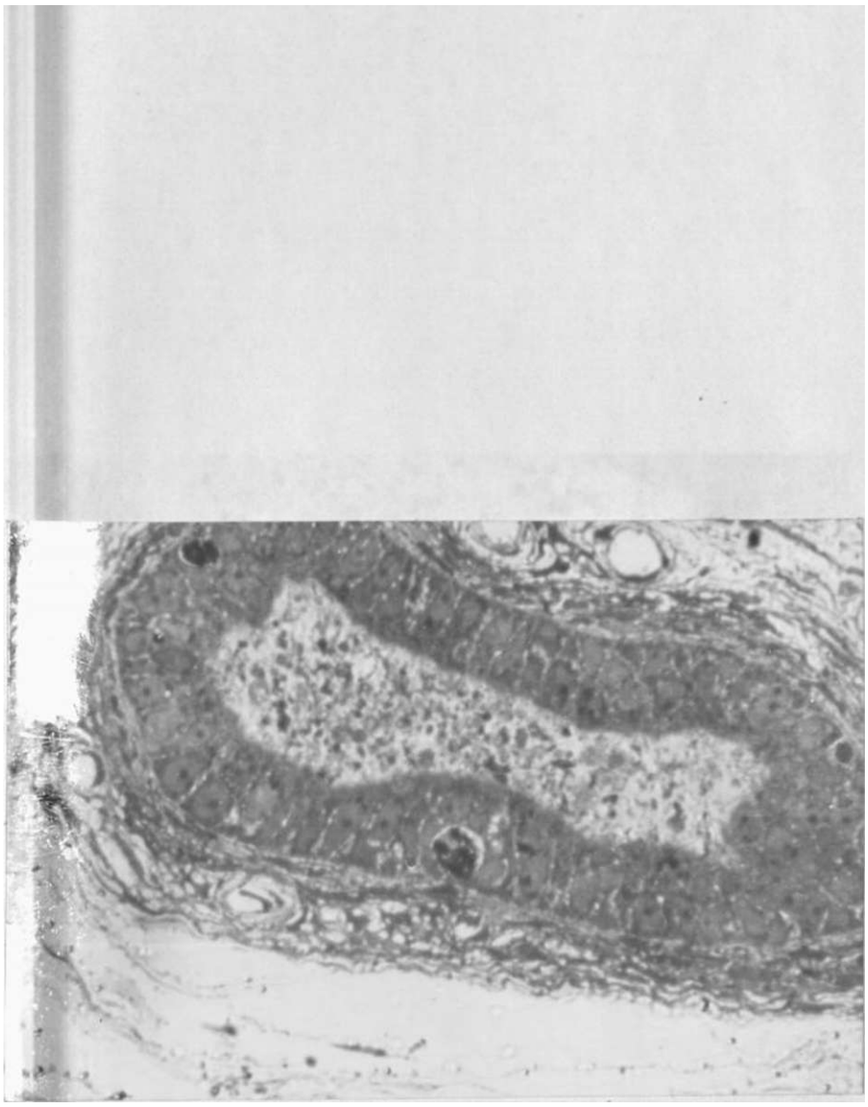


Fig. 23. An initial segment showing lymphocytes within the epithelium.

x 400 (Toluidine blue).

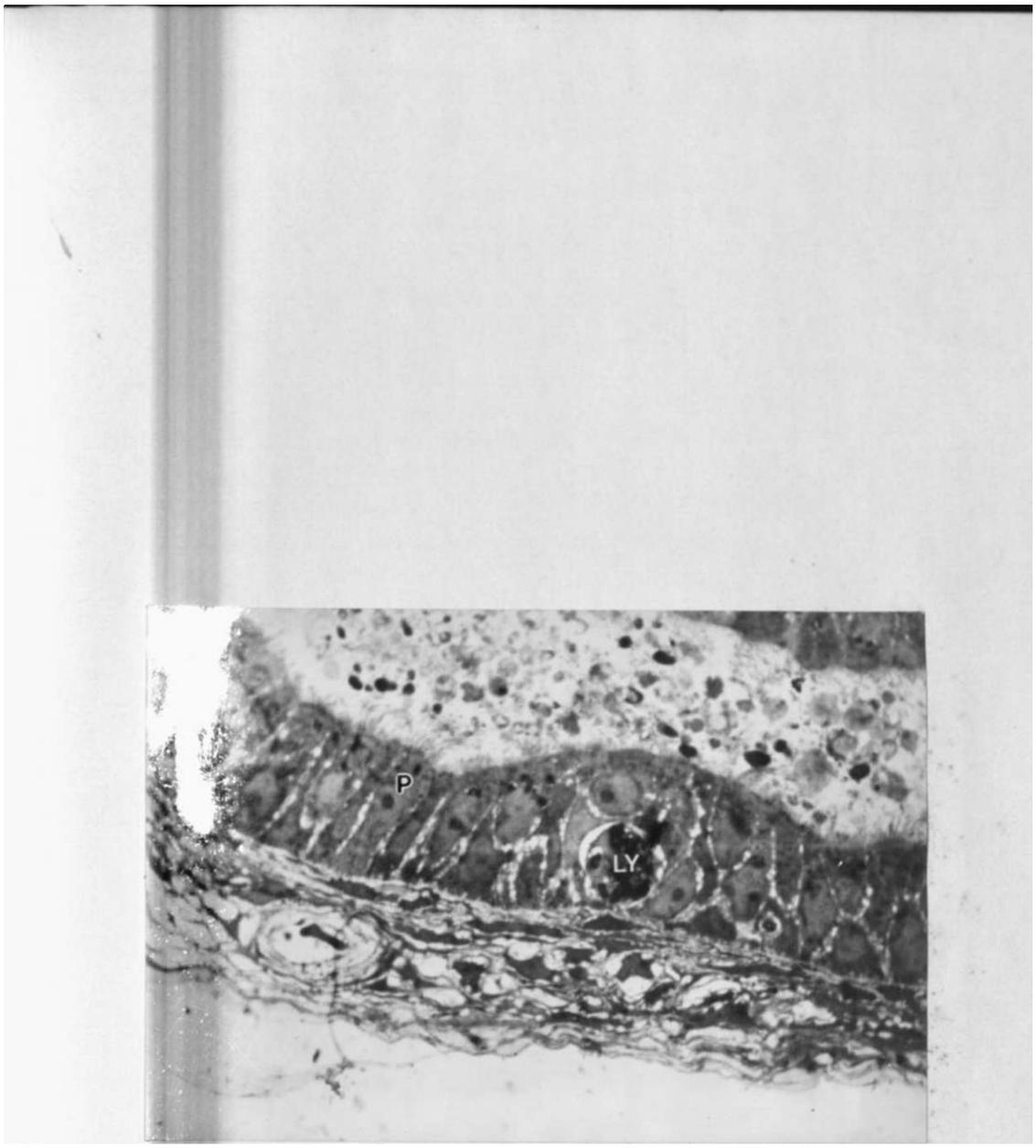


Fig. 24. A higher magnification of part of the initial segment showing the lymphocyte (LY) and the principal cells (P). Note the presence of cellular debri in the lumen.

x 1,000 (Toluidine blue).

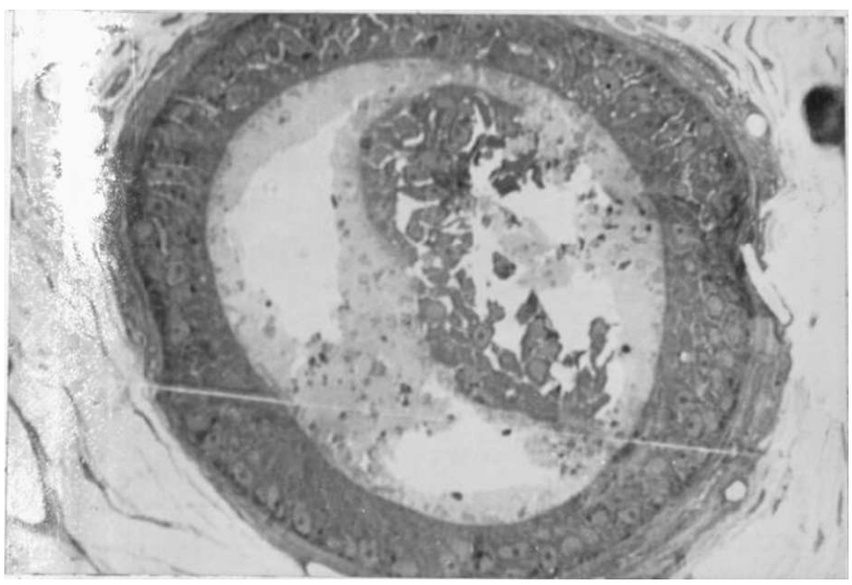


Fig. 25. The beginning of middle segment (at the caput flexure). The lumen contain no spermatozoa except the epithelial artefacts.

x 400 (Toluidine blue)

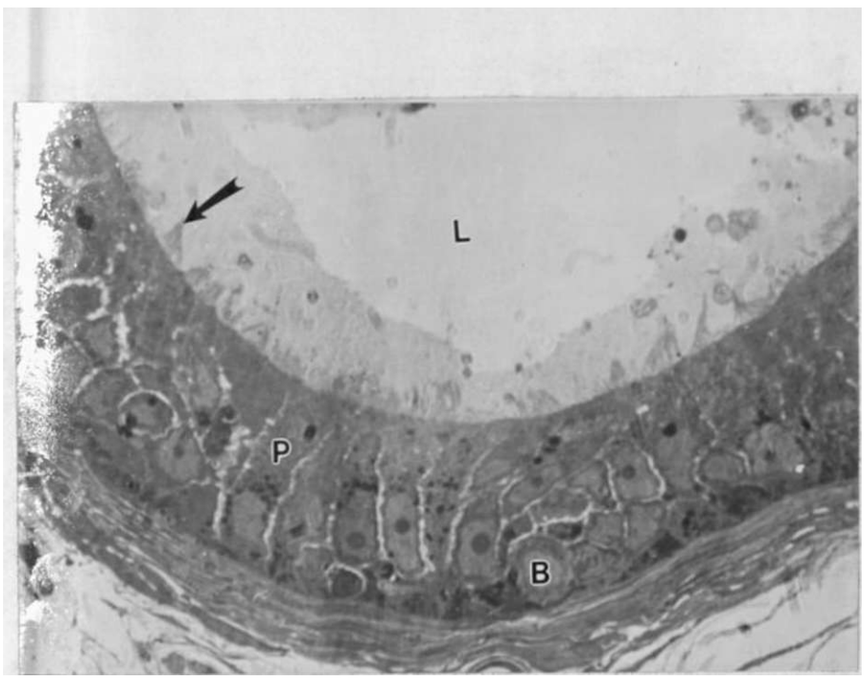


Fig. 26. A higher magnification of part of the middle segment (fig. 25) showing the principal cells (P) and basal cells (13). Note the clumping together of microvilli to form long strands of discrete groups (arrow). The lumen (L) has no spermatozoa.

x 1,000 (Toluidine blue).

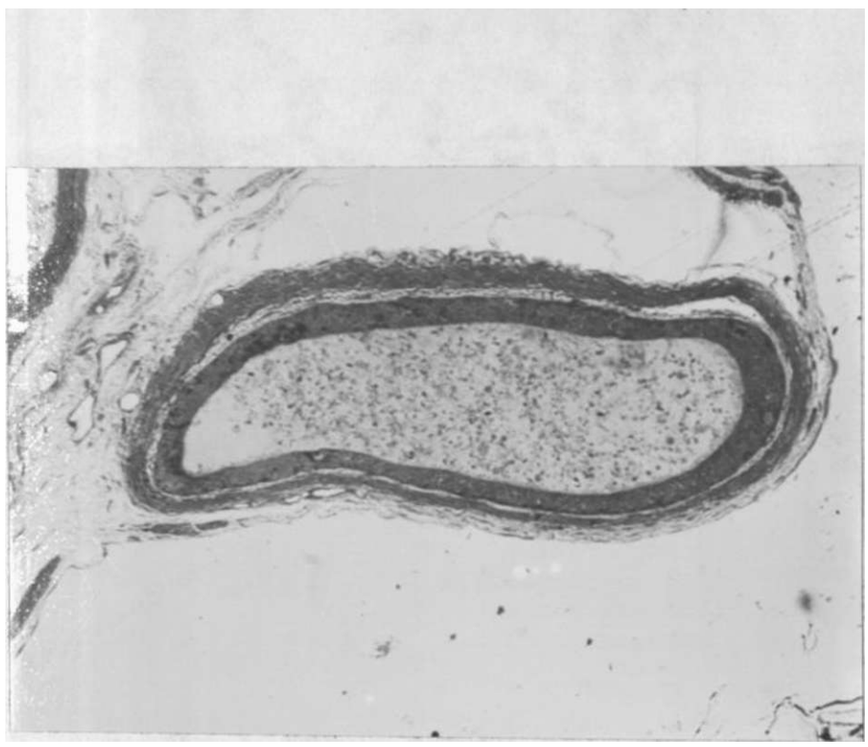


Fig. 27. Continuation of the middle segment. Note the increased luminal diameter with a slight reduction in epithelial height. There are cellular debri within the lumen.

x 250 (Toluidine blue).

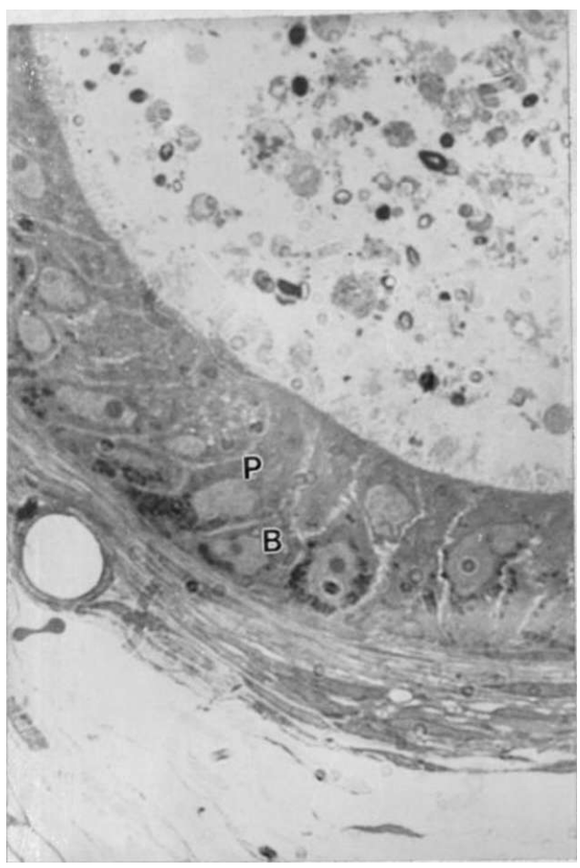


Fig. 28. A higher magnification of part of fig. 27 showing principal (P) and basal (B) cells.

x 1,000 (Toluidine blue).

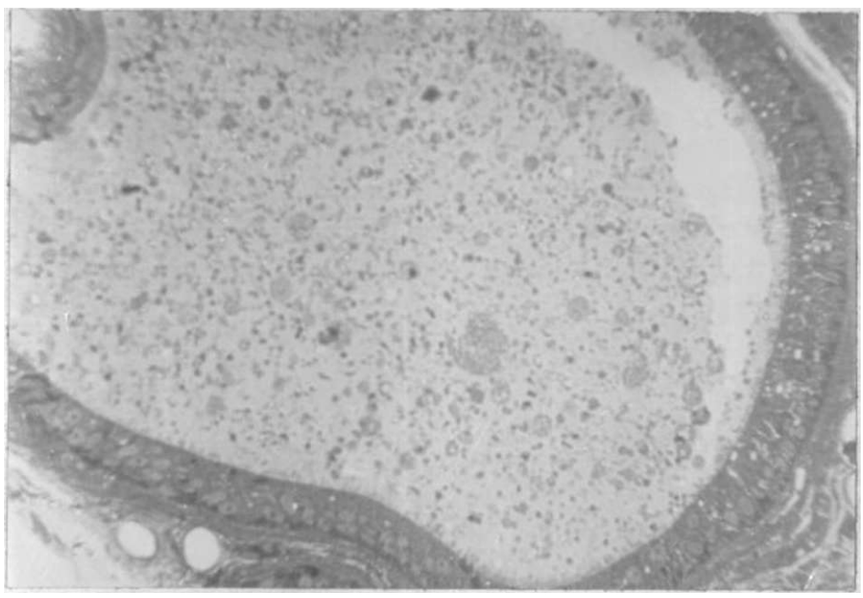


Fig. 29. The terminal segment. Note the presence of cellular debri and secretory material within the lumen.

x 250 (Toluidine blue).

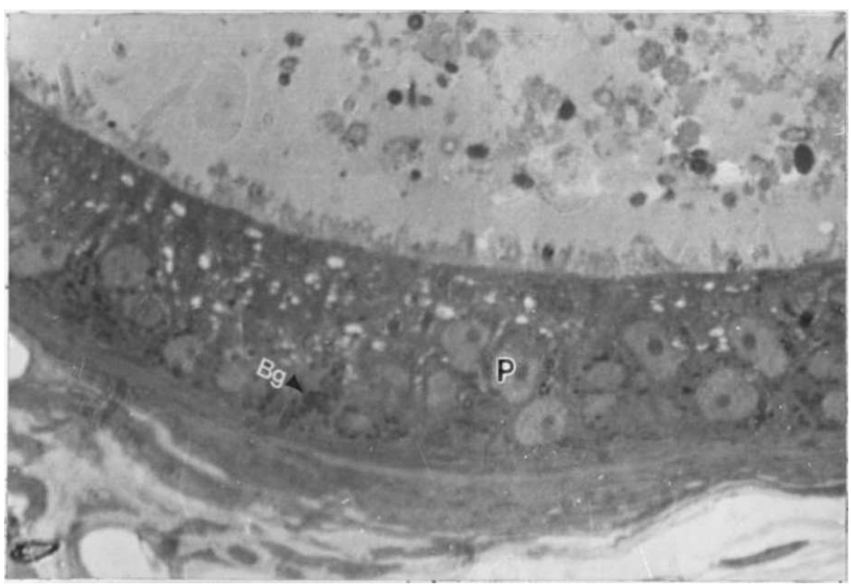


Fig. 30. A higher magnification of part of the terminal segment (fig. 29) showing principal cells (P) with several apical vacuoles and basal granules (Bg).

x 1,000 (Toluidine blue).

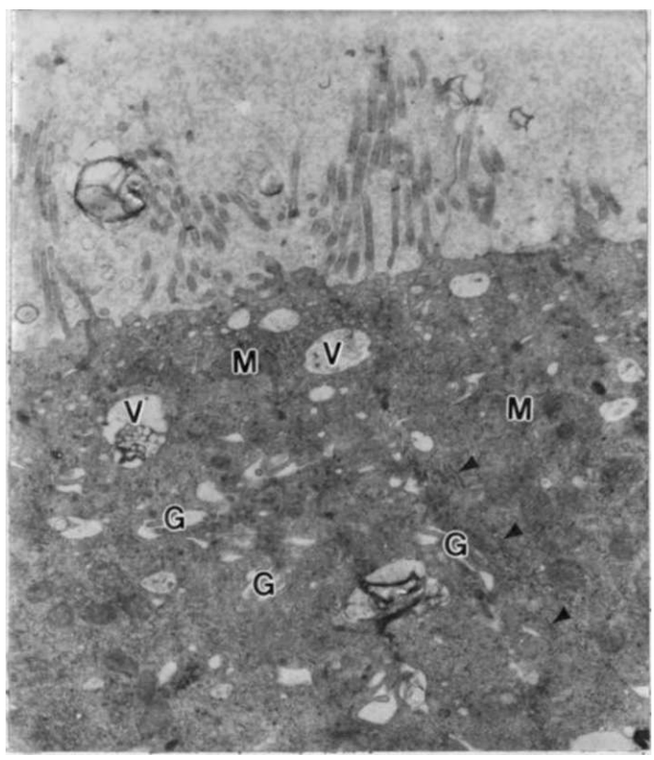


Fig. 31. An electron micrograph of the apical cytoplasm of the principal cell of the terminal segment showing several apical vacuoles (V), Golgi apparatus (G), mitochondria (M) and short strands of rough endoplasmic reticulum (arrow heads). Note the presence of microvilli on the apical surface.

x 2,950

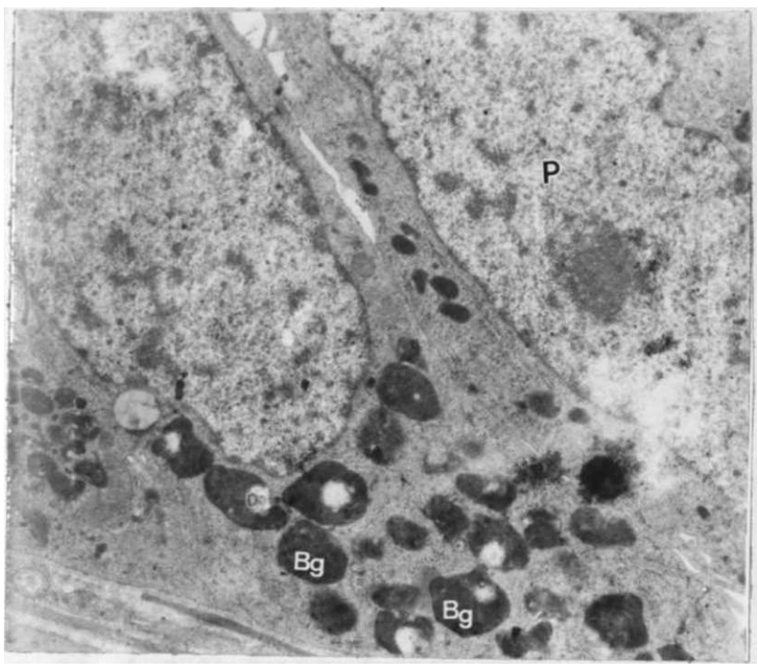


Fig. 32. An electron micrograph of the basal cytoplasm of the principal cell (P) of the terminal segment. Note the presence of the basal granules (Bg).

x 2,950.

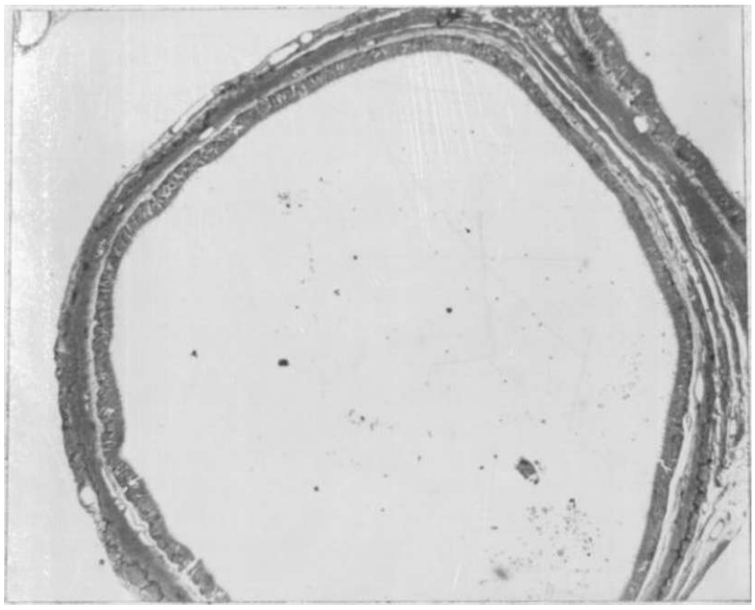


Fig. 33. A photomicrograph showing the proximal ductus deferens. Note the reduction in epithelial height compared to the terminal segment.

x 100 (Toluidine blue).

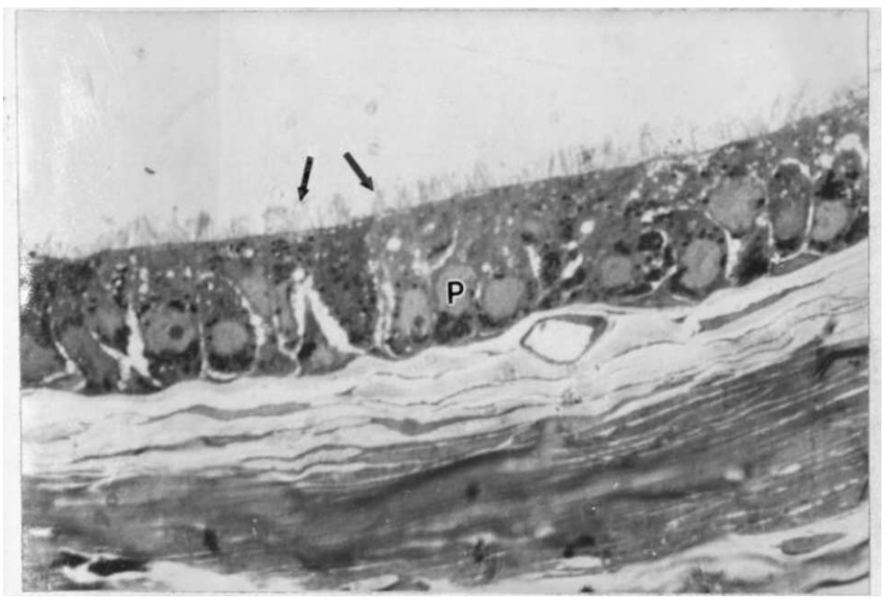


Fig. 34. A higher magnification of part of the proximal ductus deferens (fig. 33). The principal cells (P) have a reduced height compared to those of fig. 30 and the apical surface have microvilli that form brush border (arrows). Notice the presence of vacuoles in the apical cytoplasm and granules in the basal cytoplasm sometimes extending apically.

x 1,000 (Toluidine blue).

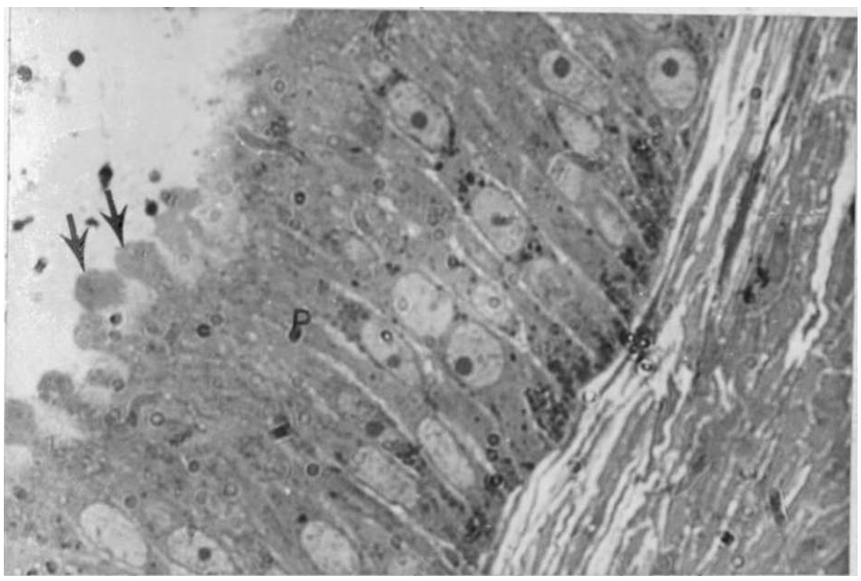


Fig. 35. The point of transition from proximal to distal ductus deferens. Note the marked increase in principal cell heights (P) and presence of a bleb-like apocrine evaginations apically (arrows) and granules basally.

x 1,000 (Toluidine blue)

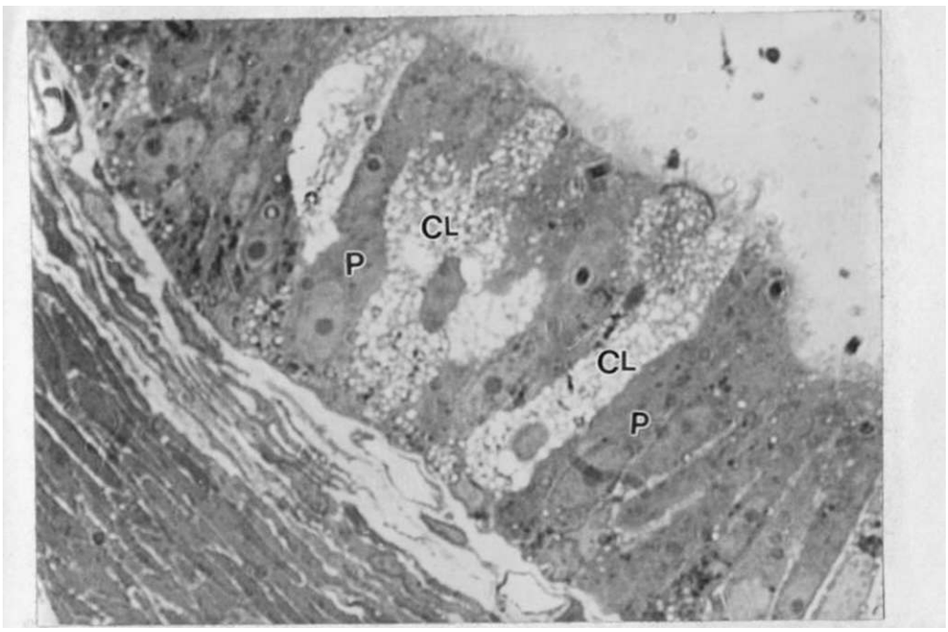


Fig. 36. The epithelium at the transition point showing presence of clear cells (CL) among the principal cells (P).

x 1,000 (Toluidine blue).

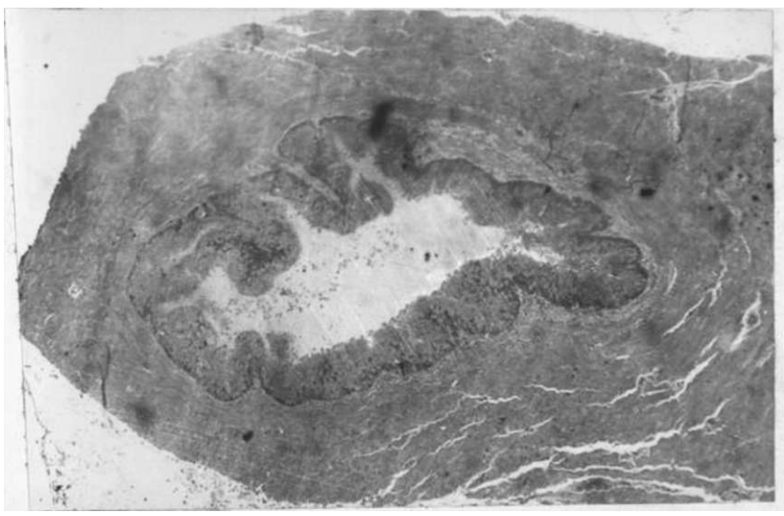


Fig. 37. A photomicrograph of the distal ductus deferens. Note the thickened muscular wall around the irregular epithelial lining. The lumen is devoid of spermatozoa.

x 160 (Toluidine blue).

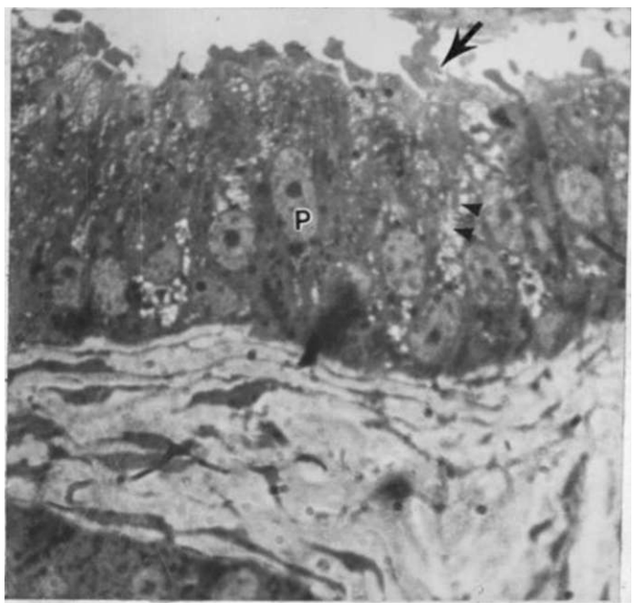


Fig. 38. A higher magnification of part of distal ductus deferens epithelium (fig. 37) showing presence of vacuoles (arrow heads) within the principal cells (P) and apocrine evaginations (arrow) on the apical surface.

x 1,000 (Toluidine blue).

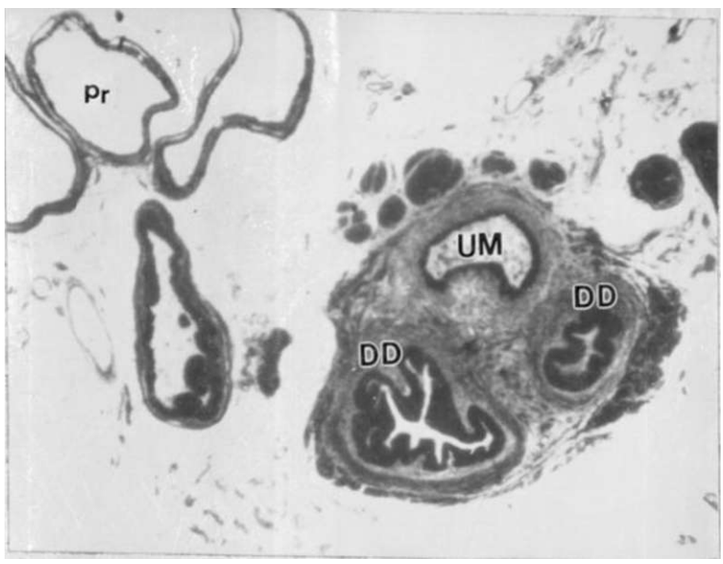


Fig. 39. A photomicrograph of the two distal ductus deferens as they approach the urethral wall. Notice that the two ducts (DD) are joined together by a connective tissue fold and a third duct, utriculus masculinus (UM), becomes evident at this stage. Follicles of prostate gland (pr) can also be seen.

x 63 (Toluidine blue).

3.2.3 Accessory sex glands and external genitalia

At the neck of the bladder on the ventral surface was a grey mass of translucent tissue lying transversely (see figs. 2a, b, c and d). This was initially thought to be the seminal vesicle but on histological examination, it was found to be a mass of fatty tissue (fig. 40). However, on either side of the distal ductus deferens and within the wall of the pelvic urethra there were seen follicles and ducts suggesting that portions of the prostate gland may be existing within the wall (figs. 39 & 41). These follicles were lined by simple cuboidal epithelia whose apical surface showed presence of several vacuoles (fig. 42).

The penis was basically of vascular type and comprised of a central lumen or urethra bounded by a transitional epithelium. Outside the epithelium was an erectile tissue consisting of carvenous spaces. On the dorsal surface, here was a system of ducts, the continuation of ducts observed in fig. 39. These consisted mainly of the central rudimentary uterus utriculus masculinus, flanked on either sides by the ejaculatory ducts and in between the ejaculatory ducts and the rudimentary uterus were ducts of the prostate gland. Further outwards, the erectile tissue was surrounded by an outer coat of circular and longitudinal muscles (figs. 43 & 44). This duct system finally coalesced to form part of the urethra at the middle of pelvic urethra so that the distal part of the pelvic urethra, penis and glans penis showed the urethra as the only opening. The tip of the penis was enveloped in a fold of skin which was lined by stratified squamous epithelium that was keratinized. Under the

epithelium were connective tissue fibres that surround several hair roots. These indicated the existence of hair on the skin of glans penis. A ventral interruption was observed on this skin. This fold of skin was the prepuce (fig. 45).

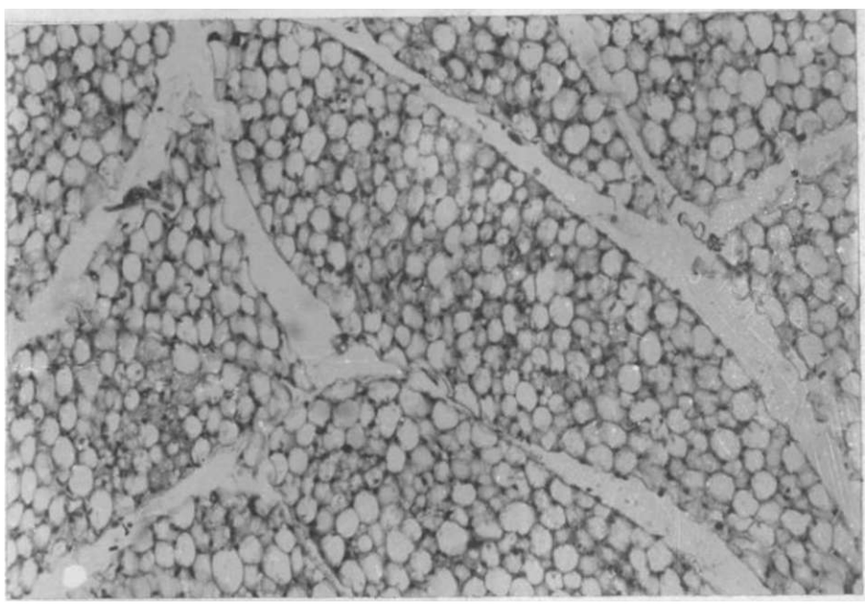


Fig. 40. A photomicrograph of adipose tissue found on the ventral surface of the bladder neck. This tissue had initially been referred to as the seminal vesicle (Hill *et al,* 1957).

x 100 (H & E).

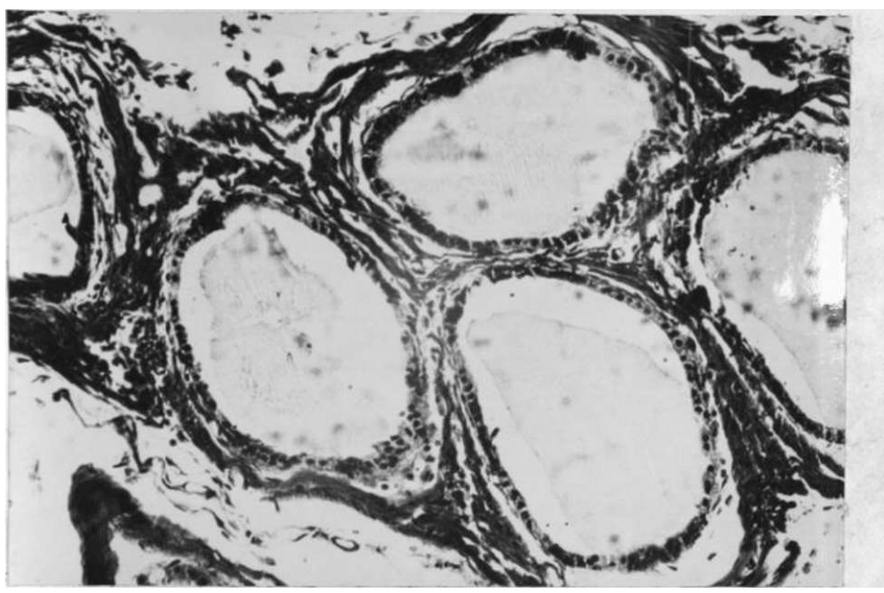


Fig. 41. Follicles of the prostate gland (see fig. 39) showing simple cuboidal epithelial lining.

x 160 (H & E).

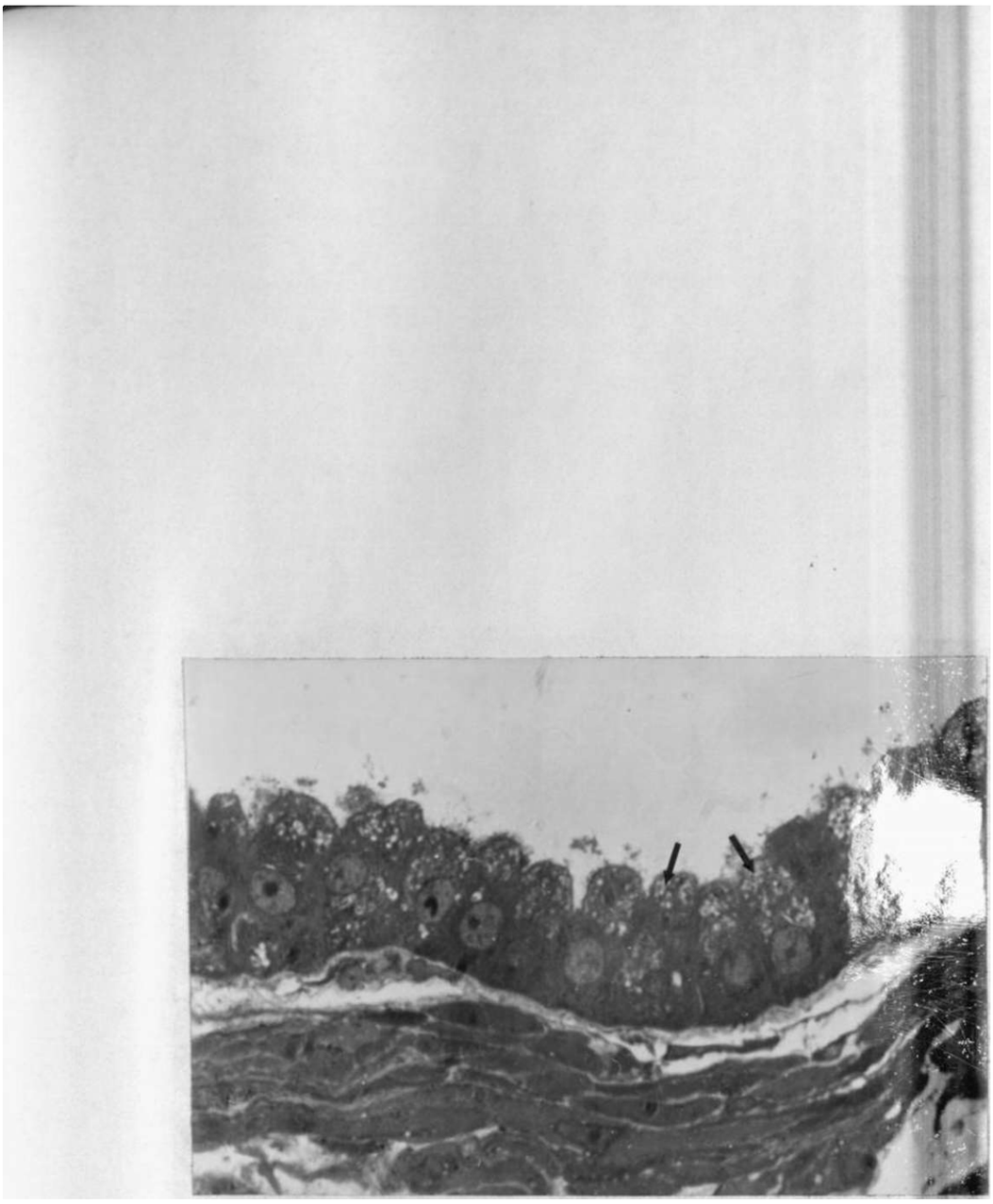


Fig. 42. Apart of prostate gland follicle epithelium comprising high cuboidal cells. Note the foamy appearance (arrows) of the apical cytoplasm of the cells due to the presence of several vacuoles.

x 1,000 (Toluidine blue)

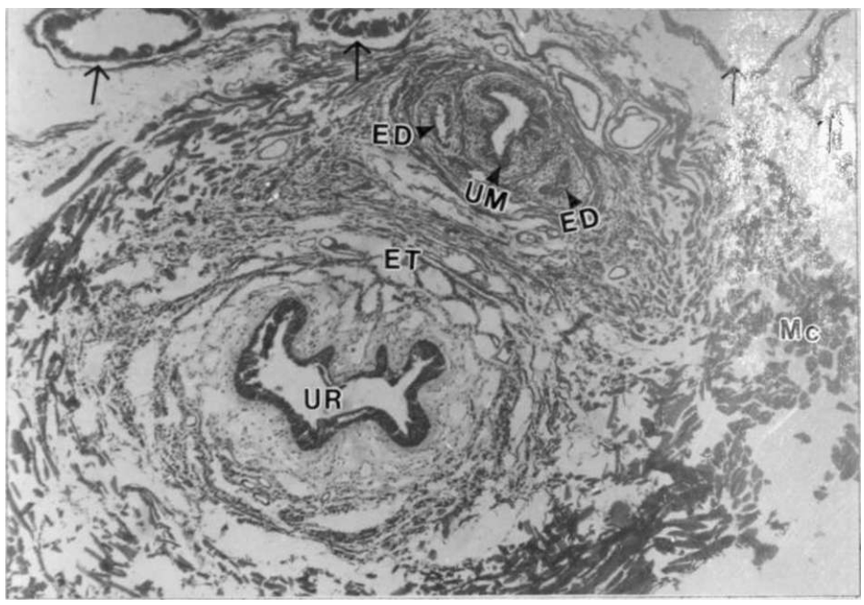


Fig. 43. A photomicrograph of the pelvic urethra (close to the urinary bladder). The urethra (UR) is surrounded by an erectile tissue (ET) and a muscular coat (Mc). Note the penetration of the ejaculatory ducts (ED) which are continuations of ductus deferens (see fig. 39) and utriculus masculinus (UM) through the dorsal wall of urethra. The arrows show follicles of prostate gland.

x 100 (H & E)

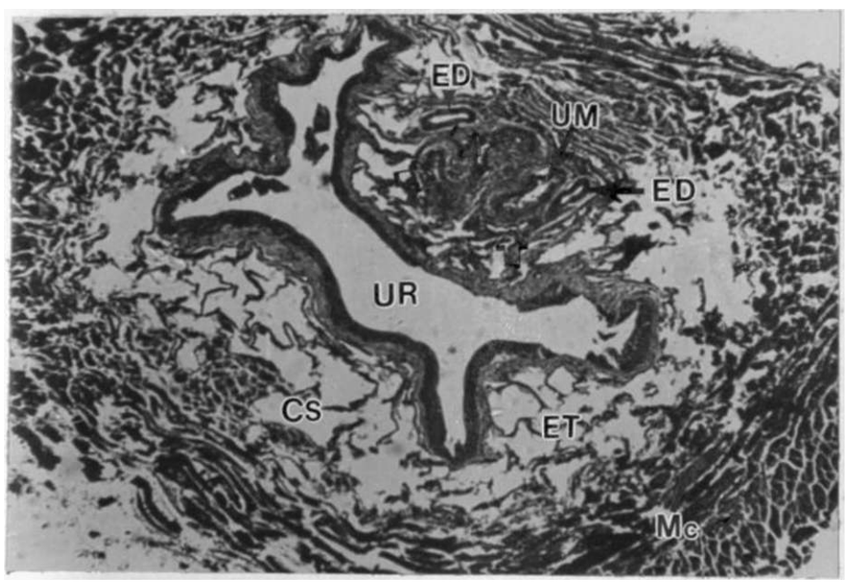


Fig. 44. A photomicrograph of the middle part of pelvic urethra. The urethra (UR) is surrounded by an erectile tissue (ET) having cavernous spaces (CS). Both the circular and longitudinal muscles (Mc) complete the urethral wall. Note that the ejaculatory ducts (ED), utriculus masculinus (UM) and prostate gland ducts (open arrows) have become smaller and closer to the urethra implying that after a short distance, they will become confluent with it.

x 100 (H & E)

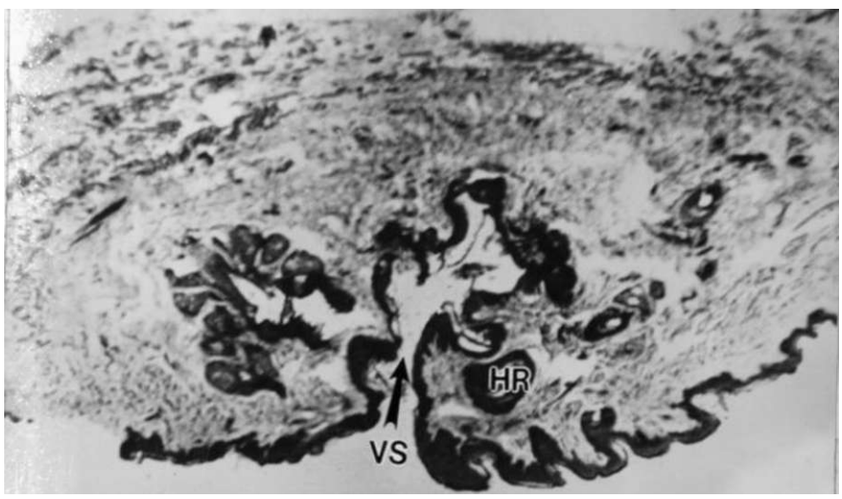


Fig. 45. The terminal part of the penis showing prepuce with a ventral slit (VS) and hair roots (HR). A stratified squamous epithelium form the outer covering of the prepuce.

x 100 (H & E)

The results (table IV) show that the *in vitro* stimulation of testicular interstitial cells from non-breeding naked mole rats using LH elicits negligible response in terms of testosterone production. Group 2 & 3 animals had testosterone levels ranging from 1.5-3.3 nmol/1. There was no significant deviation from the basal level secretion of testosterone which in the same two groups correspondingly ranged from approximately 1.65 nmol/1 to 2.80 nmol/1. However, in one group (group 1), surprisingly high levels of testosterone were obtained but with similar trend of response as in the rest of the groups (fluctuating increments). In this group, the basal secretion was 7.65 nmol/1 with the highest level of secretion recorded at 8.88 nmol/1.

When the mole rat cells were treated with a concentration of LH ranging from 0 to 300 jilU/tube, a very small increase in testosterone production was observed (groups 1 & 2). Similar results were obtained when the concentrations of stimulating LH was increased to between 0 and 2,500 ^ilU/tube where the observed response only ranged between 1.48 and 2.39 nmol of testosterone/1 (group 3). Since results of groups 1, 2 and 3 tended to suggest some response, though small, with increase in concentration of LH it was therefore necessary to examine side by side the response of naked mole rat and mice cells to a stimulation of up to 2,500 (ilU/tube and compare the magnitude. It was found that where the basal secretion in mole rat was 3 ± 0.21 nmol/1, a stimulation of cells with 2,500

|iIU/tube increased testosterone production to 4.21 ± 0.41 runol/1 (n =4). The mice Leydig cells subjected to a similar treatment showed exponentially graded increase in response ranging from 1.93 ± 0.31 to 15.99 ± 0.35 nmol/1 (fig. 48). fig. 47 gives a graphical representation of the naked mole rat and mice interstitial cell response to varying concentrations of LH. It is evident that whereas there is little response in naked mole rat preparations, those of mice show an exponential increase.

3.3.2 Plasma LH and testosterone levels

Plasma LH levels varied between 0.70 and 2.96 nIU/ml with an average of 2.0 ± 0.24 |iIU/ml (n = 9). Testosterone levels on the other hand ranged from 3.12 nmol/1 to 54.11 nmol/1 and an average of 26.09 ± 5.70 nmol/1 (n = 10). When levels of testosterone are plotted against those of LH (fig. 49), regression is described by the equation Y = 21.09X - 14.66, with a correlation coefficient of r = 0.83- The inter and intra-assay coefficients of variation for *in-vitro* and plasma testosterone were 15% at 1.15 mILVml (n = 12) and 8.33 ± 1.39% at basal secretion respectively. The latter had 10% and 5% at 3.5 nmol/1 (n = 10) respectively.

Table II: % Viability of mice and mole rat Leydig cells

ᵛSN\iinutes	0	30	60	90	120	150	180
Mice	80	85	90	85	80	80	80
NMR-Gpl	80	80	85	80	90	87	81
-Gp2	83	91	88	84	85	80	86
-Gp3	80	80	85	83	88	86	80

NB: Gp = Group

NMR = Naked mole rat

Fig. 46. MOLE RAT/MICE LEYDIG CELL VIABILITY (%)

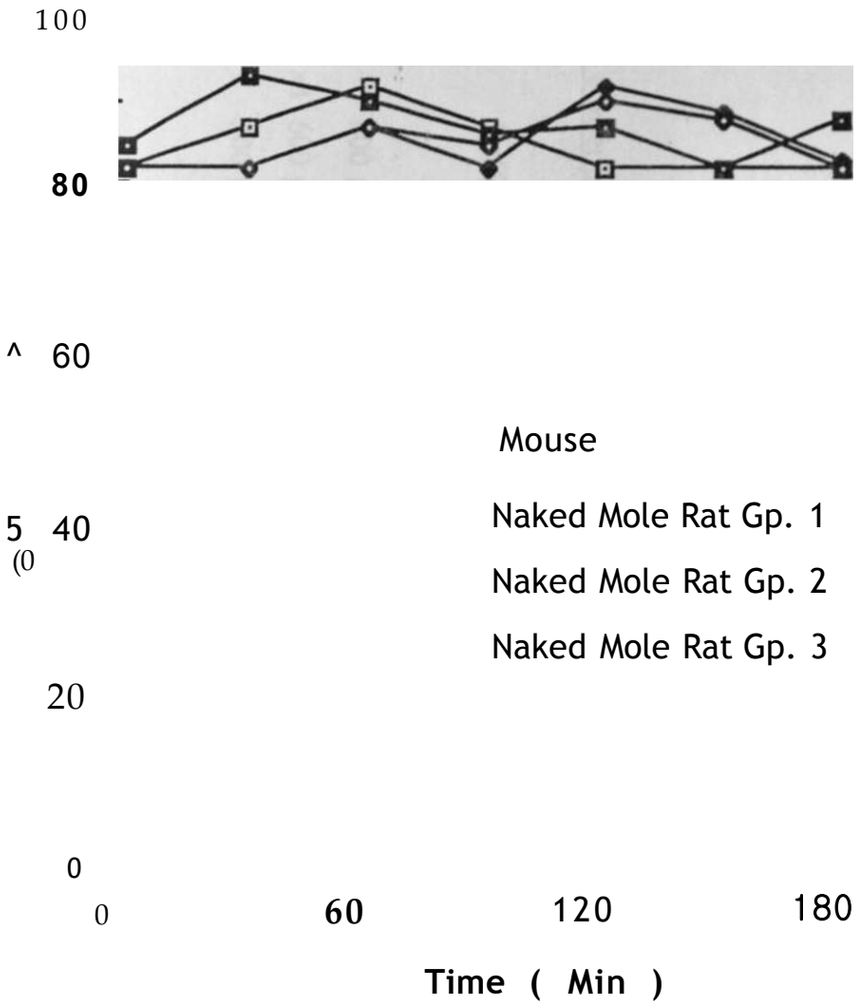


Table III: A summary of the contents of assay tubes

Labelled assay tube	Buffer	Sample or standard	3H-testosterone (working dilution)	Antiserum (Working dilution)		Charcoal Reagent
Total count (TC) x2	800 H1		100		Incubate	
Non-specific binding (NSB) x4	600		100 Hi		Overnight	200 Hi
Optimum binding (BO) x4	500 H1		100 Hi	100 Hi	at 4«C	200 Hi
Standards or sample		500 nl	100 Hi	100 Hi		200 Hi

	Testosterone (nmol/1)			
L.H. Dose (UlU/tube)	Group 1	Group 2	Group 3	Group 4
0.000	7.650	2.800	1.650	1.930
9.300	.	3.210	.	.
18.750	7.750	3.070	.	.
37.750	7.710	2.980	.	2.410
75.000	8.820	3.260	.	3.400
78.000	.	.	1.480	.
150.000	8.880	3.030	.	5.100
156.000	.	.	1.480	.
300.000	8.470	.	.	7.200
313.000	.	.	2.000	.
600.000	.	.	.	8.430
625.000	.	.	1.830	.
1250.000	.	.	2.390	.
2500.000	.		2.260	15.990

Table IV. Testosterone produced by mole rat/mice Leydig cells. Groups 1, 2 and 3 represent the months in which the mole rats were caught. Group 1 animals were caught in March, Group 2 in April, Group 3 in May. Group 4 animals were mice obtained in May. About 250,000 cells/tube were co-incubated with LH. Note that where the testosterone production in each group corresponding to LH dose is represented by a blank it means that the LH dose was not used for co-incubation.

Fiq.47 **MOLE RAT/MICE LEYDIG CELL RESPONSE TO LH**

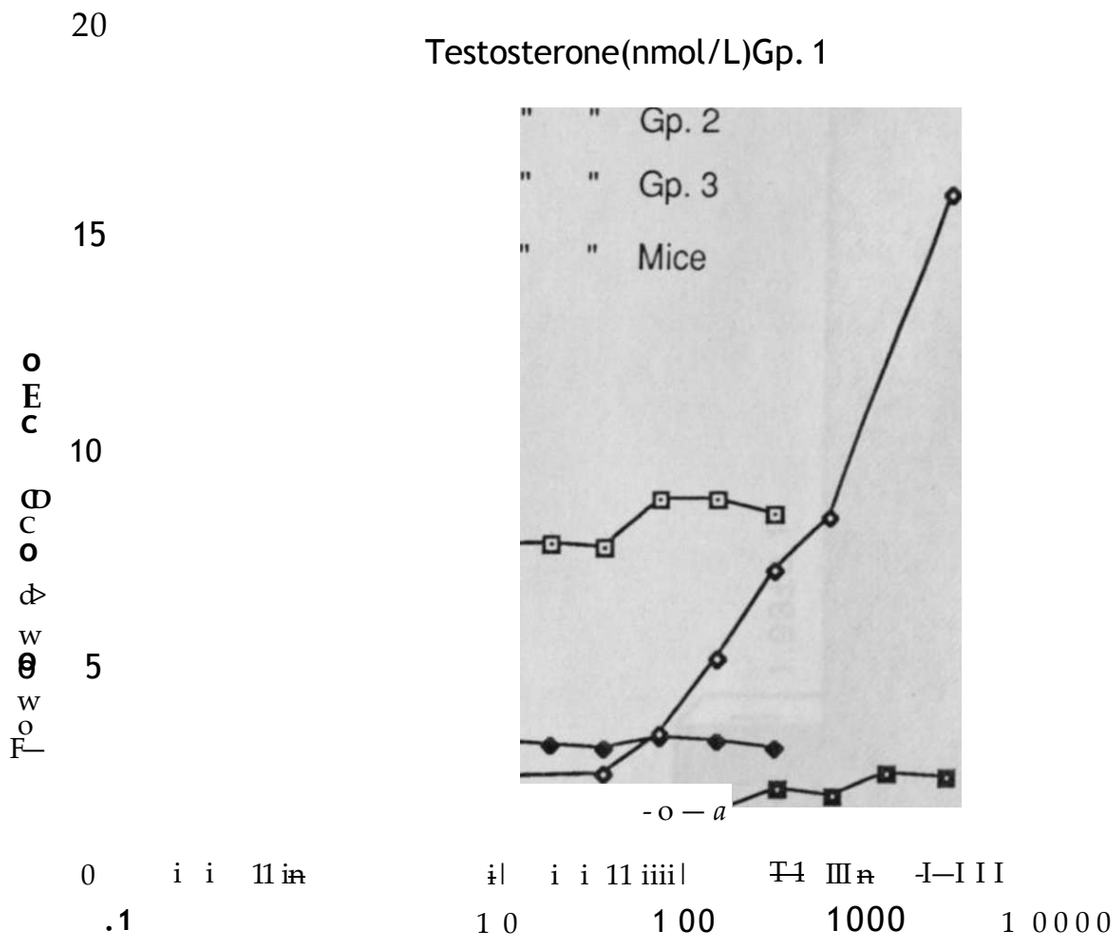


Fig. 48. MQLE RAT/MICE LEYDIG CELL RESPONSE TO HIGH I_H pflflf

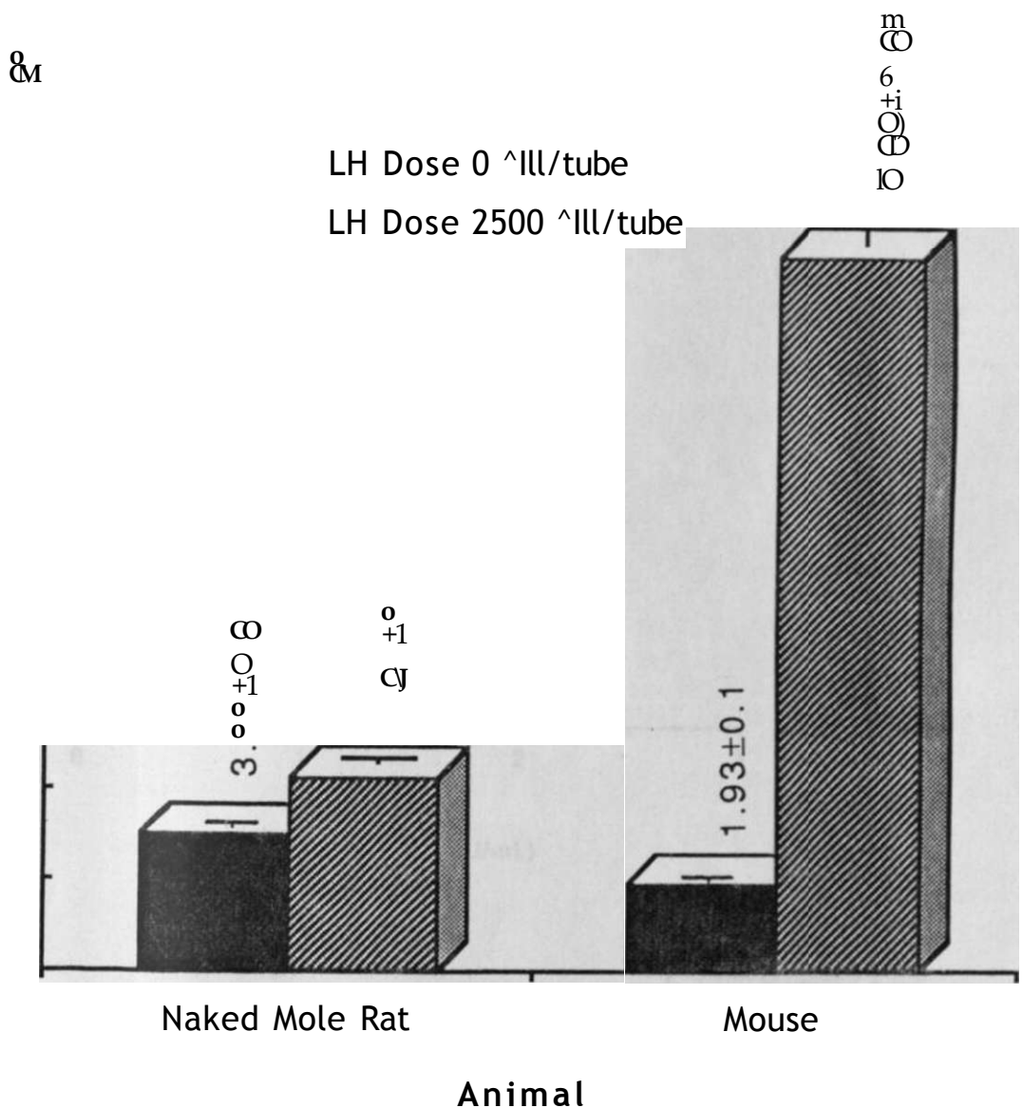
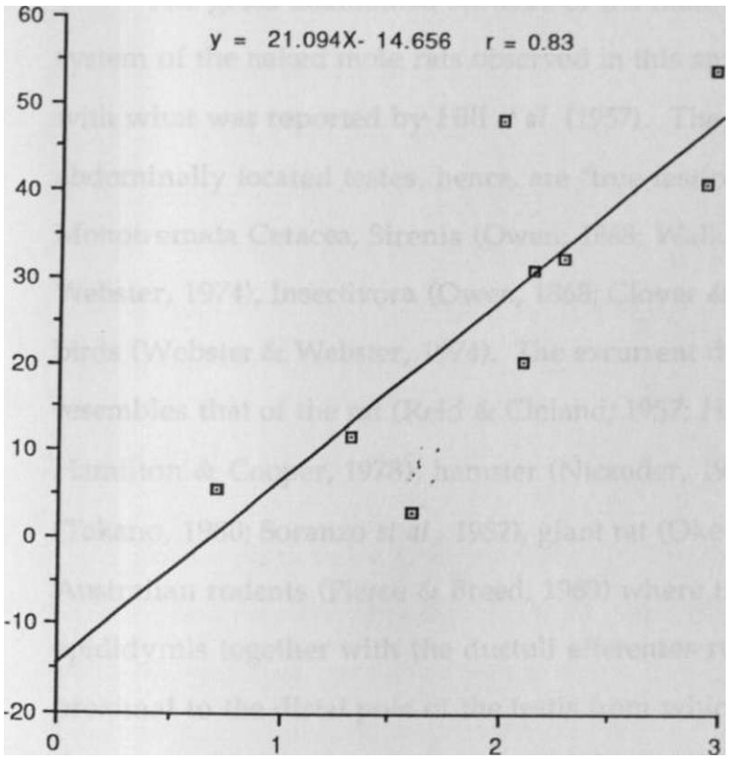


Fig. 49. PLASMA LH AND TESTOSTERONE CORRELATION



CHAPTER 4

DISCUSSION

4.1 General discussion

The gross anatomical features of the male reproductive system of the naked mole rats observed in this study largely agree with what was reported by Hill *et al.* (1957). The animals have intra-abdominal^ located testes, hence, are "true testiconda" as in Monotremata Cetacea, Sirenia (Owen, 1868; Walker, 1968; Webster & Webster, 1974), Insectivora (Owen, 1868; Glover & Sale, 1968) and birds (Webster & Webster, 1974). The excurrent duct system closely resembles that of the rat (Reid & Cleland, 1957; Hamilton, 1975; Hamilton & Cooper, 1978), hamster (Nicander, 1973), mouse (Takano, 1980; Soranzo *et al.,* 1982), giant rat (Oke *et al.,* 1988) and the Australian rodents (Pierce & Breed, 1989) where the ductus epididymis together with the ductuli efferentes run from the proximal to the distal pole of the testis from which emerges the vas deferens as the caudal continuation of the terminal segment of the epididymis. In contrast to this general disposition of the duct system, guinea pigs exhibit a rather unique arrangement where only the ductuli efferentes covers the entire length from proximal to distal pole of the testis and the rest of the epididymis begins from the distal pole (Hoffer & Greenberg, 1978). In the rock hyrax, however, only the ductuli efferentes and caput epididymis occupy this proximal and distal pole length while the distal continuation form the corpus epididymis (Glover & Sale, 1968). In this study it was

further observed that the naked mole rat has only one accessory sex gland, the prostate. Hill *et al.* (1957) reported that the naked mole rat had seminal vesicle but no prostate gland was mentioned. Observations in this study seem to show that what was reported to be the seminal vesicle is, in fact, a mass of fat deposits. One puzzling observation about this fat deposit is that it is consistently found in males and even females and occupies almost the same position. To-date only two species of mammals, spotted skunk (Mead, 1970) and the dog (Nickel *et al.,* 1973) are known to have the prostate gland only and the naked mole rat is an addition to the group. The penis is of vascular type and in this respect resembles those of other rodents, equines and carnivores but lacks os penis.

4.2 Testes

Histological and ultrastructural observation revealed the presence of a continuous sheet of Leydig cells among which are sparsely distributed seminiferous tubules with a clear evidence of spermatogenesis marked by presence of very few spermatids and spermatozoa. There are relatively little interstitial connective tissue, few interstitial capillaries and lymphatic vessels. The Leydig cells have abundant lipid droplets, sER and mitochondria. The distribution of Leydig cells in animals is species specific such that in some animals these cells are sparsely distributed yet in others such as opossum (Fawcett *et al.,* 1973; Nogueira *et al.,* 1977) and European mole rats (Suzuki & Racey, 1978) these cells are abundant. The naked mole rat falls in the latter group as observed by Fawcett *et al.* (1973) and confirmed by the present study. Such an expansive

proliferation of Leydig cells as in the latter case has been associated with production of other steroids other than testosterone. In the boar, for example, these cells have been implicated in the production of 16-androsterone which is thought to be the source of boar odour (Sink, 1967). It is not, however, clear whether these cells could also be producing other steroids in the naked mole rats. The presence of abundant lipid droplets, sER and mitochondria in Leydig cells paints a picture reminiscent of that of steroid producing cells (Christensen & Gillim, 1969; Burgos *et al.,* 1970; Christensen, 1975) which indeed they are. Since lipid droplets are known to be important in steroidogenesis (Connel & Eik-nes, 1969), their presence in large amounts in cells should indicate a state of inactivity which may be occasioned by age (prepubertal males) (Baillie, 1961; Niemi & Ikonen, 1963; Russo, 1970; Russo & De Rosas, 1971; Mugisha-Girasi *et al.,* 1979), season (in seasonal breeders) (Neaves, 1973; Lam & Farner, 1976; Leceta *et al.,* 1973) or inadequate levels of pituitary gonadotrophins (Lynch & Scott, 1951; de Krester, 1967) although in some animals the inclusions may persist even during the active phase (Suzuki & Racey, 1978; Bernard, 1986; Pudney, 1986). Limited spermatogenic activity in the seminiferous tubules leading to very few spermatids in the tubules and no spermatozoa in the excurrent ducts coupled with the known social suppression of reproduction in these animals (Jarvis, 1984) eliminates the possibility that these animals were in the phase of sexual activity. Furthermore, it is possible that a few of the animals studied could either have been in the prepubertal stage but since the abundance of lipid droplets in Leydig cells was a consistent feature in all the animals studied, it is

highly unlikely that all these animals were prepubertal. These animals are also known not 'to be seasonal breeders (Jarvis, 1984) and this further eliminates seasonal influence as a possible cause of sexual inactivity. The only likely cause of this sexual suppression leading to such accumulation of lipid droplets is inadequate level of pituitary gonadotrophins. This theory is even supported by the results of previous endocrinological studies where it was reported that male naked mole rats have low LH levels in blood (Faulkes *et al.,* 1989b).

Spermatogenic cell populations in the seminiferous tubules were predominantly spermatogonia and primary spermatocytes although a few secondary spermatocytes and very few spermatids were encountered. This further confirms observations of Jarvis (1984) where spermatogenesis was seen in males of all castes. Even in females, Kayanja & Jarvis (1971) reported presence of many primordial and primary follicles but few secondary and tertiary follicles in non-breeders. It would seem that as an extension of the effect of sexual suppression there is depressed transformation of primary spermatocytes to more advanced forms seen and hence the occurrence of very few spermatozoa in the testis of majority of males studied.

4.3. Excurrent duct system

Morphological differentiation of the epididymal duct into head, body and tail was not clear grossly although areas designated as head, body and tail were recognized on the basis of the convolutions. At histological level, the ductuli efferentes presented an epithelium

having two cell types; principal and ciliated cells which is consistent with what has been reported in other rodents (Hoffer, 1972; Hamilton, 1975; Hamilton *et al.,* 1977; Flickinger *et al.,* 1978; Oke *et al.,;* 1988; Pierce & Breed, 1989), opossum (Ladman, 1967), rabbit (Jones *et al.,* 1979) and in large domestic and wild species such as the boar, ram, bull, billy-goat and stallion (Johnson, 1978; Hemeida *et al.,* 1978; Goyal & Williams; 1988). Due to the small number of vacuoles and vesicles in this region, the absorptive role must have been at the lowest level. This can be explained by the fact that these animals are sexually inactive, hence, no serious need for such elaborate absorptive apparatus in the cells. It, however, remains to be seen what the picture would be in these animals when in full sexual activity.

The dramatic increase in principal cell heights, reduction in the diameter of the lumen, presence of apical cells and lymphocytes in the initial segment are expected observations as has been recorded in other mammalian species (Hoffer *et al.,* 1973; Hamilton, 1975; Hamilton *et al.,* 1977; Kayanja, 1989; Pierce & Breed, 1989). Because of the rarity of vacuoles and vesicles in this region it resembles that of the giant rat (Oke *et al.,* 1988) but differs in the presence of apical cells. As in the ductuli efferentes, sexual dormancy may be a factor contributing to this observed rarity of vesicles and vacuoles. In other sexually active animals there is a predominance of vesicles and vacuoles in this segment (Hoffer *et al,* 1973; Hamilton, 1975; Flickinger *et al,* 1978; Pierce & Breed, 1989). The middle segment which includes the body and part of the head followed the general

histological pattern as in other animal species where there is a general graded reduction of principal cell heights, increase in the diameter of the lumen, presence of basal cells and no apical cells. Hamilton (1975) reported in rats that apical cells are only found in the initial segment. This also applies to the naked mole rats. The presence of small supranuclear granules in some cells within this region may indicate a secretory role but their number, being too low, overules this. In the terminal segment there was no remarkable reduction in the height of principal cells although the diameter of the lumen increased. This is close to what was observed in the hopping mouse (Pierce & Breed, 1989). The lumen in this region contained no spermatozoa in all the animals studied. Due to the presence of vacuoles in apical cytoplasm and vesicles scattered within the cytoplasm it is suggested that this region is involved in absorptive or secretory function apart from storage of spermatozoa. It has been reported that in rats (Hamilton, 1975) and hamster (Vicentini & Orsi, 1989), this region has vesicles and vacuoles which confer upon it absorptive/secretory role.

On the basis of histological observations, the ductus deferens exhibited two segments namely the proximal or epididymal vas and distal segments. The proximal segment show presence of short microvilli forming brush border, low principal cell heights, vacuoles and vesicles continuing from the terminal segment. The principal cells are the predominant cells here and contain vesicles and vacuoles. A similar division of the ductus deferens has been reported in rats (Hamilton & Cooper, 1978; Kennedy & Heidger,

1979), hamster (Flickinger *et ai,* 1978) and Australian rodents (Pierce & Breed, 1989). The point of transition from proximal to distal segment is marked by an increase in principal cell heights and presence of clear cells. In the basal cytoplasm of principal cells are osmiophillic aggregates that may be ER. Similar structures have been seen in rat vas deferens and referred to as ER (Niemi, 1965). On the apical surface of these cells are exocytotic evaginations implying an apocrine mode of secretion. Niemi (1965), however, reported presence of pinocytotic funnels and vesicles and disintegration of apical cytoplasm suggesting fluid absorption. In addition, protein absorption has also been reported in the vas deferens (Friend & Farquhar, 1967). Clear cells are tall cells with cytoplasm filled with vacuoles. In many animal species, clear cells have been encountered in the terminal segment (Hamilton, 1975; Hamilton *et ai,* 1977; Flickinger *et ai,* 1978; Oke *et ai,* 1988; Pierce & Breed, 1989; Vicentini & Orsi, 1989). Kennedy & Heidger (1979), however, reported presence of clear cells in the proximal segment near the terminal segment of the epididymis. The naked mole rat therefore is the only animal where the clear cells have been seen close to the distal segment of vas deferens. In the distal segment, there is a marked increase in the thickness of the surrounding muscle coat, the lumen is irregular due to the undulating pattern of the epithelium and the principal cells are tall with numerous vacuoles in basal and apical cytoplasm. All these features are commonly encountered in several animals but the presence of these vacuoles suggest an absorptive role in this area. The absorptive role of ductus deferens has been documented (Niemi, 1965; Friend & Farquhar, 1967; Hamilton,

1975; Hoffer, 1976). One cell that has been reported in rats (Niemi, 1965; Hamilton, 1975) but which has not been seen in the mole rats is the pencil cell.

4.4 Accessory sex glands and external genitalia

The prostate gland follicles of the naked mole rat are lined by simple cuboidal epithelium. The epithelial cells are mainly high cuboidal with foamy appearance of the apical cytoplasm. The foamy appearance is largely due to the presence of vesicles and vacuoles which suggests an active secretory role of these cells. It has been stated previously that the epithelial cells of prostate gland when in a secretory phase exhibit numerous vesicles and vacuoles in the apical cytoplasm (Brandes, 1974b). It appears therefore that even though the naked mole rats used in this study were non-breeders their prostate glands were, nevertheless, actively secreting. This is not unusual because even in the testis limited spermatogenic activity has been described, suggesting that a correspondingly active prostate gland may be existing. The mole rat penis as mentioned before is of vascular type. On the dorsal wall of the urethra is a rudiment of Mullerian duct, the utriculus masculinus. This is part of the Mullerian duct that fails to regress in male animals giving the implication that these are primitive animals. Such ducts have also been seen in many other animals including the rock hyrax (Glover & Sale, 1968) and dogs (Dellmann & Wrobel, 1976). This rudimentary duct is flanked on either side by ejaculatory ducts which are continuations of the ductuli deferens. The penis is

enclosed terminally by the prepuce that has a sparse distribution of hair.

4.5 Endocrinology

The results obtained during *in vitro* stimulation of Leydig cells of the naked mole rat by LH showed that the cells respond to a very small extent when exposed to this gonadotrophin. this may be due to inadequate levels of LH used, saturation of receptor sites on the plasma membrane, inadequate intracellular enzyme activation or a combination of these factors. When the cells were subjected to the normal graded LH doses that usually leads to an overt response in mice Leydig cells (0.093 - 3 mlU/ml) there was no significant increase in testosterone secretion from the basal level. A stimulation of mice Leydig cells using a similar dose led to a steady increase in the amount of testosterone. This indicates that the naked mole rat cells were not responding to LH level that would otherwise cause marked stimulation in mice. When the mole rat cells were further exposed to a relatively high dose of LH (25 mlU/ml) there was still no marked response whereas the mice cells registered a clear upsurge in testosterone production. This reflects massive deficiency of the mole rat cells to respond to co-incubation with LH. It is, therefore, probable that the receptor sites on the plasma membrane of the Leydig cells are readily saturated by low LH levels such that additional treatment with LH yield very little response.

Regarding the control of steroid production in other steroidogenic tissues such as corpus Iuteum, it has been reported that the stage of cellular maturity may influence the response to stimulatory agents (Alila *et al.,* 1981). It is also possible that in this case, the lack of stimulation might be influenced by this factor as well. However, there is need to confirm this with serial studies using tissues of progressive ages. Another possibility is that the cells may actually be producing testosterone but this is rapidly converted into metabolites not detectable by the method employed or there may be inhibition of enzymes in the testosterone synthetic pathway such as cholesterol side chain cleavage enzyme (CSCC) or 3B-Hydroxysteroid dehydrogenase (3B-HSD). Removal of the inhibition would then lead to reactivation of the enzymes. These aspects were not considered in this study but would soon be given a follow-up.

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It is important to mention at this stage that the media used in these experiments has been shown to be adequate for such investigation in mice. Similarly, in studies involving mice Leydig cells, 250,000 cells were found to be adequate for such investigations. However, due to the scarcity and difficulty in obtaining mole rat tissues, it is relatively difficult to establish the optimal cell numbers and media conditions for studying the mole rat cells. In our study, we assumed that the media and number of cells used for the former would also be adequate for the mole rat. However, further studies are needed to establish whether this is the case. We are presently in the process of carrying out such experiments with the aim of

optimising and standardising our mole rat system for further studies.

When plasma LH and testosterone concentrations were determined, they were found to be 2.0 ± 0.24 mIU/ml which was considered low (Faulkes *et al,* 1990a). The present findings indicate that LH levels in mature wild caught male naked mole rats are also low. This is in total agreement with findings of Faulkes *et al* (1989b). On the other hand, the plasma testosterone levels are high as depicted by the value obtained. This, however, contradicts the previously observed low urinary testosterone (Faulkes *et al,* 1989b). The low levels of LH in plasma seem to suggest that gonadotropin releasing hormone (G_nRH) production is reduced as had previously been suggested in the female naked mole rats (Faulkes *et al,* 1990a) and the common marmoset (Abbott *et al.,* 1989) or it may be due to a negative feedback mechanism to the hypophysis resulting from the high plasma testosterone levels. Faulkes *et al* (1990b), reported that the socially induced block to ovulation in non-breeding female naked mole rats may be due to inhibition of hypothalamic G_nRH secretion lending immense support to the former suggestion in the males. Removal of breeding female and male(s) appears to get rid of this suppression resulting in increased LH production and after a short while increased testosterone level. The observed high plasma testosterone level may be explained as a cumulative effect of the large volume of Leydig cells in these animals as found by Fawcett *et al* (1973). Moreover, it had been postulated that the massive volumes of Leydig cells as encountered in the boar could be a source

of androgens that lead to the boar taint (Sink, 1967). There was a strong positive correlation between plasma LH and testosterone (r = 0.83) indicating that the Leydig cells may be highly sensitive to these low levels of LH and that contact between LH and plasma membrane receptor sites quickly leads to saturation of the latter and a rapid response by testosterone production. Although the levels of the testosterone in mole rats.iS considered high when compared to other animals, it would seem that it is not high enough to elicit spermatogenesis in the former.

The phenomenon of sexual suppression is known to exist in other animals apart from the naked mole rat (Hearn, 1983; Pandey & Pandey, 1986; Abbott *et al.,* 1989). It is thought that the source of the suppression stimuli in the naked mole rats are the females of the same (Jarvis, 1984) as is seen in the common marmoset (*Calithrix jacchus*) (Hearn, 1983; Abbott *et al.,* 1989). Whereas in the former group of animals both the males and females are suppressed (Jarvis, 1984), in the latter group only the females appear to be suppressed (Hearn, 1983; Abbott *et al.,* 1989). In wild mice, however, the suppression of male sexual maturity is due to the presence of mature wild mice in the neighbourhood (Pandey & Pandey, 1986). This seems to suggest that social suppression of breeding or sexual maturity may originate from either female or male animals. On the other hand, exposure of the female prairie voles (*Microtus ochrogaster)* to male urine induces increased secretion of luteinizing hormone releasing hormone (LHRH) and norepinephrine from the

olfactory bulb, hence, increasing LH levels in blood (Dluzen *et al.*, **1981).**

In conclusion, male non-breeding naked mole rats have testes with a large volume of interstitial tissue within which are few seminiferous tubules. The interstitial tissue mainly comprise of interstitial cells of Leydig with little connective tissue fibres and few blood and lymphatic capillaries. The Leydig cells have numerous lipid droplets, sER and elongate mitochondria but the cells, apparently, do not respond to *in-vitro* stimulation by LH. The seminiferous tubules show all stages of spermatogenesis with the predominant forms as spermatogonia and primary spermatocytes. Secondary spermatocytes, spermatids and spermatozoa are very few. This shows that there is a slow-down (arrest) in further development of primary spermatocytes to mature forms. Reproductive suppression, therefore, occurs both at the level of Leydig cells and seminiferous tubules.

Morphology of the excurrent duct system, accessory sex glands and external genitalia is, in many ways, similar to other rodents. Only one accessory sex gland, the prostate, is present. The os penis (bacculus) is not present in the naked mole rat.

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