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Ultrastructural changes in the prostate gland of a seasonally breeding mammal, the grey squirrel (*Sciurus carolinensis* Gmelin)

ANDREW A. SIWELA* AND W. H. TAM

Department of Zoology, University of Western Ontario, London, Ontario N6A 5B7, Canada

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INTRODUCTION

The fine structure of the prostate gland has been studied in detail only in a few domestic species such as man (Brandes, Kirchheim & Scott, 1964; Fisher & Jeffrey, 1965), dog (Seaman & Winnell, 1962), rat (Dahl, Kjaerheim & Tvester, 1973), mouse (Brandes & Portela, 1960) and rabbit (Nicander, Ploen & Larsson, 1974). Similarly, observations on ultrastructural changes resulting from a brief duration of experimentally induced androgen deprivation have been confined only to rats (Dahl & Kjaerheim, 1973; Brandes, 1974) and dogs (Ofner, Leav & Cavazos, 1974). There is no report on changes in the fine structure of the prostate as a consequence of reduced androgen levels over a prolonged period, such as can be found during the sexual quiescence of seasonal breeders. The aim of this study is to document the ultrastructural changes that occur in the prostate gland of the grey squirrel (*Sciurus carolinensis*, Gmelin) as it passes from the sexually active into the inactive phase. The periods of breeding and of sexual quiescence in grey squirrels in Ontario are known to occur between January–June and July–November, respectively (Siwela & Tam, 1983). That the weight of the prostate gland changes gradually from 225 mg/100 g body weight during the breeding season to 50 mg/100 g body weight during the sexually inactive period has also been reported (Siwela & Tam, 1983).

MATERIALS AND METHODS

Grey squirrels were either live-trapped unharmed with traps of the wire cage type or were shot throughout the year in the immediate suburbs of London, Ontario, Canada. Criteria of sexual maturity (depending on body weight and the occurrence of spermatogenesis during the breeding season, or the presence of spermatogenic debris of the previous breeding season during the sexually inactive phase) have been reported elsewhere (Siwela & Tam, 1981). In the case of the squirrels that were shot, the prostate gland was immediately removed and processed in the field. Animals that were live-trapped were taken back to the laboratory and killed with an overdose of diethyl ether on the same or on the following day. After removal, the prostate was placed either in 2–5 % glutaraldehyde in 0·1 M Sorensen's phosphate buffer (pH 7·4) and maintained at 4 °C, or in Bouin's fixative. The glutaraldehyde-fixed tissue was cut into 1 mm cubes and immersed in fresh glutaraldehyde for two hours. After

* Present address: Department of Biology, University of Zambia, P.O. Box 32379, Lusaka, Zambia.

several rinses in buffer, the tissue was post-fixed in 1 % phosphate-buffered osmium tetroxide for one hour, passed through a buffer rinse, dehydrated in alcohol and propylene oxide, and then finally embedded in Epon 812. Sections (1–2 μm thick) were cut on a Reichert OM-U3 ultramicrotome, mounted on glass slides and stained in 0·5 % toluidine blue in 0·25 % sodium borate (pH 7·8) for examination by light microscopy. Thin sections were mounted on uncoated copper grids, stained with aqueous uranyl acetate and lead citrate, and then examined with a Philips 201 electron microscope. The tissue fixed in Bouin's fluid was embedded in paraffin, sectioned at a thickness of 8 μm and stained with haematoxylin and eosin for observation with the light microscope.

The presence of acid phosphatase was demonstrated by the modified Gomori method (Holt, 1959). After fixation and rinse, the tissue was incubated in a Dubnoff metabolic shaking incubator for 60 minutes at 32 °C, using sodium β-glycerophosphate as substrate. Control sections were incubated in the absence of the substrate. After post-fixation, thin sections were cut and viewed unstained with the electron microscope.

RESULTS

Light microscopy

The prostate gland of the grey squirrel was a single lobed, compact structure. Internally, it was composed of numerous identical lobules, each of which was made up of many tubulo-alveolar secretory units (Fig. 1). In mature animals, secretory droplets could be seen in the lumen of the secretory units during the sexually active months of January–June. The secretory epithelial cells could be divided into two morphological types. In glutaraldehyde-fixed tissue, 'Type I' cells had larger nuclei and lightly staining cytoplasm. 'Type II' cells were darkly staining and had smaller and denser nuclei (Fig. 2). In material preserved in Bouin's fluid, although the characteristics of both nuclei remained the same, the difference in cytoplasmic staining intensity of the two cell types could hardly be observed. Both morphological types were columnar cells set at right angles to the longitudinal axis of the lumen of the secretory unit. Prostatic involution began in July and by August and September

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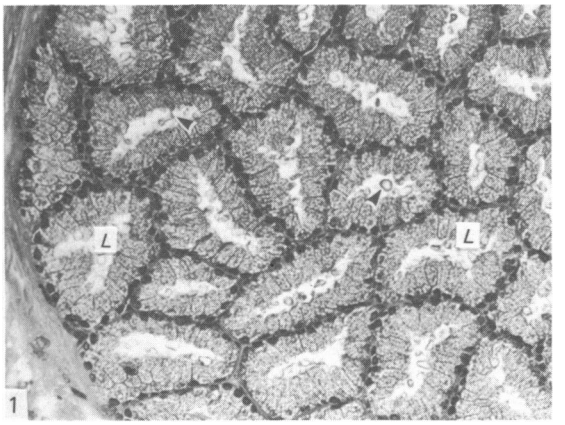
Fig. 1. The prostate gland of a sexually active mature male obtained in February. This photomicrograph of a single lobule shows that each lobule is subdivided into a number of secretory units or acini, each with a central lumen (*L*) surrounded by tall columnar epithelial cells with a basal nuclei. Secretory droplets (arrowheads) can be seen. × 340.

Fig. 2. In glutaraldehyde-fixed and toluidine blue-stained tissue. two morphological types of cells can be seen in the secretory epithelium. 'Type I' cells (arrowheads) are less numerous and their cytoplasm is lightly stained. 'Type II' cells (arrows) are more numerous and their cytoplasm appears to be more dense and contain many vacuoles. × 800.

Fig. 3. The fully regressed prostate gland of an adult obtained in September. The lobules (*LB*) resemble solid masses of cells. The lumina of the secretory units are completely occluded. × 660.

Fig. 4. One of the distinguishing factors between 'Type I' (*I*) and 'Type II' (*II*) cells is the nucleus. 'Type I' cells have a much larger nucleus with a narrower, peripheral rim of heterochromatin and prominent nucleoli. The smaller and more irregular outlined nucleus of the 'Type II' cells has a wide band of peripheral heterochromatin and darker nucleoplasm. The vacuoles in 'Type II' cells give them a spongy appearance. However, both cells abut the basal membrane (*BM*) and stretch out to the lumen of the secretory unit. *LI*, lipid droplet. × 9000.

Fig. 5. In the subcellular region as in all other parts of these cells, rough endoplasmic reticulum (*RER*) and free ribosomes (arrowheads) are the prominent features of 'Type I' cells. Unlike other species, cisternae of the granular endoplasmic reticulum are typically narrow in grey squirrels, even at a time of secretory activity. A few mitochondria (*M*) can also be found. × 27200.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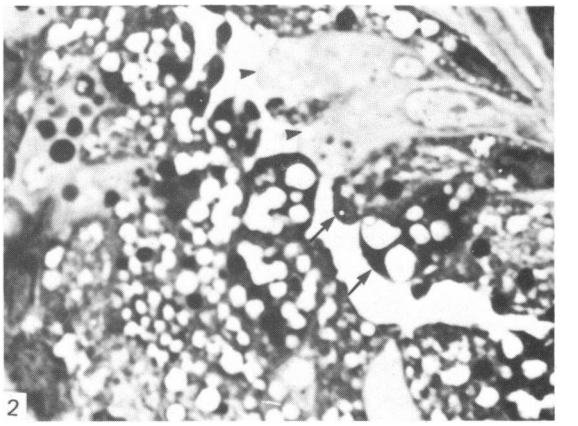
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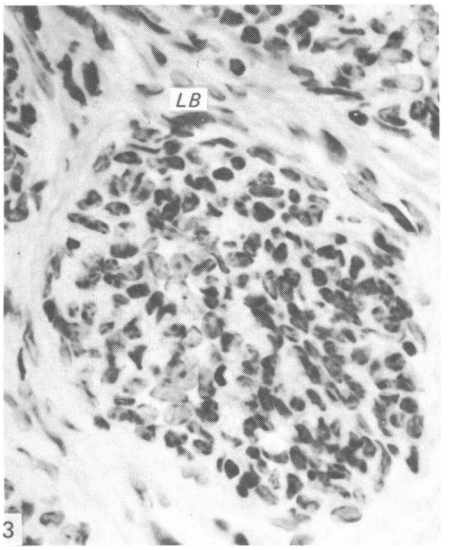
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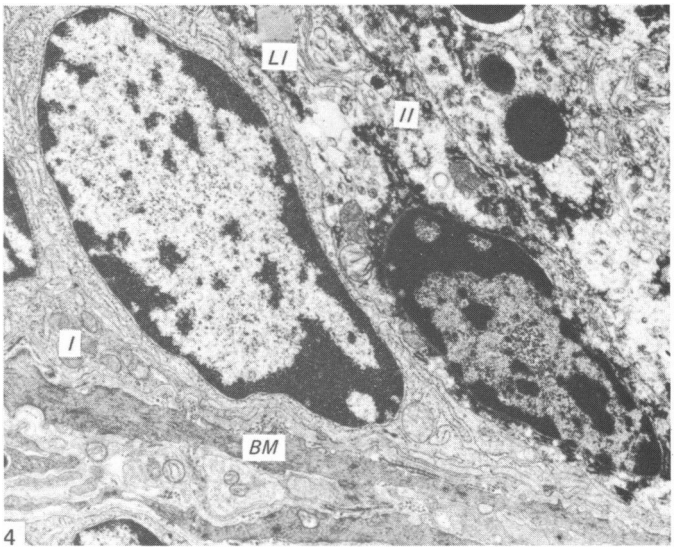
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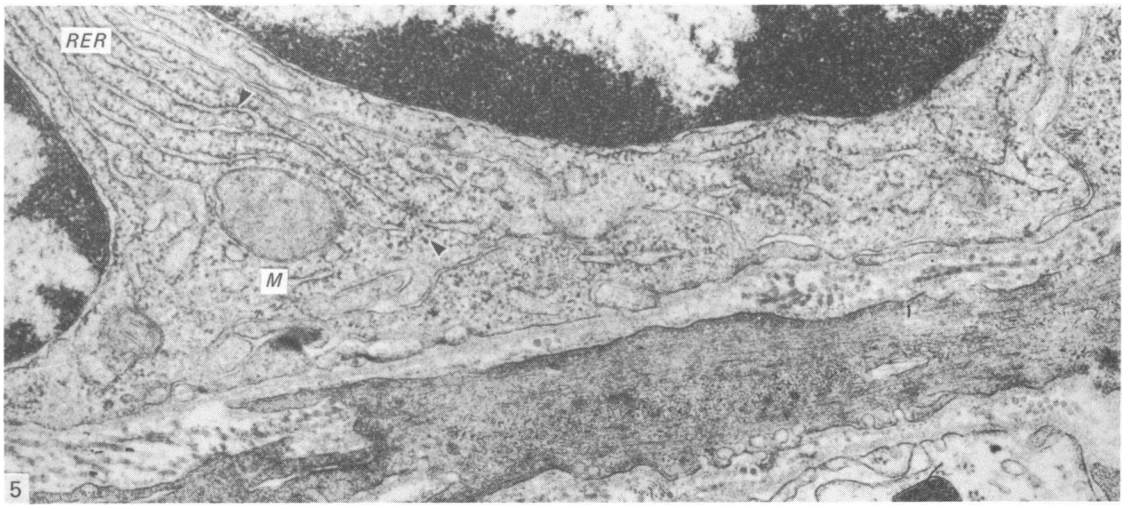
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the process was complete. The lumina of the secretory units were obliterated and the lobules resembled solid masses of atrophic cells (Fig. 3). Mitotic activity could be observed in the lobules throughout October and December, but due to the atrophic conditions of the cells it could not be seen whether mitosis occurred in one or both types of cell. This wave of mitotic activity was followed by the very slow functional recovery of the tubulo-alveolar secretory units. Lumina began to reappear by November, but the epithelial cells, still lacking their full complement of cytoplasm, were cuboidal in shape. The epithelial cells typically became tall columnar in form by December, but secretory droplets were not seen until January.

Ultrastructure of the active gland

The 'Type I' cell had a large elongated basal nucleus, surrounded by a thin layer of cytoplasm at the sides and on its basal aspect (Figs. 4, 5). Most of the cytoplasm was supranuclear, where the rather simple Golgi apparatus was confined (Fig. 6). Small secretory vesicles, apparently formed from the Golgi apparatus, and some lipid droplets could also be seen. More prominent were the numerous large osmiophilic secretory granules (or dense bodies) found in this region (Fig. 7). They condensed into smaller granules as they approached the luminal border where their membranes appeared to fuse with the plasma membrane before the granule contents were finally released by exocytosis (Fig. 8). Granular endoplasmic reticulum and free ribosomes were abundant in all parts of the cell. Acid phosphatase activity could be demonstrated in the granular endoplasmic reticulum (Fig. 9).

'Type II' cells were more abundant than 'Type I' cells, but their nuclei were smaller and had a rather irregular contour (Fig. 4). The Golgi apparatus was more elaborate and appeared as stacks of dilated sacs (Fig. 10). Scattered throughout the cytoplasm were numerous secretory vesicles, containing a finely granular material. Numerous vacuoles were also found in the cytoplasm and these vacuoles gave the 'Type II' cells a spongy appearance. Lipid droplets were more abundant than in 'Type I' cells. Dense bodies, granular endoplasmic reticulum and free ribosomes were seldom encountered in 'Type II' cells. However, the frequency of occurrence of mitochondria seemed to be similar in both types of cells. Acid phosphatase activity was histochemically demonstrable in secretory vesicles and vacuoles as well as in dense bodies of 'Type II' cells (Figs. 9, 11).

Ultrastructure of the inactive gland

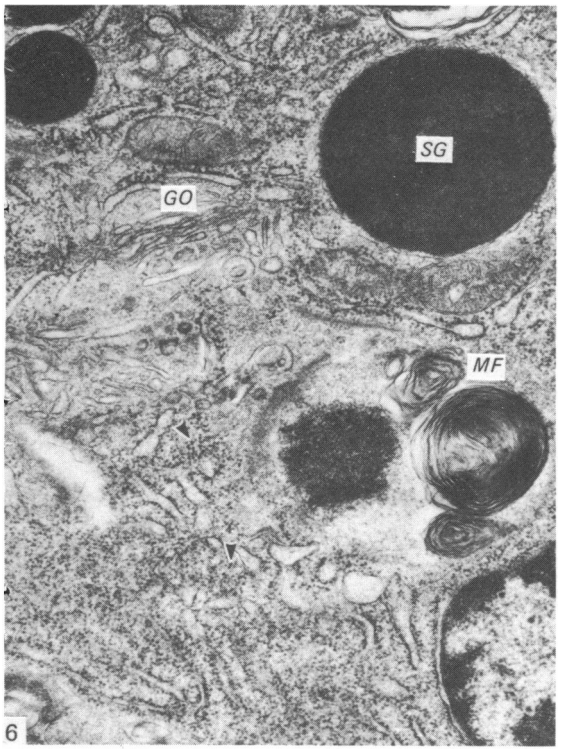
Both types of cell could be clearly distinguished during the sexually inactive months of July–December (Fig. 12). The characteristics of the two nuclei remained

Fig. 6. Electron micrograph of the supranuclear region of a 'Type I' cell. Occasional myelin figures (*MF*) are encountered. However, granular endoplasmic reticulum, ribosomes (arrowheads), a few Golgi membranes (*GO*) and an abundance of osmiophilic secretory granules (*SG*) are characteristic of this region. Secretory vesicles are usually seen adjacent to the Golgi apparatus. × 16500.

Fig. 7. A photomicrograph showing the numerous osmiophilic secretory granules (*SG*) found in the supranuclear region of 'Type I' cells. *LY*, lysosomes. × 10500.

Fig. 8. The osmiophilic secretory granules gradually reduce in size as they approach the luminal end of 'Type I' cells. Secretory granules (*SG*) in the process of being released into the lumen (*L*) of the acinus can be seen in this micrograph. Some of the vesicles may become confluent with each other before releasing their contents (arrowheads). *MV*, microvilli. × 10400.

Fig. 9. In the actively secreting prostate gland, acid phosphatase (arrowheads) can be demonstrated in the granular endoplasmic reticulum of 'Type I' cells (*I*), and in the dense bodies (*DB*), secretory vesicles and vacuoles (*VA*) of 'Type II' cells (*II*). × 28400.



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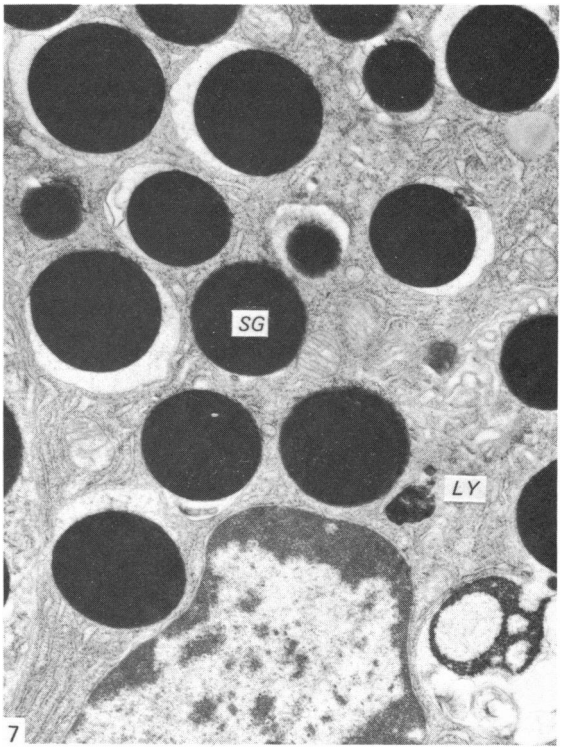

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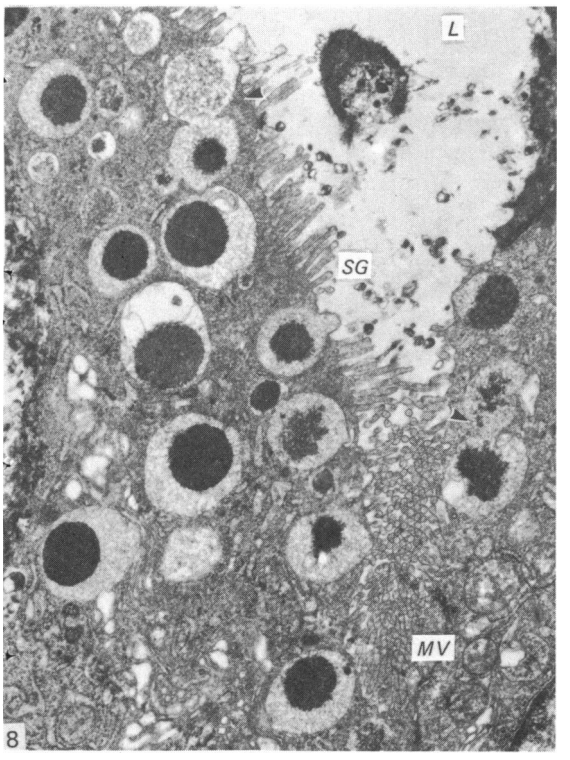

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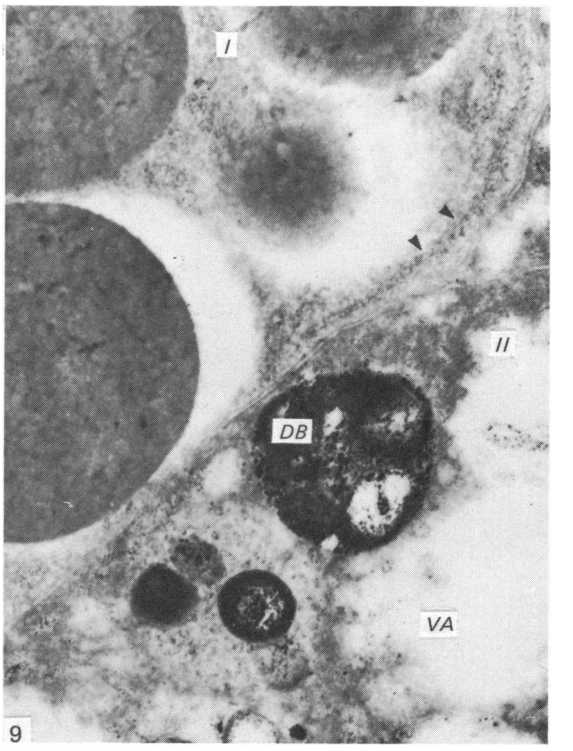

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the same. The cytoplasm of 'Type I' cells remained pale while that of 'Type II' cells continued to be dark in glutaraldehyde-fixed tissue. However, in both cases, the organelles associated with secretory activity had almost completely disappeared. In 'Type I' cells, granular endoplasmic reticulum was rare; if found, it was reduced to small vesicles rimmed with ribosomes. Osmiophilic secretory granules and the Golgi apparatus could no longer be found. In 'Type II' cells, the Golgi apparatus and the numerous secretory vesicles of the active gland were now absent. In both types of cell, the greatly reduced cytoplasm was represented by a narrow band surrounding the nucleus and by thin cytoplasmic strands leading from the nucleus. Interspersed amongst the two types of cell were occasional macrophage-like cells containing cellular debris and numerous lysosomes and autophagic vacuoles. A few cells, containing membrane-bound structures with a lipid-like content were also encountered. These cells had relatively long spindle shaped nuclei and very few cytoplasmic organelles. During the non-secretory period, acid phosphatase could not be demonstrated in either 'Type I' or 'Type II' cells, but was detected in the lysosomes and autophagic vacuoles of the macrophage-like cells.

Ultrastructure of the immature gland

'Type I' and 'Type II' cells could be distinguished even in immature grey squirrels (Fig. 13), by the large nucleus and, in glutaraldehyde-fixed material, the pale cytoplasm of 'Type I' cells, as compared with the heavily heterochromatin laden, intensely staining nucleus and the darker cytoplasm of 'Type II' cells. They were similar to the cells found in the adult during the non-secretory period in that there were very few organelles. However, immature gland cells differed markedly from those of the inactive adult in that they were cuboidal, instead of elongate and atrophic as in the inactive adult.

DISCUSSION

By using more than one method of fixation and staining and by studying the ultrastructure of the squirrel prostate gland, two morphological forms of secretory cells can be observed instead of the single uniform epithelial cell described earlier by Mossman, Hoffman & Kirkpatrick (1955). The two morphological forms of epithelial cell differ from one another in many respects. Throughout the year, the two nuclei are always different and the two types of cytoplasm demonstrate different

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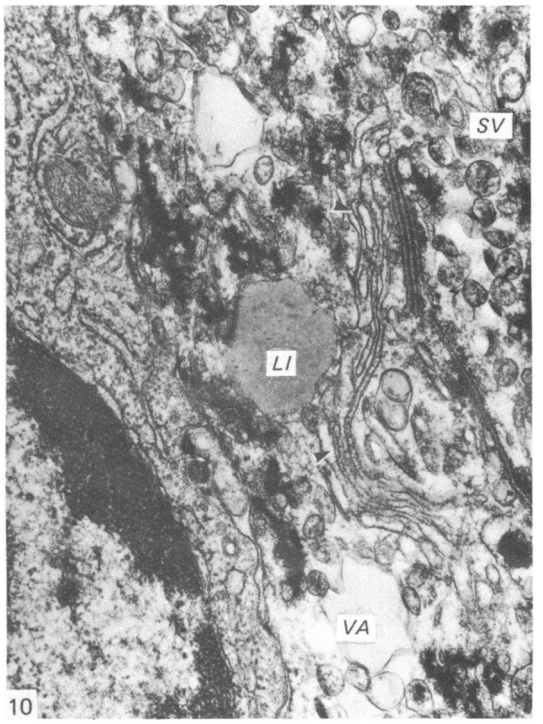
Fig. 10. The supranuclear region of a 'Type II' cell contains vacuoles (*VA*), lipid droplets (*LI*), numerous small secretory vesicles (*SV*) and a relatively more substantial Golgi apparatus (arrowheads). Large osmiophilic secretory granules are rare. A portion of a 'Type I' cell can be seen at the left hand side of the picture. × 24700.

Fig. 11. Electron micrograph showing acid phosphatase activity in the secretory vesicles (*SV*) and the walls (arrowheads) of the vacuoles (*VA*) in 'Type II' cells of a sexually active animal. × 65000.

Fig. 12. Electron micrograph showing a portion of the secretory acinus in a fully regressed prostate. 'Types I' (*I*) and 'II' (*II*) cells can easily be distinguished by features of the nucleus and cytoplasm. The lumen (*L*) and the microvilli (*MV*) of both types of cells are greatly reduced. Note the elongated shape of both types of cells and the absence of organelles in their cytoplasm. Tight junctions (arrowheads) are more evident in the regressed gland. × 8260.

Fig. 13. Even in the immature squirrel, 'Type I' (*I*) and 'II' (*II*) cells are easily distinguishable by the nuclei and the paler (Type *I*) and denser (Type *II*) nature of the cytoplasm. No secretory activity nor organelle associated with such activity can be seen. Also, these cells are not elongated and therefore they cannot be atrophic adult cells. × 10300.



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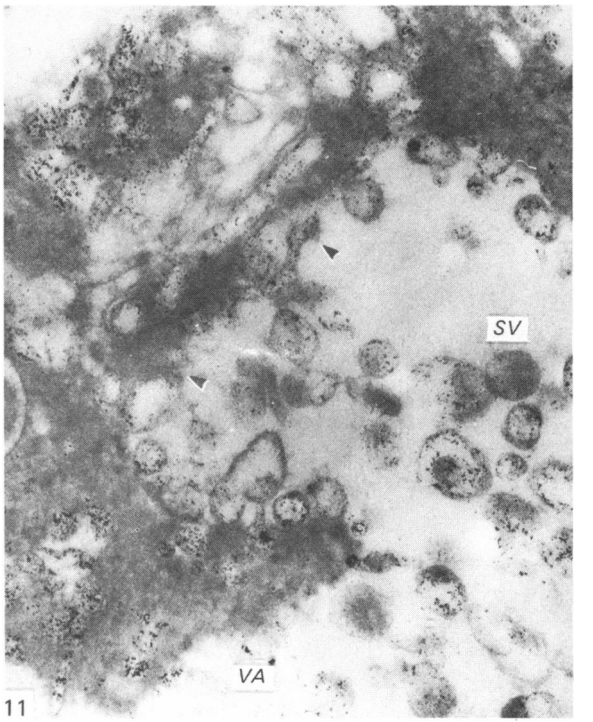

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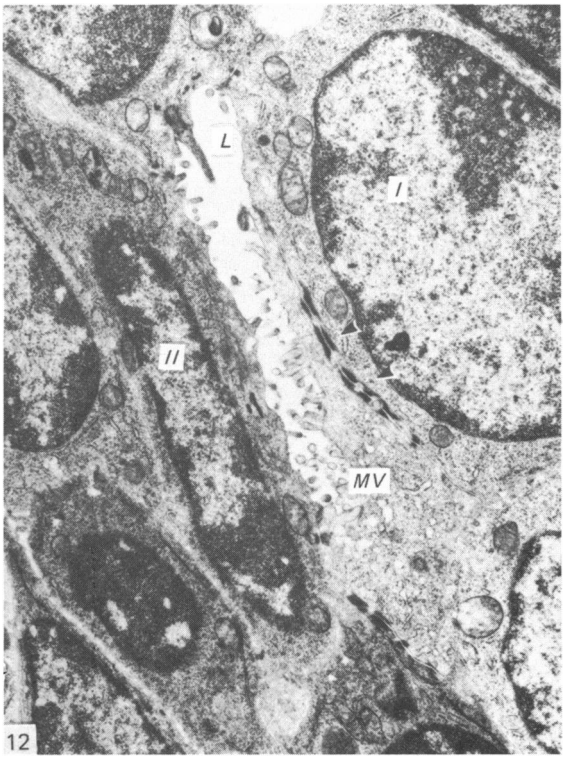

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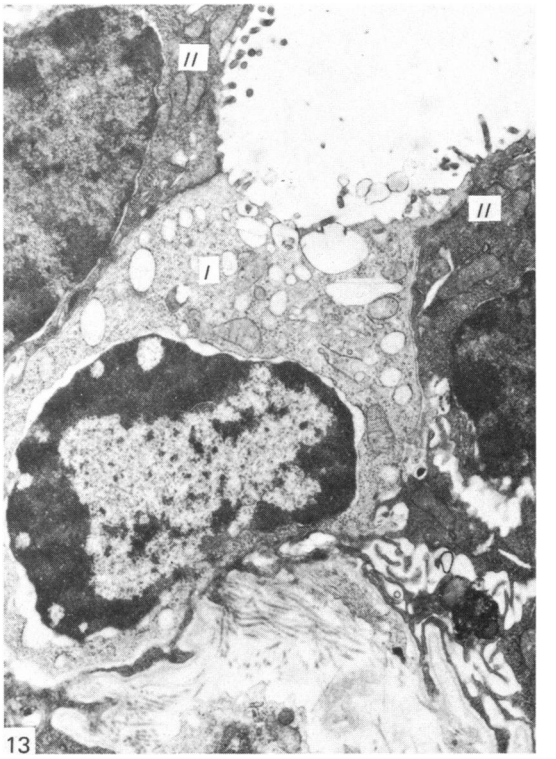

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staining properties after glutaraldehyde fixation. These basic differences are also found in immature animals and in mature grey squirrels during the sexually quiescent period. In the sexually active phase, both forms of cell develop further qualitative and quantitative differences in organelles, vacuolation and secretory activity. The 'Type I' cells, rich in granular endoplasmic reticulum and ribosomes, are probably responsible for secretions of a protein nature. The more substantial Golgi complex and abundant secretory vesicles of the more numerous 'Type II' cells are most likely involved in the synthesis of carbohydrates needed for their secretory products (Whaley, Dauwalder & Kephart, 1972).

There are two possible explanations to account for the observed differences in morphology of the secretory cells. The first possibility is that the two forms are two stages of a single cell type, the morphological difference reflecting a different functional status. If this is the case, then the turnover rate of the 'Type I' phase, either on conversion to the 'Type II' phase or when eliminated by degeneration, must be very rapid since only a small number of 'Type I' cells can be seen. The grey squirrel is then remarkable in that it would be the first animal to provide evidence that there are two well demarcated phases in the life span of its secretory cells. The second possible explanation is that there are two distinct populations of secretory cells, each with its own characteristic morphology and secretion. The greater number of 'Type II' cells could be explained by the fact that carbohydrates are generally held to be the major component of seminal plasma (Mann, 1969). If there are two types of secretory cells within the single lobed gland, the prostate gland of the grey squirrel is unique in that its fine structure is intermediate between that found in the rat and in man. The rat prostate gland is divisible into several lobes, each with its own cell type (Brandes & Groth, 1961; Schrodt, 1961) and its own peculiar concentrations of fructose and zinc (Gunn & Gould, 1957). On the other hand, the single lobed human prostate gland seems to have only one type of secretory cell, since the basal cells, normally never having access to the lumen of the acinus, are believed to be non-secretory and are regarded as reserve cells proliferating to replace secretory cells in response to certain stimuli (Spring-Mills & Hafez, 1980). No basal or reserve cell has been seen in the secretory epithelium of the grey squirrel.

The regressed prostate glands investigated in this study were taken from animals in the middle of the non-breeding season when circulating androgens have already been at their lowest level for at least a month (Siwela & Tam, 1981). Thus, the reported progressive reduction of the granular endoplasmic reticulum and the Golgi apparatus (Helminen & Ericsson, 1972; Dahl & Kjaerheim, 1973) and the rearrangement of granular endoplasmic reticulum into whorls (Brandes, 1974) during the initial phase of androgen withdrawal as seen after castration have not been observed here. In many respects, the non-secretory squirrel prostate gland observed in this study resembles the gland in rats 30 days after castration. While organelles associated with secretory activity have almost all disappeared, lysosomes and autophagic vacuoles are commonplace as are also macrophage-like cells. However, in the grey squirrel both types of regressed secretory cell appear in the form of greatly elongated strands. This has not been reported for castrated rats (Helminen & Ericsson, 1972). In squirrels, no ostensible difference is observed in the nuclei of secretory cells as the animals pass from the sexually active to the inactive state. This again is different from the prostate gland of castrated rats, where nuclei are reported to undergo regional redistribution of heterochromatin and to possess a more irregular outline (Brandes, 1974).

No attempt has been made in this study to distinguish between secretory and non-secretory acid phosphatase activity. The location of acid phosphatase in granular endoplasmic reticulum and inside secretory vesicles only during the sexually active season when the circulating androgen level is high (Siwela & Tam, 1981), and the demonstration of this enzyme only in lysosomes and autophagic vacuoles during sexual quiescence clearly show that acid phosphatase in the grey squirrel is androgen-dependent and that it has the same role in secretion and phagocytosis as in rats (Helminen & Ericsson, 1972). It is possible that in the grey squirrel whole cells are phagocytosed by the macrophage-like cells during the non-secretory period. However, with the techniques used in this study, it is not possible to decide whether there is any tendency to selectively phagocytose any one cell 'type'.

The wave of mitotic activity observed during the initial phase of recovery obviously serves to replace lost cells. It occurs at a time when the squirrel prostate gland regains the ability to metabolise testosterone into androstanediols (Siwela & Tam, 1981), which have been reported to induce prostatic hyperplasia in dogs (Farnsworth, 1980). It is probable that this annual replacement of cells makes special reserve cells unnecessary in seasonal breeding animals. Yet, the replacement process is slow, lasting from October to December, and the prostate is not secretory until January, two months after spermatozoa are present in the seminiferous tubules of the testis and one month later than the appearance of spermatozoa in the epididymis (Siwela & Tam, 1983). The slow functional recovery of the prostate may be a means, through the lack of seminal plasma, to ensure that fertile mating is not possible before the most appropriate time for the survival of the young has arrived.

SUMMARY

Grey squirrels were obtained from the wild every month for two calendar years. The prostate gland was found to be a single lobed structure, subdivided into many lobules each of which was composed of numerous secretory acini with central lumina. Secretion was active throughout the breeding season (January to June), but the gland became atrophic from July to September, and recovered between October and December. Two 'types' of secretory cells were observed in the secretory epithelium throughout the year in both adults and juveniles. During the secretory period 'Type I' cells were characterised by a large nucleus and abundant granular endoplasmic reticulum, ribosomes and secretory granules. The vacuolated 'Type II' cells were more abundant and possessed a smaller nucleus, more substantial Golgi apparatus and numerous secretory vesicles. In the typically atrophic gland almost all organelles associated with secretory activity disappeared, but both types of cells could still be distinguished by their peculiar nuclei and even by their characteristic light and dark cytoplasm in tissue fixed in glutaraldehyde. Recovery of the prostate gland was preceded by a wave of mitotic activity lasting from October to December. However, secretory activity was not resumed until the following January. The two morphological forms were either two functional phases of a single cell type or two distinct populations of secretory cells. Whichever may be the case the prostate gland of the grey squirrel is unique. No other animal has yet been observed to possess secretory cells capable of passing from one morphological and functional phase to the next. If there are two types of secretory cell within this single lobed structure, the organisation of the grey squirrel prostate gland differs from that in the rat and in man where each lobe contains only one single type of secretory cell.

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