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Fine structure of human seminal vesicle epithelium

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The ultrastructure of the seminal vesicle has been extensively studied only in small laboratory animals. A number of investigations with the electron microscope have been carried out on the epithelium of the seminal vesicle in rodents (Fujita, 1959; Deane & Porter, 1960). In addition, Schantz (1964) has described the fine structure of the posterior lobe of the rabbit's prostrate, a part of the organ which has been regarded as homologous with the seminal vesicle of other mammals (Davies & Mann, 1947).

The seminal vesicle is one of the few male secondary sex organs not yet examined in man; Horstmann (1962) has described the ultrastructure of the epididymis and Brandes, Kirchheim & Scott (1964) that of the prostate.

Following the recent descriptions of nuclear bodies in the human and canine epididymis (Horstmann, Richter & Roosen-Rungen, 1966; Horstmann, 1965, respectively) particular attention has been focused on the epithelial cell nuclei in this study.

MATERIALS AND METHODS

Fresh surgical material has been used for this investigation. Specimens were obtained at cystectomy or retropubic prostatectomy in patients who had not had prolonged hormonal treatment and who had no clinical history of prostato-vesiculitis. The ages of the subjects were 54, 55, 56, 57, 57, 59, 62, 72 and 78 years.

Small pieces of tissue were fixed in 3·5% phosphate-buffered glutaraldehyde, washed overnight in buffered sucrose rinse (Sabatini, Bensch & Barrnett, 1963) and then postfixed in 1% osmium in acetate buffer. Alternatively pieces of tissue were fixed in 2% osmium tetroxide in phosphate buffer (Millonig, 1962) with or without the addition of 10% sucrose (Deane, 1963). The specimens were then dehydrated with alcohol, passed through propylene oxide and embedded in Araldite; thin sections were mounted on uncoated grids, stained with a saturated solution of uranyl acetate in methanol (Barnett & Palfrey, 1965) or with lead citrate (Venable & Coggeshall, 1965) and examined with a Metropolitan-Vickers E.M. 6 electron microscope. Parallel light-microscopical examinations were carried out in each case to make sure that the specimens did not show any sign of inflammation or neoplastic infiltration.

OBSERVATIONS

As can be seen from Fig. 1, only two types of cells, principal and basal, are discernible in epithelium of the human seminal vesicle.

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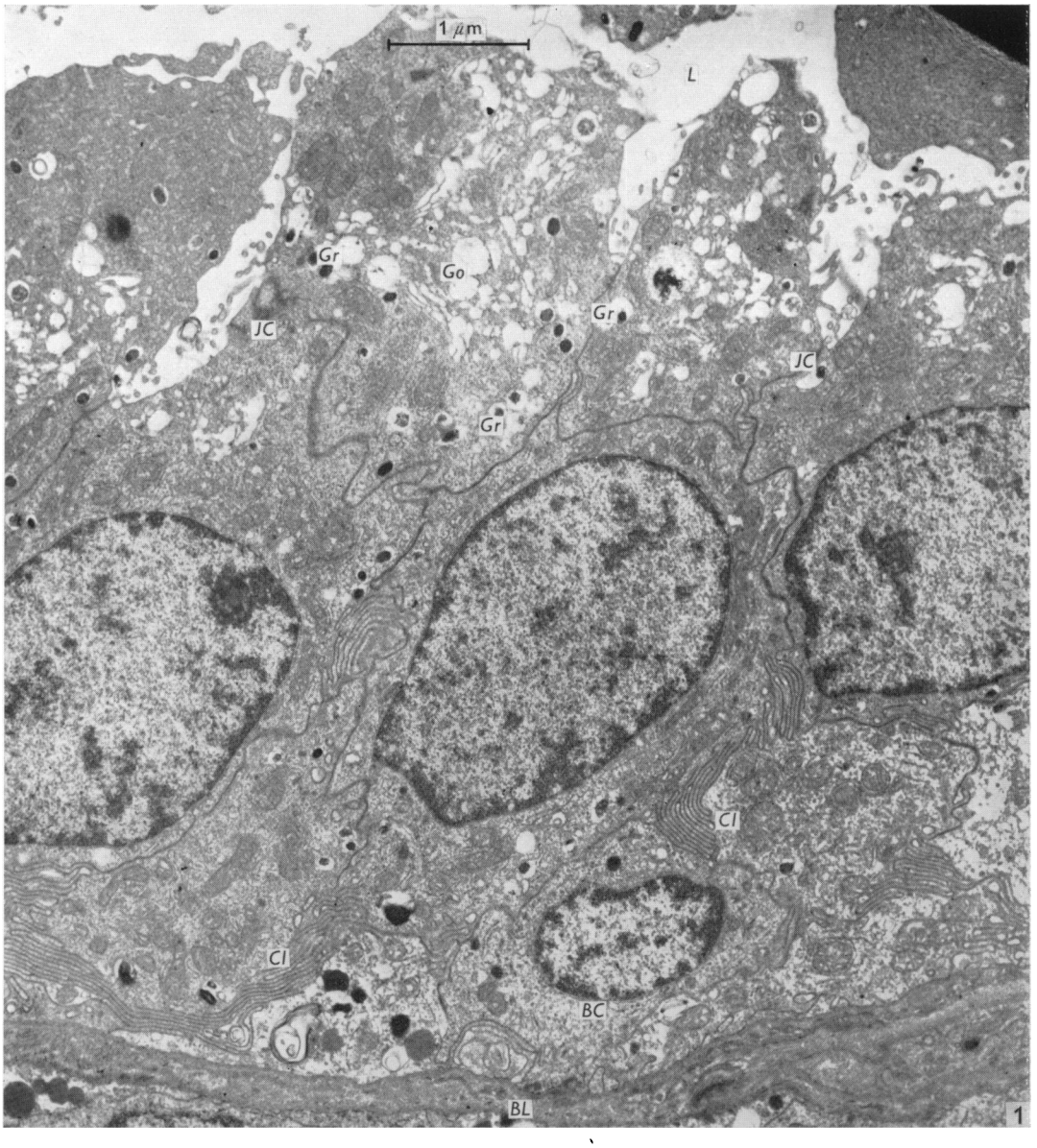


All the photographs are of human seminal vesicle epithelium. During preparation tissues were glutaraldehyde-osmium fixed except for those depicted in Figs. 10 and 12 which were fixed in osmium. Figs. 1–4, 6, 10 and 12 are from sections which were stained with uranyl-acetate; in the remaining cases, lead-citrate has been used.

Fig. 1. General view of the epithelium showing several principal cells; a small basal cell (*BC*) is discernible near the basal lamina (*BL*). Cellular interdigitation (*CI*) basally, and junctional complexes (*JC*) apically are present between the epithelial cells. The apical parts of the principal cells which bulge into the lumen (*L*) contain many secretory granules (*Gr*), especially around the Golgi apparatus (*Go*).

Principal cells

The principal cells are slender and columnar, showing 5–6 irregular sides in oblique or cross-section (Fig. 4). Their long axes appear to be oblique or even parallel to the direction of the lumen (Fig. 2), for these cells are seldom observed in all their

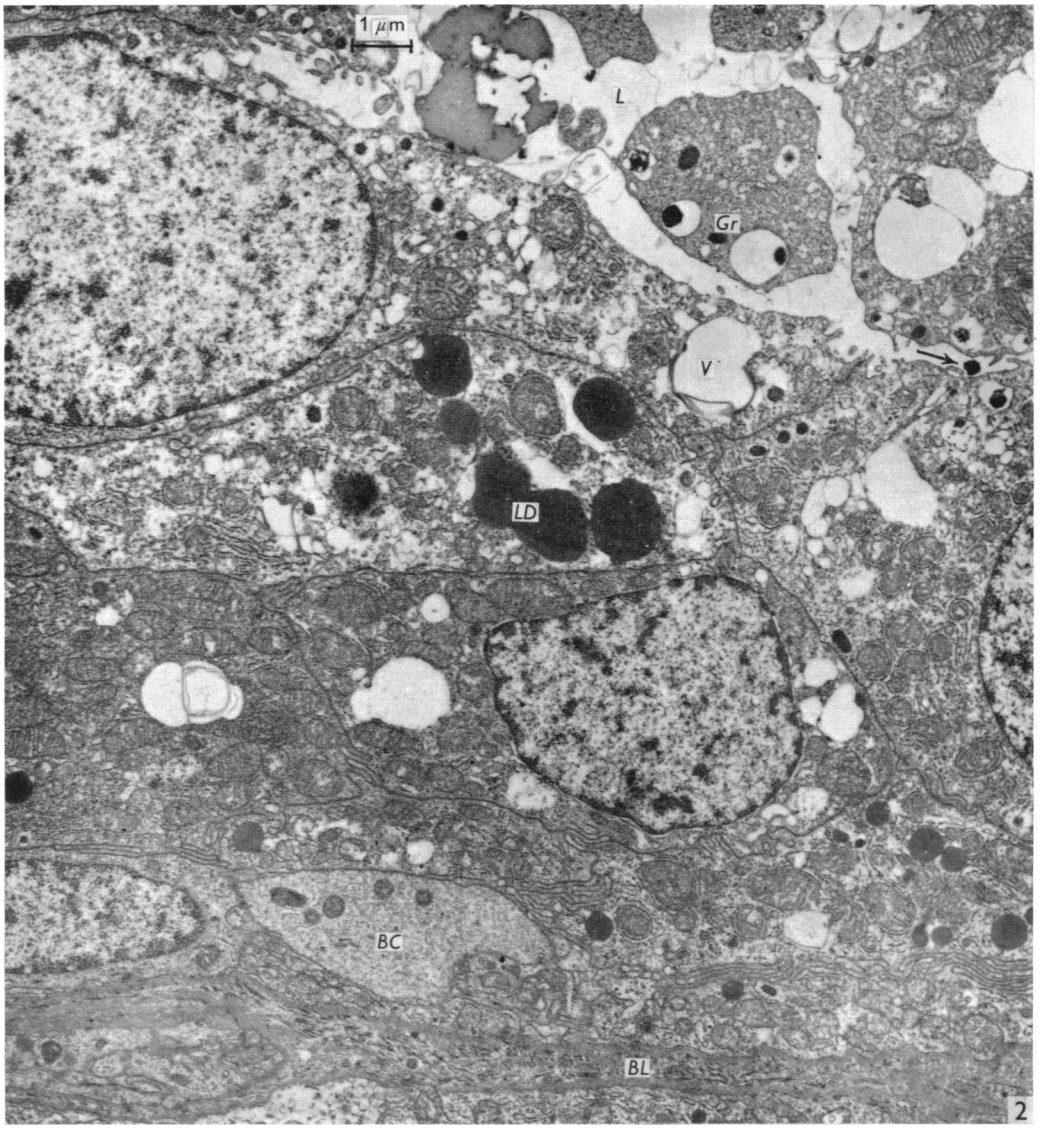


Fig. 2. The lumen (*L*) is reduced to a narrow canal extending between the apical parts of the principal cells, some of which are shown in cross-section. A discharged secretory granule (arrow) and a mass of amorphous material lie free in the cavity of the lumen. Secretory granules (*Gr*), large vacuoles (*V*), and lipid droplets (*LD*) are seen in the cytoplasm of the principal cells. Portions of basal cells (*BC*) may be observed near the basal lamina (*BL*).

length, from the basal lamina to the lumen. Their apical portions, conical or club-shaped, project and bulge into the lumen (Figs. 1, 2, 3). Intercellular canaliculi are present between the lateral walls of these apical processes (Figs. 1, 2, 5). When two

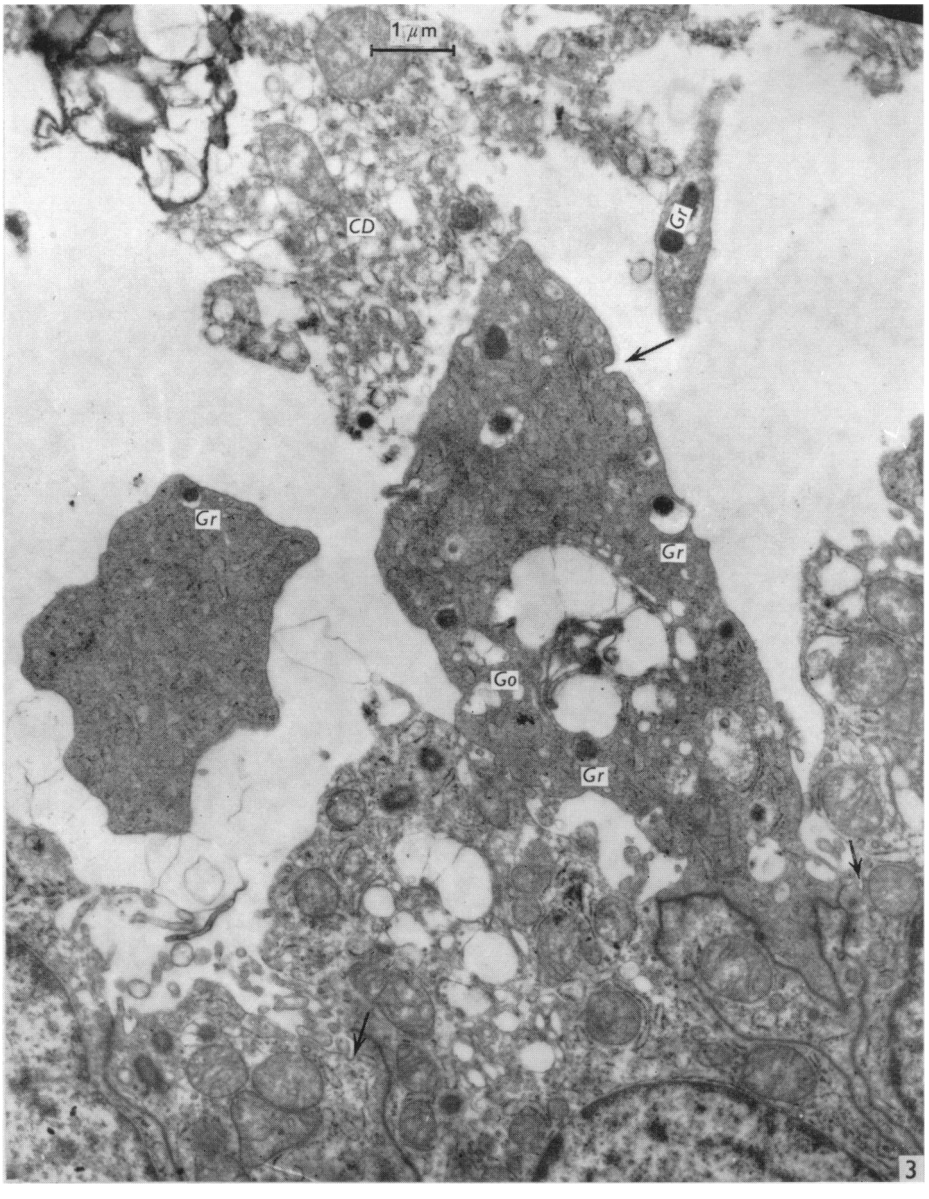


Fig. 3. Cellular debris (*CD*) and free mitochondria more or less degenerated are present in the cavity of the lumen. A dilated Golgi apparatus (*Go*) is seen in a prominent apical portion of a principal cell. Note that dense amorphous material is evident between the Golgi cisternae; it possibly represents a stage of formation of the secretory granules (*Gr*). The arrows indicate regular invaginations of the apical membrane probably related to pinocytosis.

mucosal folds are adjacent, the lumen is then reduced to a narrow canal extending between the apical parts of the principal cells by means of the intercellular canaliculi (Figs. 1, 2, 5). The apical surface of the principal cells shows twisted, microvilli-like, cytoplasmic projections (Figs. 5, 6, 11). In the basal part of the epithelium the cells' surfaces are closely related to one another by extensive cytoplasmic interdigitations. These features are observed in both oblique (Figs. 1, 4) or cross-sections, particularly where three or four cells are in apposition. In the subapical part of the epithelium,

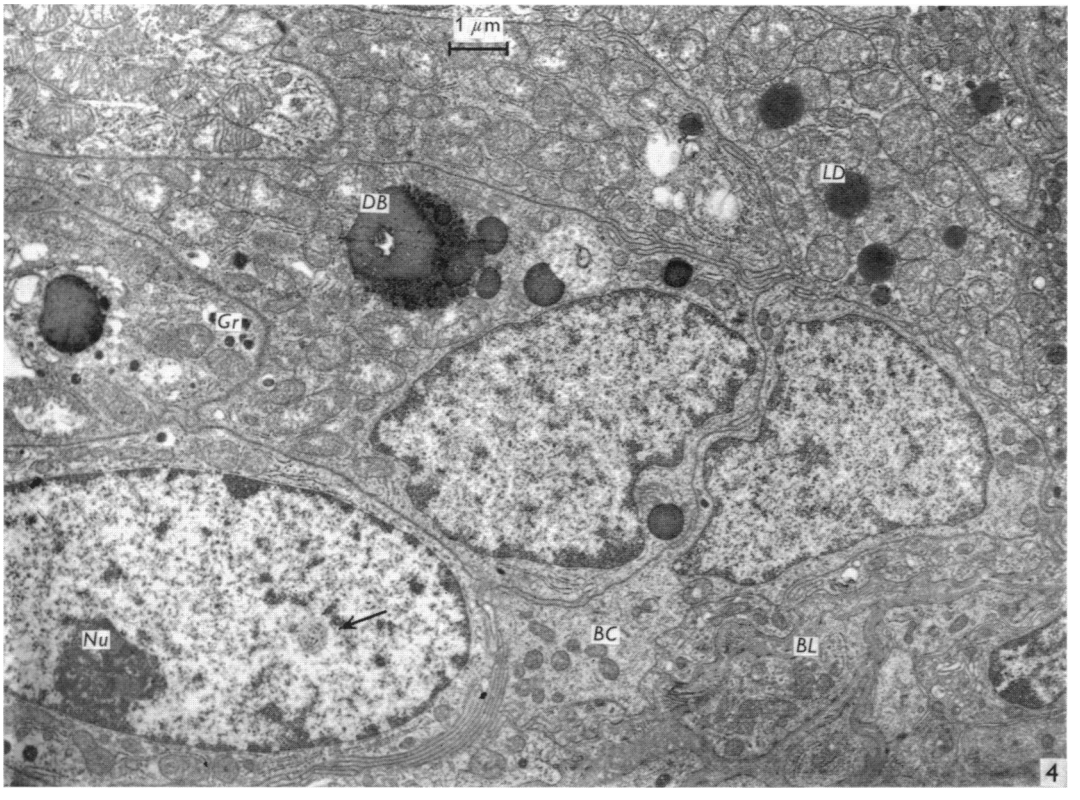
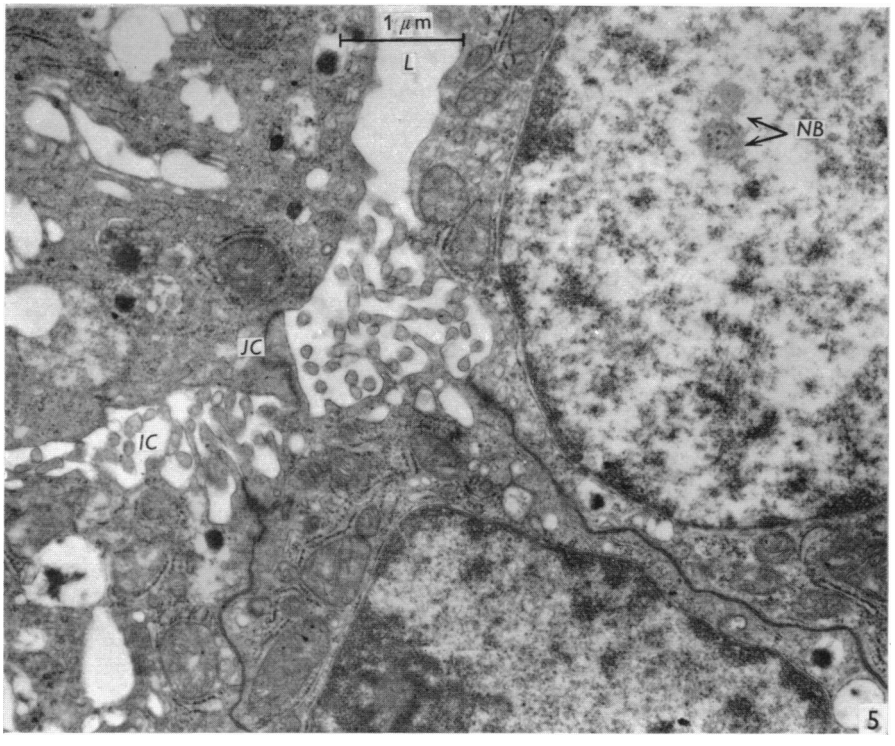


Fig. 4. A basal cell (*BC*) is present near the basal lamina (*BL*). The cytoplasm of the principal cells, which is extremely rich in mitochondria, contains dense bodies (*DB*), small secretion granules (*Gr*) and lipid droplets (*LD*). A nucleus shows a large nucleolus (*Nu*) and a nuclear body (arrow).

the lateral membranes of the columnar cells are joined together by junctional complexes (Figs. 1, 3, 6, 7, 11) with tight junctions (zonulae occludentes), intermediate junctions (zonulae adherentes) and desmosomes (maculae adherentes).

The nuclei are placed at different heights in the epithelium. They are large and ovoid or spherical in shape; sometimes nuclei with irregular outlines and deep fissures are found, especially in the oldest subjects (Fig. 8). The chromatin tends to assume a marginal position, being condensed along the nuclear membrane (Figs. 1, 7). When cut in the appropriate plane the nuclei exhibit one or more large and complex nucleoli (Figs. 4, 5, 7). Spheroidal inclusions with diameters of about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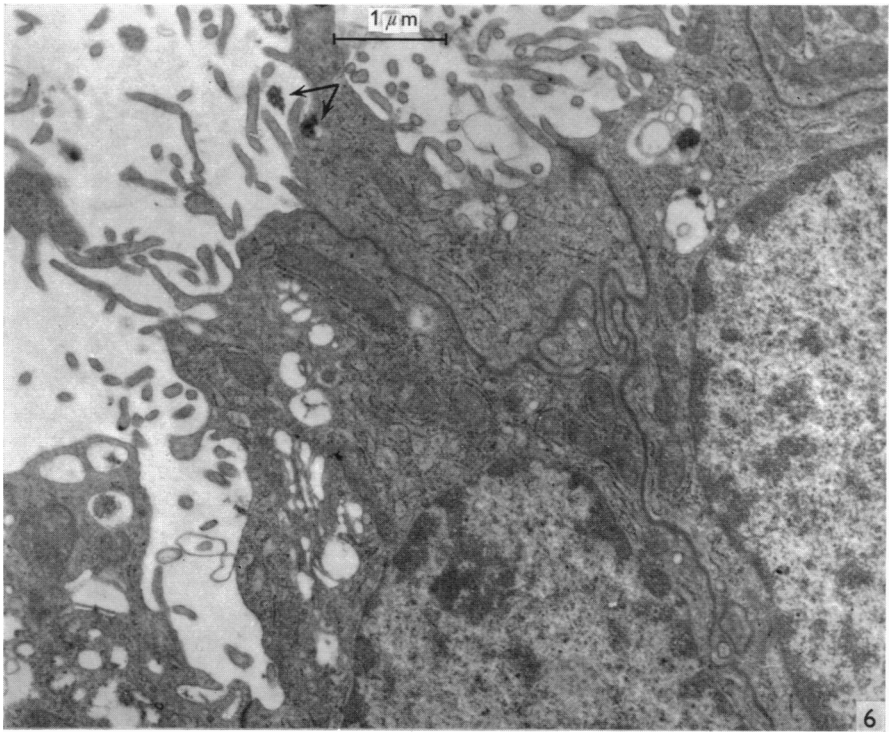
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0·2–0·4 μm may be distinguished from the common components of the nucleoplasm (Figs. 4, 5). They appear as membranaceous structures arranged concentrically, or as rosettes of delicate filaments (Figs. 9, 10). Their centre is usually represented by a granular particle with a diameter of 100–150 Å; less often, particular matter with the same appearance is observed in an eccentric position in the body (Figs. 5, 9). These inclusions do not show any topographical *rapport* with the nucleoli or with the nuclear membrane. The nuclear envelope shows pores with a diameter of 500–700 Å; the outer nuclear membrane is studded with ribosomes.

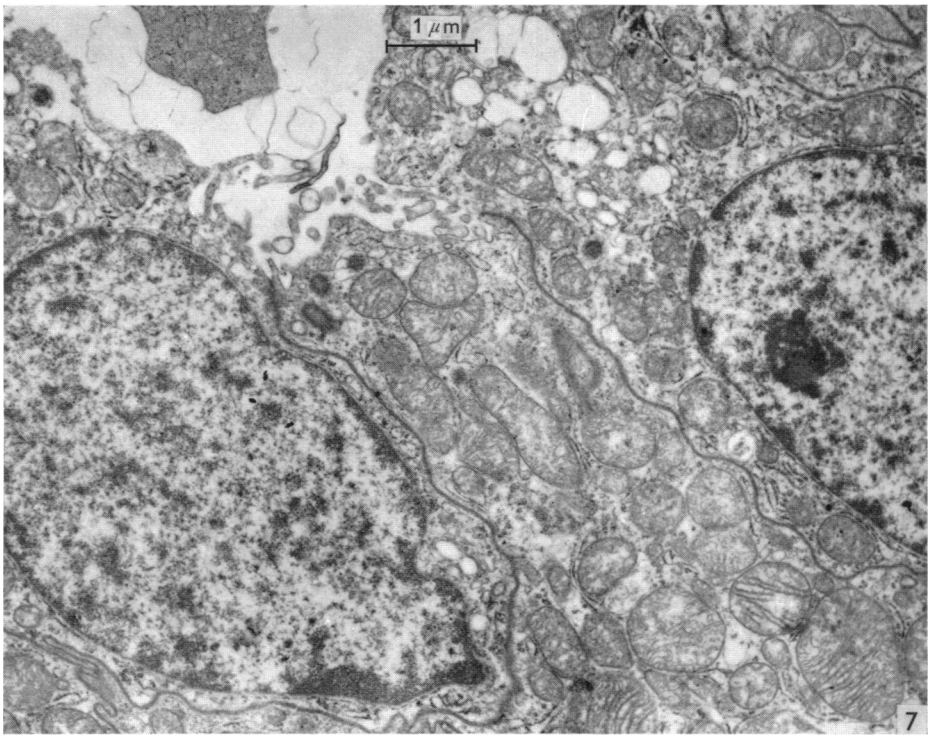
The principal cells contain a very large number of mitochondria. They are situated throughout all the cytoplasm, being particularly numerous in the perinuclear regions. In several cells these organelles occupy most of the cytoplasm (Figs. 4, 7). They have a spherical or ovoid shape and are of variable size; a few reach a diameter of 2 μm. The cristae are well developed and tend to traverse the entire breadth of the organelle (Figs. 7, 11). The mitochondrial matrix is of rather low electron density and shows a finely granular appearance. Mitochondrial granules are seldom seen and, if present, are inconspicuous. Very often cisternal profiles of the granular endoplasmic reticulum are seen wrapping around the mitochondria (Figs. 8, 11). Rough-surfaced endoplasmic reticulum is abundant in these cells. It consists of cisternae, frequently dilated, which are placed irregularly throughout all the cytoplasm including the apical part (Fig. 11).

In the specimens fixed in glutaraldehyde and postfixed in osmium, the cisternae are dilated and contain a flocculent material of low electron density (Fig. 11). Using osmium alone, the appearance of the cisternae is similar. The attempts to reduce this distension using a hypertonic fixative have not been successful; sucrose at a concentration of 0·3 M produces a nearly complete destruction of the reticulum (Fig. 12). The cytoplasm of the principal cells also contains a number of free or clustered ribosomes. The smooth reticulum, although present, is observed infrequently in these cells.

Golgi membranes are situated mainly in the supranuclear zone and may occasionally be seen in other regions. Usually they form typical structures consisting of parallel laminae, small vesicles and vacuoles of various sizes (Fig. 11). The vacuoles may contain dense secretory granules which rarely fill them completely. The interval between the secretory granule and the vacuolar wall varies greatly. In the apical cytoplasm, two or more secretory vacuoles may occasionally coalesce (Figs. 2, 5). The granules show rather irregular outlines and are small, rarely exceeding a diameter of 0·2 μm (Figs. 1, 2, 3, 5). Dense material with a similar appearance to that forming the secretory granules is often seen at the luminar surface of the cell, especially between the microvilli (Figs. 2, 6). The secretion of the granules into the lumen seems to take place by means of the fusion to the vacuolar wall with the cellular membrane (Figs. 3, 6). In addition to the secretory granules, many lipid droplets are present in these cells, particularly in their basal

Fig. 5. An intercellular canaliculus (*Ic*), communicating with the lumen (*L*) passes between the principal cells. A junctional complex (*JC*) is observed between two opposite cells across the canaliculus. Two filamentous nuclear bodies (*NB*) are present in the same nucleus.

Fig. 6. Many twisted irregularly spaced microvilli are seen on the luminal border. Discharged secretory material is also observed (arrows). Glutaraldehyde-fixation; uranyl acetate stain.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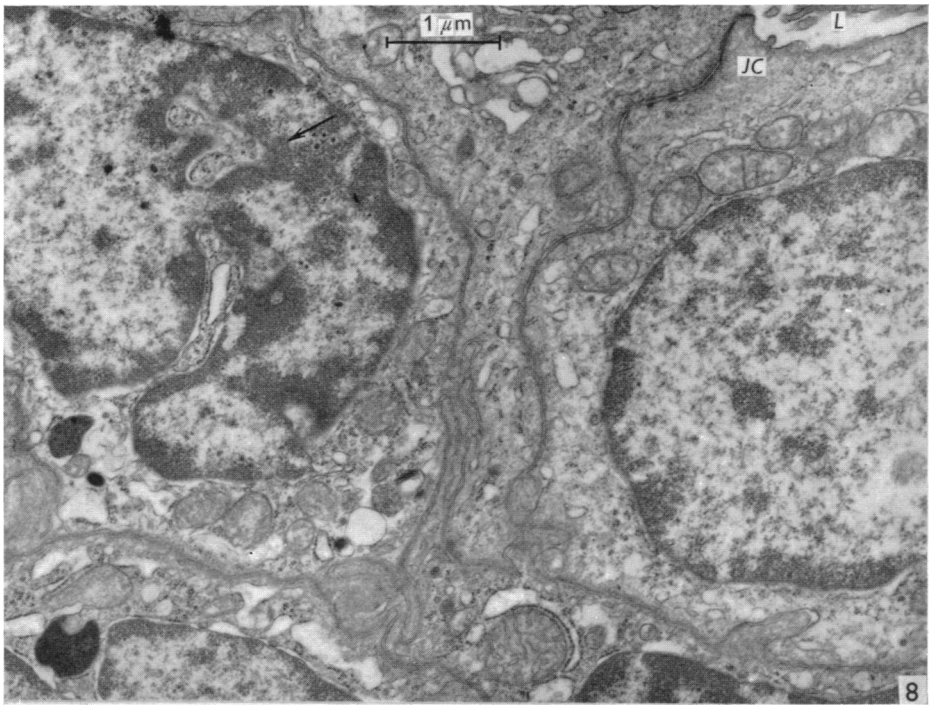
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portion (Figs. 2, 4). They are distinguished by their spherical shape, high electron density and lack of a limiting membrane. A third type of inclusion seen in the principal cells is in the form of irregular dense bodies of various shape and size. These bodies occur frequently in the basal region of the cells (Fig. 4) but they are present also in the apical cytoplasm.

Particularly evident in these cells are structures usually associated with the process of absorption. They consist of a number of micropinocytotic vesicles and vacuoles (Fig. 11) lying just beneath the apical border. Invaginations of the cellular membrane are observed (Fig. 3). The frequency of these findings varies from cell to cell, being possibly related to different functional states.

Basal cells

The basal cells are small stellate cells occasionally present in the angle between the bases of two principal cells (Figs. 1, 2, 4). Their cytoplasm is more compact than that of the principal cells with fewer mitochondria and other organelles. Of the three types of inclusions present in the principal cells, only lipid droplets are observed in the basal cells. The nucleus is very rich in particulate chromatin and usually lacks a nucleolus. In spite of their basal position in the epithelium, many of them do not lie on the basal lamina in all their length, but are in contact with it only by means of short cytoplasmic digitations (Fig. 1, 4).

The basal lamina (Figs. 1, 4) is a moderately dense band having a thickness of about 500 Å; it follows the basal contour of the epithelium invariably. A thick layer of dense connective tissue which contains numerous blood vessels lies subjacent to it.

Content of the lumen

Apart from the secretory granules, recognizable only near the cellular surface, the content of the lumen is represented by masses of dense, amorphous substance (Fig. 2) and by debris of cellular origin (Fig. 3). The latter consists mainly of fragments of cytoplasm, not surrounded by a membrane, and of free organelles in particular mitochondria, more or less degenerated (Fig. 3).

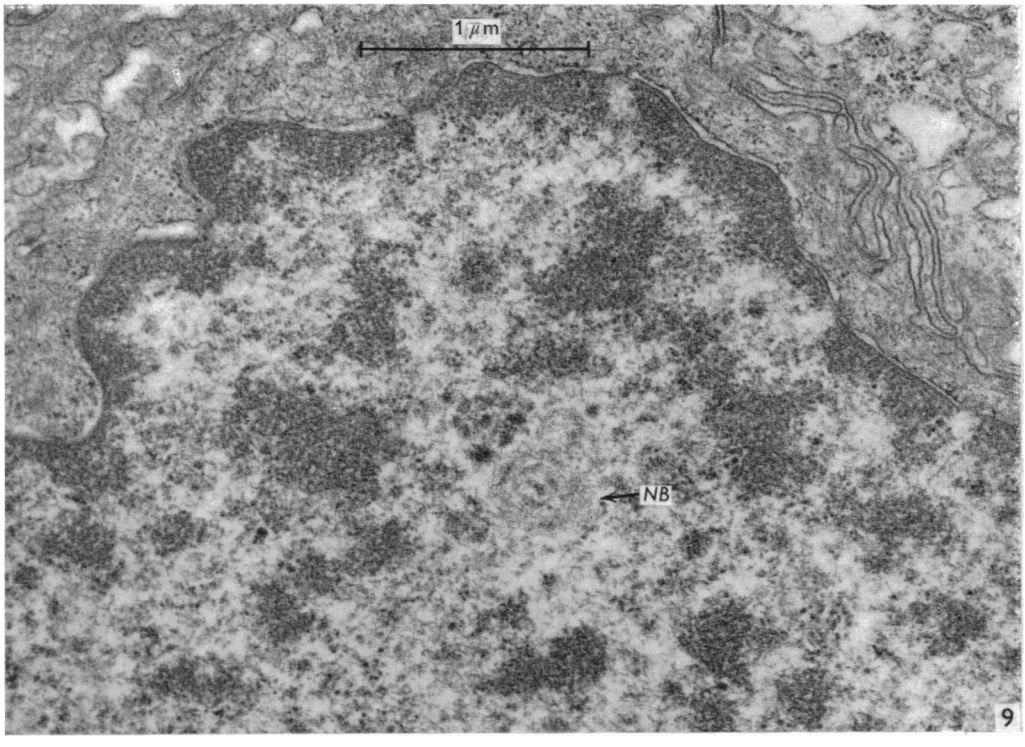
DISCUSSION

Although the fine structure of the human seminal vesicle epithelium is similar to that described for rodents many distinctive characteristics have been observed.

The most striking of these is represented by the ruffled, irregular appearance of the epithelium caused by the projections of apical portions of cells into the lumen. This feature in man appears far more evident and extensive than in the homologous organs in the mouse (Deane & Porter, 1960; Toner & Baillie, 1966), hamster (Cavazos, Belt, Sheridan & Feagans, 1964) and rabbit (Schantz, 1964). It seems to be related to a functional state of cellular activity, since the bulging apical portions

Fig. 7. Apical cytoplasm of a principal cell packed with mitochondria. Glutaraldehyde-osmium fixation; lead citrate stain.

Fig. 8. Deep fissures (arrows) in the nucleus of a principal cell. A complete junctional complex (*JC*) is seen subjacent to the lumen (*L*). 72-year-old subject.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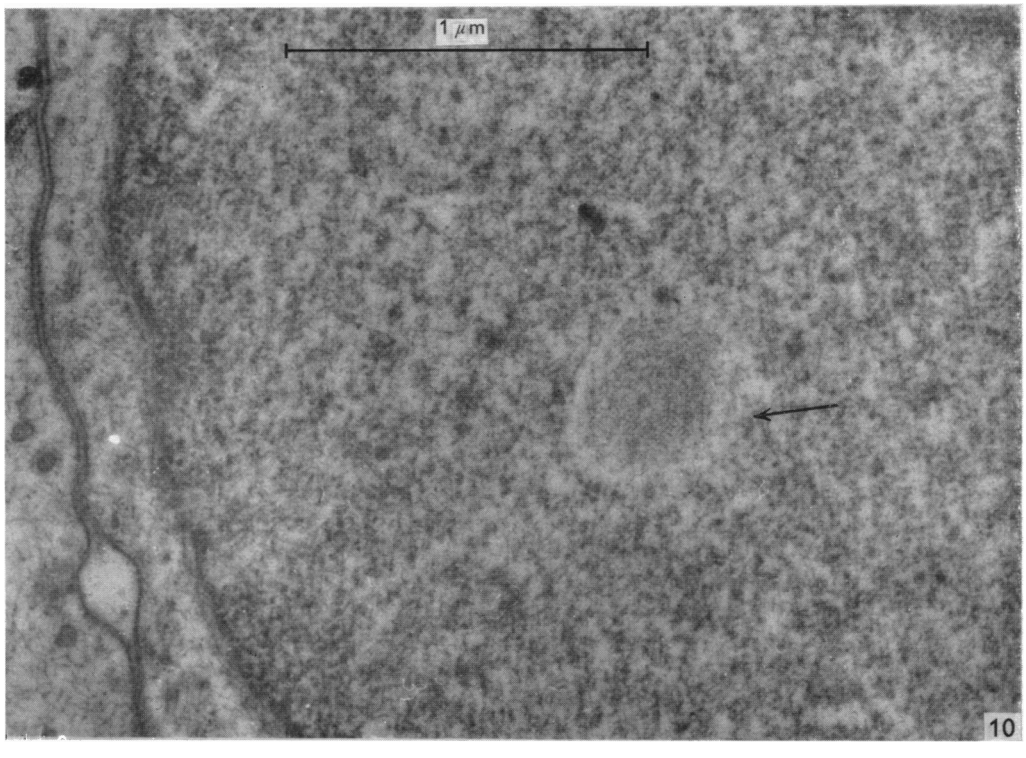
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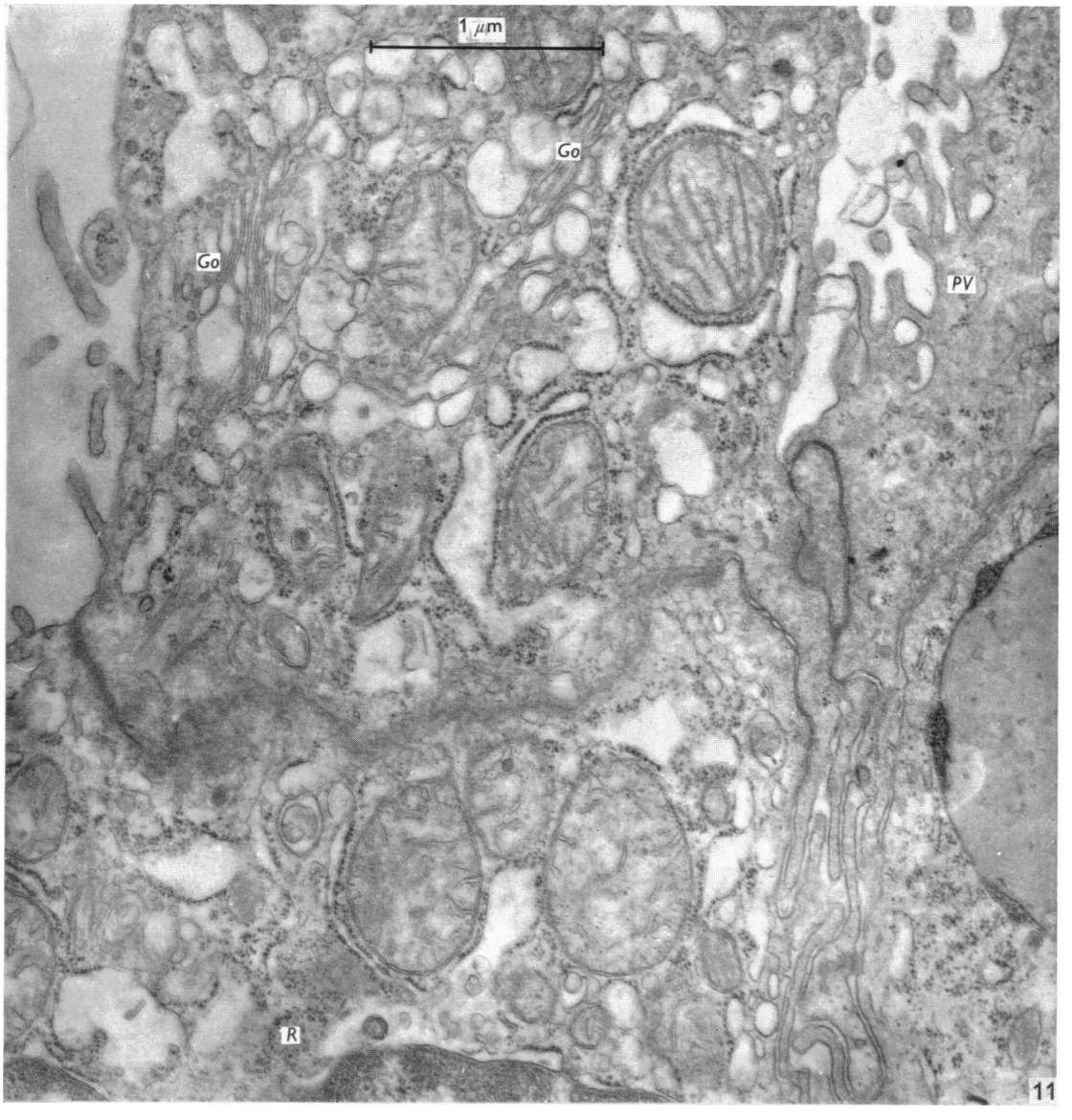

Fig. 11. Detail of the supranuclear cytoplasm of principal cells showing the close relationship between the granular endoplasmic reticulum and the mitochondria. Cisternae of the Golgi apparatus (*Go*) and free ribosomes (*R*) are also present. Beneath the apical membrane pinocytotic vacuoles (*PV*) can be seen.

Fig. 9. Rosette-shaped nuclear body (*NB*) in the nucleus of a principal cell. Glutaraldehyde-osmium fixation; lead citrate stain.

Fig. 10. Nuclear bodies with the structure shown in this picture (arrow) are the type more commonly observed.

usually contain numerous secretory granules. Moreover, the epithelium of the oldest subjects, which shows little evidence of secretory activity, has a far more regular aspect, the apical parts of the principal cells being much less prominent. This finding at the ultramicroscopical level is analogous to the appearance of the mucosa *in toto*; light microscopists (Kurosawa, 1930; Vitali-Mazza, 1956; Nillson,

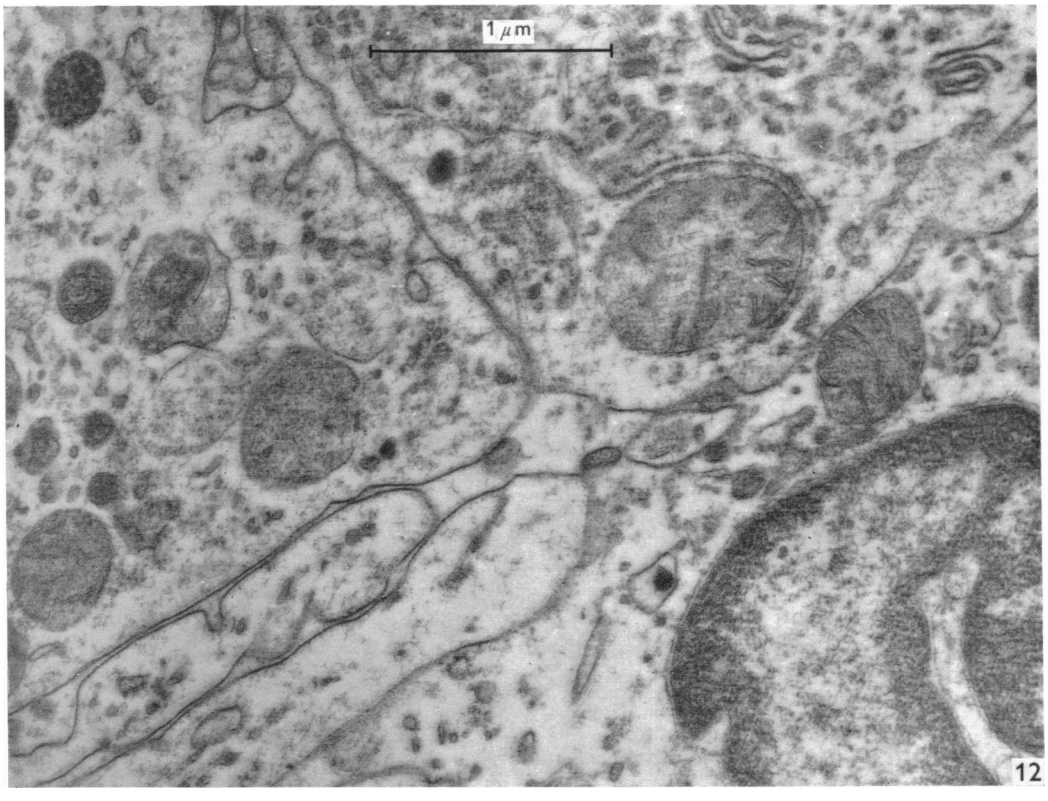

Fig. 12. Cytoplasm of principal cells after fixation with osmium containing 10% of sucrose. The disruption of the endoplasmic reticulum is evident.

1962) have demonstrated that the mucosal relief decreases greatly with age. The extensive projection of the principal cells into the lumen could also produce an increase of surface area related to absorption processes. Considerable evidence of pinocytosis is observed; similar findings have been reported by various authors in rodents (Deane & Porter, 1960; Deane, 1963; Cavazos *et al.* 1964; Deane & Wurzelman, 1965). In the epithelium of developing and adult mice, Deane & Wurzelman (1965) consider it to be evidence of a process of constant resorption of secreted material. A similar process of resorption seems likely to occur in the human seminal vesicle, for signs of active secretion and an extensive secretory area accompany only a small storage capacity (Mann, 1964).

The presence in the lumen of material consisting of cellular debris and cytoplasmic

organelles has been reported previously only in the developing epithelium (Deane & Wurzelman, 1965). According to Mann (1964, p. 38) it represents a feature typical of secretion, common to all the male secondary sex organs. Attempts to correlate these findings with signs of apocrine or holocrine secretion were inconclusive, possibly because of the surgical origin of the specimens studied. Occasional signs of cellular degeneration, such as the presence of cells with swollen mitochondria, may represent simply the effect of cellular anoxia by ligature of blood vessels during operations. It should be noted that coexisting pathological states of the bladder or prostate have little detrimental effect on the morphology of the seminal vesicle. In an investigation carried out on autopsy cases with large prostatic adenomata, Nillson (1962) was able to exclude pathological variations in the morphology of the seminal vesicle.

The very large number of mitochondria present in most principal cells represents another distinctive character. Although in the rodents abundant mitochondria are present (Orlandini, 1964*b*, 1966; Cavazos *et al.* 1964), their number is even greater in the human epithelium. These organelles evidently play an important role in the functional activity of the seminal vesicle since Orlandini (1964*a*, *b*, 1966) and Allison (1964) describe a depletion of mitochondria in the castrated rat; this finding, however, has not been confirmed by Toner & Baillie (1966) in the mouse.

The large amount of rough-surfaced endoplasmic reticulum confirms the experimental evidence that the seminal vesicle is concerned with the synthesis and secretion of proteins (Porter & Melampy, 1952; Wilson, 1962; Kochakian, 1964). In man the endoplasmic reticulum is seldom observed in the form of concentric parallel layers of cisternae as reported in rodents (Deane & Porter, 1960; Szirmai & van der Linde, 1962, 1965) and as seen in the bull (Riva, unpublished observation). The cisternae of the rough-surfaced endoplasmic reticulum are frequently dilated, showing a flocculent content of very low electron density. The degree of dilatation is not comparable with the coarse distension of the ergastoplasmic channels observed by Deane (1963) in the mouse. This author interprets the distension as an osmotic effect occurring during fixation, and postulates that substances with osmotic activity —for example, fructose or its precursors—were present inside the cisternae. She was able to obtain a marked regression of the distension, without any cellular shrinkage, using a fixative made hypertonic with sucrose (0·3 M). In the present study a fixative containing the same molarity of sucrose did not produce a satisfactory morphological result in the human tissue, particularly with regard to the endoplasmic reticulum. The significance of this finding is obscure; it may indicate, *inter alia*, a different osmotic activity in the content of the endoplasmic reticulum in man and mouse. Certainly it has been observed using biochemical methods (Mann, 1964, chapter 4) that the secretions of the seminal vesicle in the two species are not identical, although similar. Both contain fructose, but no citric acid is found in the human secretion.

Like the homologous organs of the hamster (Cavazos *et al.* 1964), the rat (Allison, 1964; Orlandini, 1964*b*, 1966) and the mouse (Toner & Baillie, 1966) the principal cells of the epithelium of the human seminal vesicle contain numerous, irregular, dense bodies. Although they are generally regarded as lipofuscin granules (Cavazos *et al.* 1964; Orlandini, 1966) there is still some discussion about their histochemical identification, especially in the mouse (Toner & Baillie, 1966). Histochemical in-

vestigations now in progress may clarify their nature and possible significance in the human seminal vesicle.

No nuclear inclusions have been recorded in the epithelial cells of the homologous organs in rodents and rabbits. A nuclear body similar to those present in the human epithelium, although twice the size, has been observed in the bull seminal vesicle (Riva, unpublished). Apart from the findings of Horstmann (1965) and Horstmann *et al.* (1966) in the canine and human epididymis, few descriptions of nuclear bodies whether in plant (Lafontaine, 1965) or animal cells are found in the literature. They have been noted in the human skin (Brody, 1962), rat kidney (Latta & Maunsbach, 1962; Farquhar & Palade, 1962), hamster liver (Jones & Fawcett, 1966) and in various parenchymal and interstitial cells of both the male and female calf (Weber & Frommes, 1963). In addition, nuclear inclusions have been observed in neoplastic cells (Bernhard & Granboulan, 1963).

Weber & Frommes (1963) who have reported the occurrence of these bodies in the calf, find them to be particularly well developed in the parenchymal cells of the adrenal cortex. These authors describe the inclusions as spheroidal bodies having a diameter of 0·8–1·2 μm and consisting of a fibrillar outer portion and a central-core area of varying size composed of electron-dense particulate matter. They also note a close topographical relationship with the nucleolus. On a morphological basis the bodies described by Weber & Frommes are indistinguishable from those seen in the bull seminal vesicle.

Examining their properties in the adrenal gland of calf, Weber, Whipp, Usenik & Frommes (1964) note that they are not seen at any mitotic stage and that the inclusions present in the nuclei of the zona fasciculata undergo structural changes after treatment with ACTH. Horstmann (1965) finds three types of nuclear inclusion in the epithelium of the canine epididymis, of which one is similar to, but roughly half the size of the analogous bodies described by Weber & Frommes (1963) in the calf. Horstmann considers this type to be the forerunner of the homogeneous globules also demonstrated with the electron microscope which, as 'nuclear spheres', were first noted by Hammar (1897) in the dog's epididymis.

Nuclear bodies and homogeneous globules have also been observed by Horstmann *et al.* (1966) in the epithelial cells of human epididymis. Although homogeneous globules have not been found in the course of present investigations, the nuclear bodies seen in the human epididymis are very similar to those present in the epithelium of human seminal vesicle.

Comparing the morphological characteristics of the nuclear bodies in all animals investigated, it may be speculated that these inclusions possess dimensions and structure peculiar to the species or at least the genus. Such an observation requires for its confirmation the comparative study of a wide range of animals.

SUMMARY

Two types of cells, principal and basal, are discernible with the electron microscope in the human seminal vesicle epithelium.

The principal cells are slender columnar cells. Their apical portions, endowed with microvilli, project into the lumen, bulging in it. The cytoplasm of the principal

cells contains numerous mitochondria and a well-developed rough-surfaced endoplasmic reticulum. The Golgi apparatus, situated in the apical portion, is characterized by a number of secretory vacuoles. These vacuoles may contain a dense homogeneous granule, usually much smaller than the vacuole itself. Apart from nucleoli, one, or more seldom two, inclusions with a filamentous appearance are often observed in the nuclei of the epithelial cells. The inclusions do not show any relationship with the nucleolus or with the nuclear membrane.

The basal cells are small stellate cells, occasionally present in the angle between the bases of two principal cells. They are distinguished by a more compact cytoplasm with few cytoplasmic organelles.

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