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Ultrastructural Localization of the Prostasome – an Organelle in Human Seminal Plasma

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ABSTRACT

Secretory granules and vesicles were demonstrated within human prostatic cells and in the acinar lumen. In size and ultrastructure the granules and vesicles were the same as those previously isolated from human prostatic fluid and seminal plasma. "Prostasomes" is suggested as the designation for these secretory granules and vesicles. As a rule they were found in storage vesicles within the secretory cells.

Two mechanisms for translocation of the prostasomes from the cell interior to the acinar lumen are described. One mechanism involves exocytosis with binding of storage vesicles to, and fusion with the plasma membrane, resulting in release of prostasomes directly into the acinar lumen.The other mechanism implies displacement of storage vesicles in toto from the cell interior to the acinar lumen. This process differs from exocytosis and is here designated "diacytosis". Both phenomena appear to be of roughly equal frequency.

INTRODUCTION

Secretory granules and vesicles in human prostatic fluid and seminal plasma were described in a series of papers (4,15,16,20,21). These organelles ranged in size from 20 to 150 nm and they were surrounded by tri-, penta or multilaminar membrane structures (15). When ultracentrifuged in a silica gradient they gave rise to a distinct band (15,16). An Mg^{2+} and Ca^{2+} -dependent ATPase activity was firmly linked to the membranes surrounding the organelles (15). This finding of a relatively high ATPase

activity in prostatic fluid and seminal plasma has been confirmed by others (2,5,10,11). The secretory granules and vesicles were found to emanate from the prostatic gland (4,15,16,20). Consequently, their translocation from the interior of the prostatic cell to the prostatic fluid and seminal plasma involved some mechanism or mechanisms.

The present report concerns electron microscopy of specimens from human prostatic tissue for study of secretory granules and vesicles within the prostatic cells. Another objective was to gain further insight into the intracellular organization of these organelles and mechanism underlying their translocation.

MATERIAL AND METHODS

Specimens of prostatic tissue were obtained from two patients in connection with transvesical operation for prostatic hyperplasia. Concomitantly with enucleation of the adenomas, additional tissue specimens were taken from areas which apparently were not involved in the adenoma growth.

The specimens were first fixed in 3% glutaraldehyde (17). They were post-fixed in 1% osmium tetroxide solution buffered with veronal acetate at pH 7.2-7.4 (12) and rendered isotonic (19). The fixation periods were 1.5 h in both the glutaraldehyde and the osmium tetroxide solution. The specimens were dehydrated in a graded series of ethanol. The temperature was maintained at 4°C during fixation, post-fixation, rinsing in Tyrode's solution and dehydration to the stage of 95% ethanol. The specimens were embedded in Epon and sectioned on an LKB Ultrotome. After staining with uranyl acetate (3) and lead citrate (14) the sections were examined in a JEOL JEM 100 C electron microscope.

For the statistical analysis of the size of the secretory granules and vesicles, the methods described by Scheffé (18) and Guenter (8) were used.

RESULTS

The abundance of storage vesicles in the prostatic secretory cells can be seen in Fig 1. The storage vesicles were bounded by a unit membrane (Figs 2-4). They could occur singly, or the membranes of adjacent vesicles could have

fused to form "compound" structures (Figs 2-4 and 6). The storage vesicles were seen to contain electron dense material as well as secretory granules and vesicles. In the following account the secretory granules and vesicles will be referred to as "prostasomes".

The representation of prostasomes and electron dense substance varied in the storage vesicles. Accordingly, some vesicles contained only prostasomes (Figs 2-4), others only electron dense material (Figs 2 and 5), while a more heterogeneous category contained prostasomes as well as electron dense material (Fig 2). Finally, some storage vesicles were seen to contain another, smaller storage vesicle.

The prostasomes displayed tri-, penta- and multilaminar membrane structures (Figs 3 and 4). Presence of prostasomes was not confined exclusively to the storage vesicles; they occasionally occurred free in the cytoplasm (Fig 5). Some vesicles close to the periphery of the cells (Fig 6) resembled those denoted as coated vesicles (13). The prostasomes were of uniform size, irrespectively of location - in the storage vesicles, free in the cytoplasm or in the acinar lumen or if they were recovered on preparative ultracentrifugation of prostatic fluid or seminal plasma (15). Table 1 summarizes the statistical data from measurements of prostasomes from different locations. The mean values were similar and the analysis revealed no significant intergroup differences. Hence, analysis of variance resulted in a low F-value being 0.30 ($p= 0.74$) and the median for the diameter of intracellular prostasomes was 125 nm, i.e. close to those of the prostasomes in acinar lumen and in prostatic fluid (100 and 123 nm, respectively) (Table 1).

Storage vesicles were observed in the acinar lumen together with abundance of prostasomes (Figs 1, 2 and 6). These vesicles agreed in shape and appearance with those in pellet I material (Fig 10), recovered at a considerably lower speed than the prostasomes (in pellet II) when subjected to differential centrifugation (15). The extracellular storage vesicles regardless if they were found in acinar lumen or in pellet I material contained prostasomes and electron

Table 1

Diameter (nm) of Prostasomes in Different Locations (Series 1 - 3)

Series	N	Mean (nm)	SD	Range	Arithmetic median (nm)
1. Intracellular	26	158.6	101.0	58.3 - 466.7	125
2. Extracellular: acinar lumen	51	140.7	99.4	33.3 - 500.0	100
3. Extracellular: pellet II recovered from prostatic fluid and seminal plasma	49	152.6	110.4	40.0 - 493.3	123

Analysis of variance: P = 0.74

dense material and were bounded by a unit membrane. Some of the storage vesicles had the same intact appearance as those found intracellularly, while others displayed discontinuities in the enveloping unit membrane.

The prostasomes in the acinar lumen exhibited tri-, penta- or multilaminar membrane structures. Electron dense substance was also present in the acinar lumen.

Two behavioral patterns of the storage vesicles in relation to the prostatic cell plasma membrane were discernible. In one pattern the entire storage vesicle was translocated from one compartment to the other through the plasma membrane. Fig 6 shows storage vesicles in an intermediate position, between the intracellular and extracellular spaces. The prostatic cell plasma membrane here displays a discontinuity, with conspicuous gaps on both sides of the storage vesicle (Fig 7). This vesicle is surrounded by a continuous unit membrane. To denote this type of passage through the plasma membrane, we use the term "diacytosis".

The other type of interaction between storage vesicles and prostatic cell plasma membrane is illustrated in Figs 8 and 9. It is characterized by apparent emptying of the content of storage vesicles, during fusion, directly into the acinar lumen. This mechanism agrees with the process conceptually described as exocytosis (6). In our observations, diacytosis and exocytosis, representing different modes of interaction, could co-exist in the prostatic secretory cells and showed approximately equal frequency.

Numerous microvilli were present on the secretory cell surfaces (Fig 5). In areas where storage vesicles lay close to the cell periphery, the plasma membrane bulged towards the acinar lumen and displayed few or no microvilli (Figs 2, 4, 6 and 8).

DISCUSSION

This study demonstrated, within human prostatic cells, membrane-enclosed secretory granules and vesicles of the type previously isolated in pellet II material recovered from human prostatic fluid and seminal plasma (4, 15, 20). We hereafter refer to these granules and vesicles as "prostasomes". Though generally found within the storage

vesicles inside the prostatic secretory cells, prostasomes occasionally appeared free in the cytoplasm. The storage vesicles probably are synonymous with previously described secretory vacuoles in normal and hyperplastic prostatic tissue (1, 7, 9).

Our observations further traced some of the events in the translocation of these storage vesicles, and thereby of the prostasomes, from the interior of the cell to the exterior, as represented by the acinar lumen. This translocation was seen to be accomplished by two distinct modes of interaction with the prostatic cell plasma membrane. One type of interaction resulted in exocytosis, and the other in what we describe as "diacytosis".

Exocytosis is assumed to involve binding of the storage vesicles to, and fusion with the plasma membrane, in which process apposing membranes of storage vesicles and prostatic cell boundary interact. Discrete pores, through which the content of the storage vesicles is discharged, may thereby be generated. The normal pattern of release seems to involve an initial fusion between the plasma membrane of the prostatic cell and the membranes of the most peripherally located storage vesicles. In addition, deeper lying storage vesicles appear to become involved through fusion with the membranes of adjacent storage vesicles. The resultant formation of "compound" storage vesicles may be equivalent to that reffered to as "compound" exocytosis in other secretory cell systems (6).

Some distinct observations form the basis for our proposal of an alternative secretory mechanism, here denoted as diacytosis, in the prostatic cell: (1) a conspicuous discontinuity of the plasma membrane to permit passage of the storage vesicles (Fig 7); (2) absence, in such circumstances, of indications of fusion between storage vesicle membranes and cell boundary; (3) abundance of storage vesicles containing prostasomes in the acinar lumen. This third observation tallies with the finding of storage vesicles in pellet I material from human prostatic fluid when subjected to differential centrifugation (Fig 10). These observations indicate that the storage vesicles are displaced <u>in toto</u> from the interior of the cell into the acinar lumen. We suggest

that this process differs from exocytosis and may appropriately be named diacytosis. In the studied human prostatic tissue, both phenomena seemed to occur with approximately equal frequency.

We found no structural arrangements suggesting predetermined loci for secretion, whether by exocytosis or diacytosis. The earliest observed ultrastructural event in the secretory process seemed to be bulging of the plasma membrane above peripheral storage vesicles (Figs 2, 4 and 8). Increased prominence of such bulging was apparently associated with a diminuition of surface microvilli. The increment in membrane required to cover the bulges may therefore be recruited from these microvilli.

As regards the mechanism of bulge formation, we have relatively little basis for a commentary. The bulges could conceivably have resulted from an active contractile process within the cell that forced the storage vesicles against the plasma membrane. We have, in fact, observed structures appearing as microfilaments in this prostatic tissue material. Alternatively the bulges may arise from osmotic swelling of the storage vesicles within their membranes, following altered permeability. This change could possibly be induced by a protein kinase-catalyzed phosphorylation reaction. Such a reaction, with significant effects on membrane thickness of the prostasomes, has recently been described (21).

It would thus seem evident that the unusual occurrence under physiological conditions of intact organelles in an extracellular fluid is explained by the fact that another bigger organelle (storage vesicle) containing the smaller ones (prostasomes) exists in the secretory cells of the prostate. Hereby, a preferential discharge of free intact prostasomes into prostatic fluid and semen is possible.

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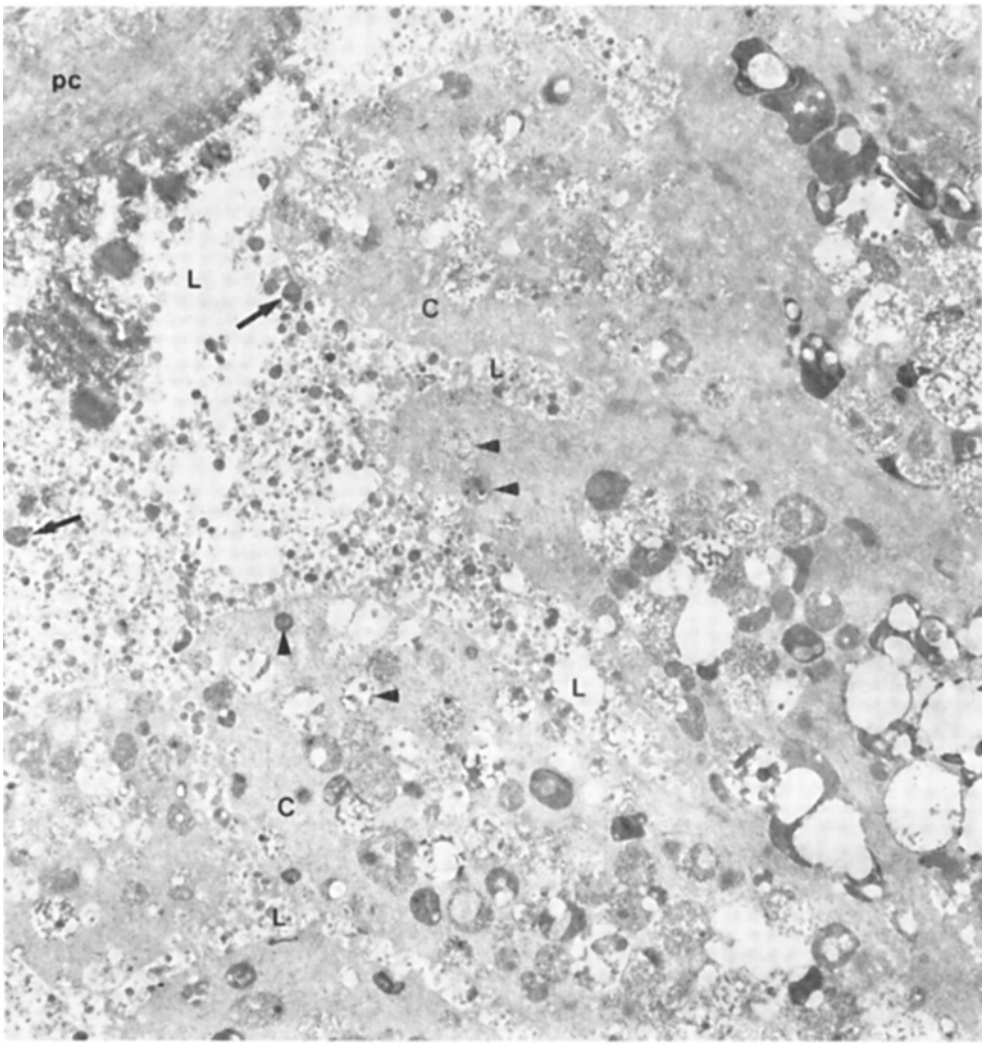


Fig 1. Survey of prostatic cells and acinar lumen. The cells (C) contain numerous storage vesicles (arrow-head). The acinar lumen (L) is filled with prostasomes, storage vesicles (arrow) and lamellar prostatic concretion (pc). (orig. mag. x 16 400).

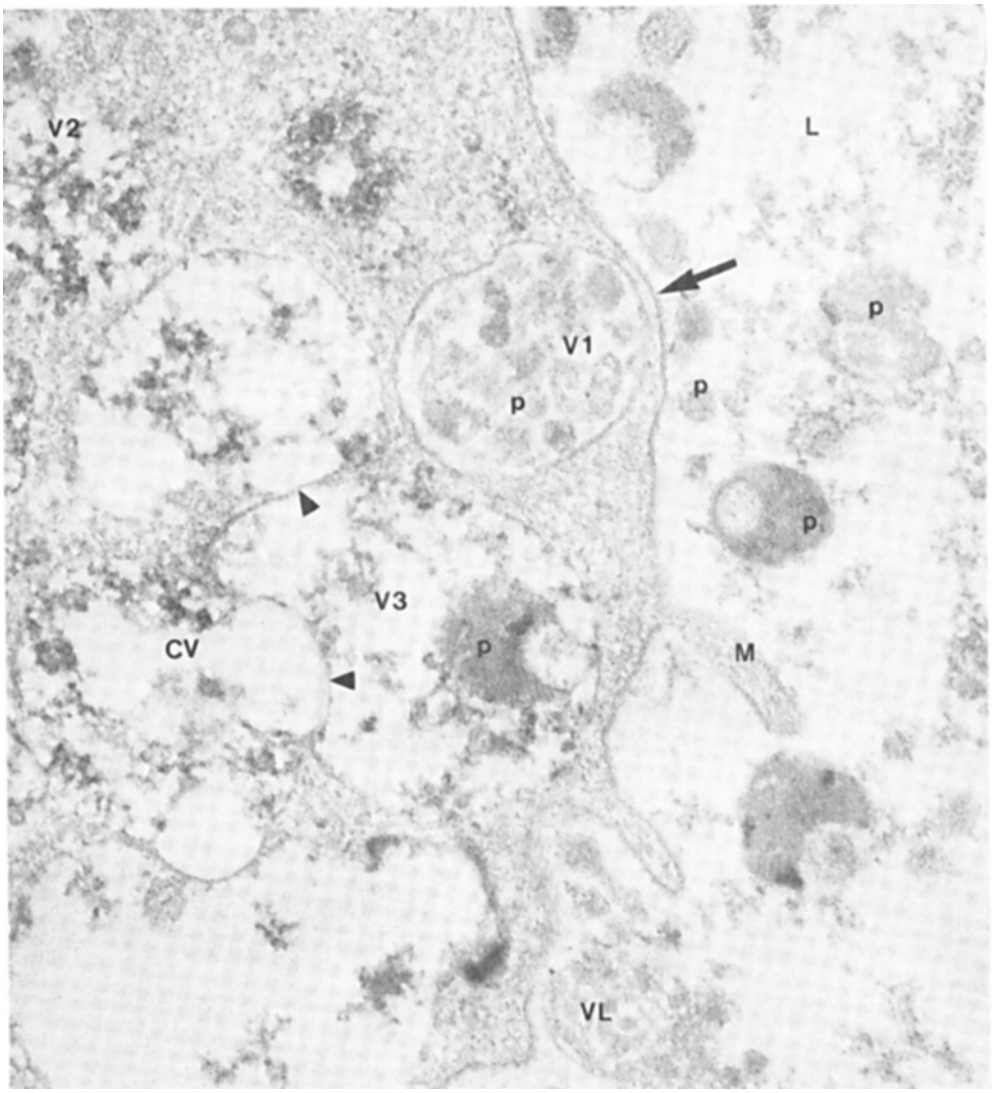


Fig 2. Prostatic secretory cell containing single storage vesicle (V1), storage vesicles with fusion of their adjacent membranes (arrow-head) and "compound" storage vesicle (CV). The storage vesicles contain only prostasomes (V1), only electron dense substance (V2), or both prostasomes (p) and electron dense substance (V3). There are few surface microvilli (M). Where the storage vesicle (V1) lies close to the cell periphery, the plasma membrane (arrow) bulges towards the acinar lumen (L). The acinar lumen contains storage vesicle (VL) and prostasomes (p) similar in size and ultrastructure to those in the intracellular storage vesicles. (orig mag x 74 000).

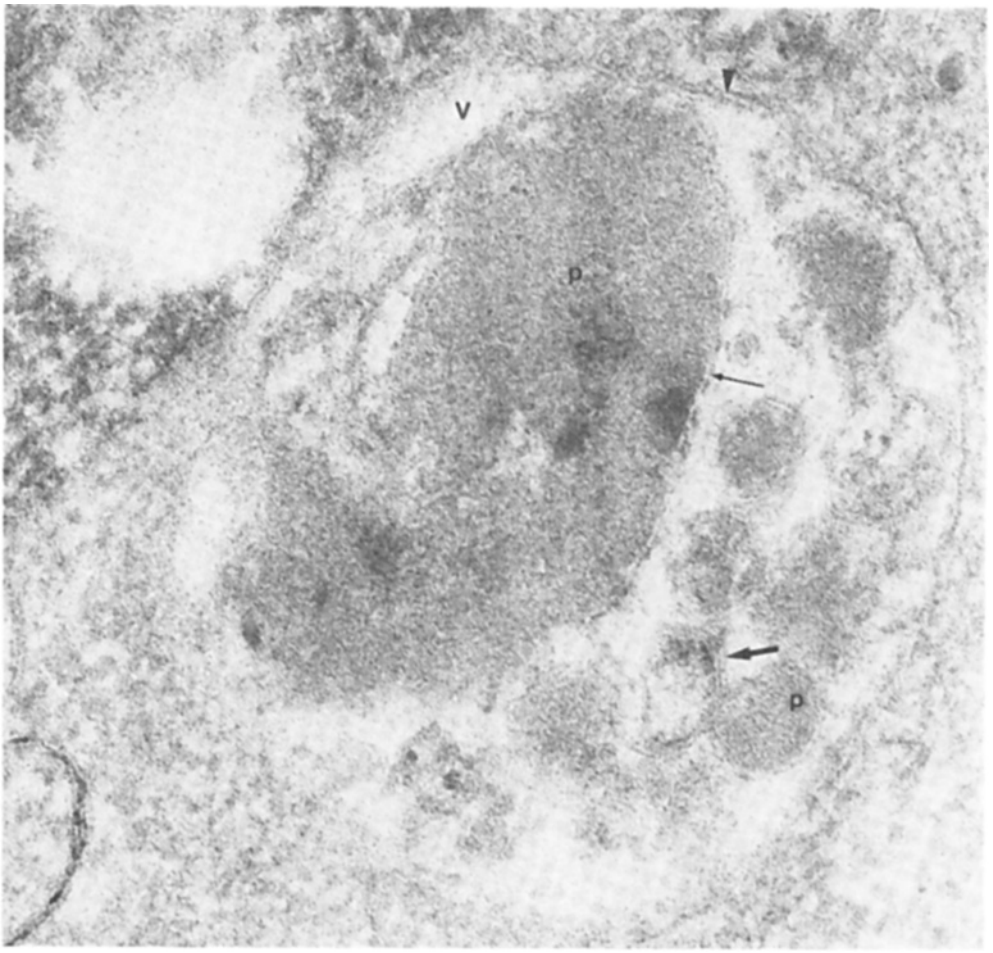


Fig 3. Storage vesicle (V) deep in a prostatic secretory cell. The vesicle is enclosed in a unit membrane (arrow-head) and contains varying-sized prostasomes (p). The prostasomes are bounded by a trilaminar (thin arrow) or pentalaminar (thick arrow) membrane. (orig mag x 216 000).

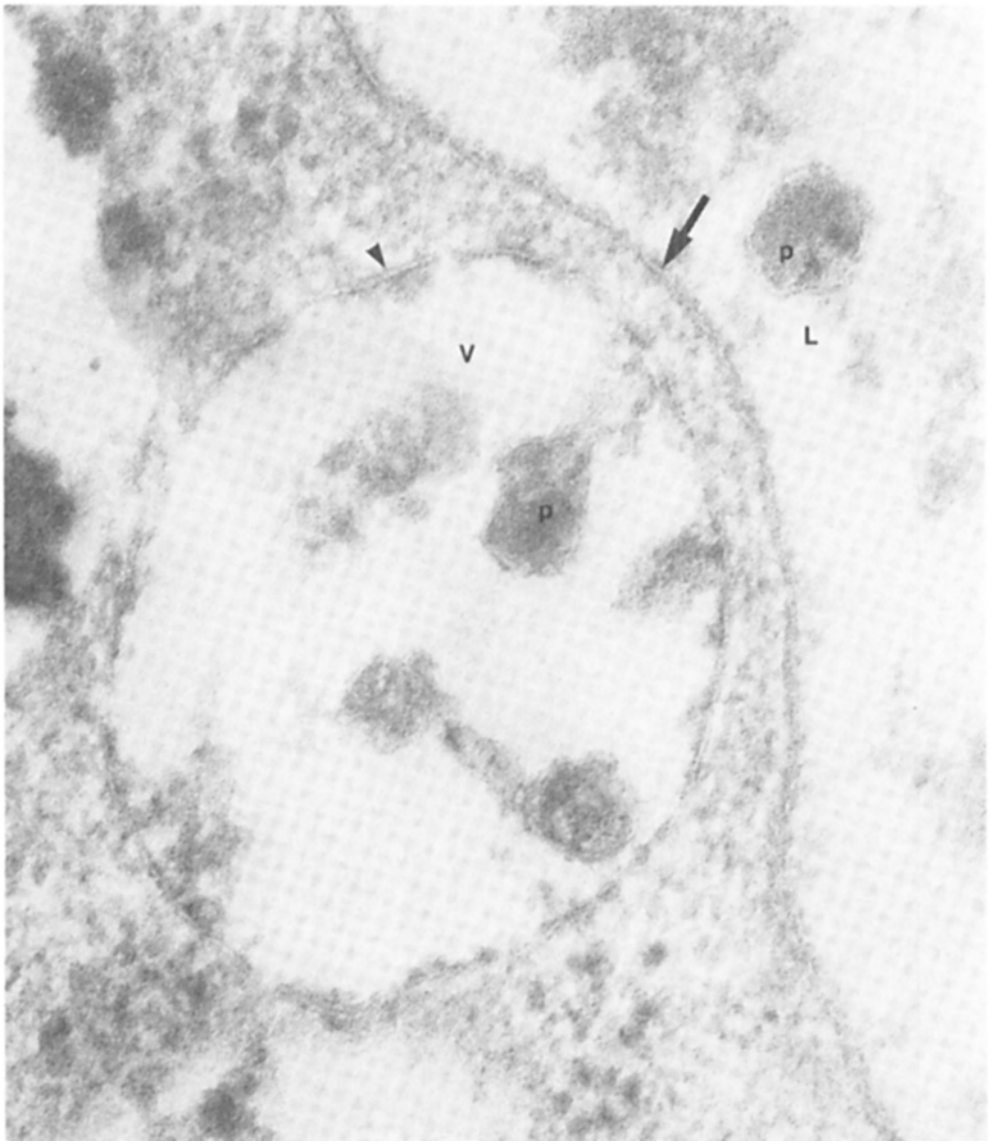


Fig 4. Storage vesicle (V) close to the periphery. The vesicle is enclosed in a unit membrane (arrow-head). Above the storage vesicle the plasma membrane (arrow) bulges towards the acinar lumen (L). The prostasomes (p) in the acinar lumen have the same size and ultrastructure as those in the intracellular storage vesicle (V). (orig mag x 230 000).

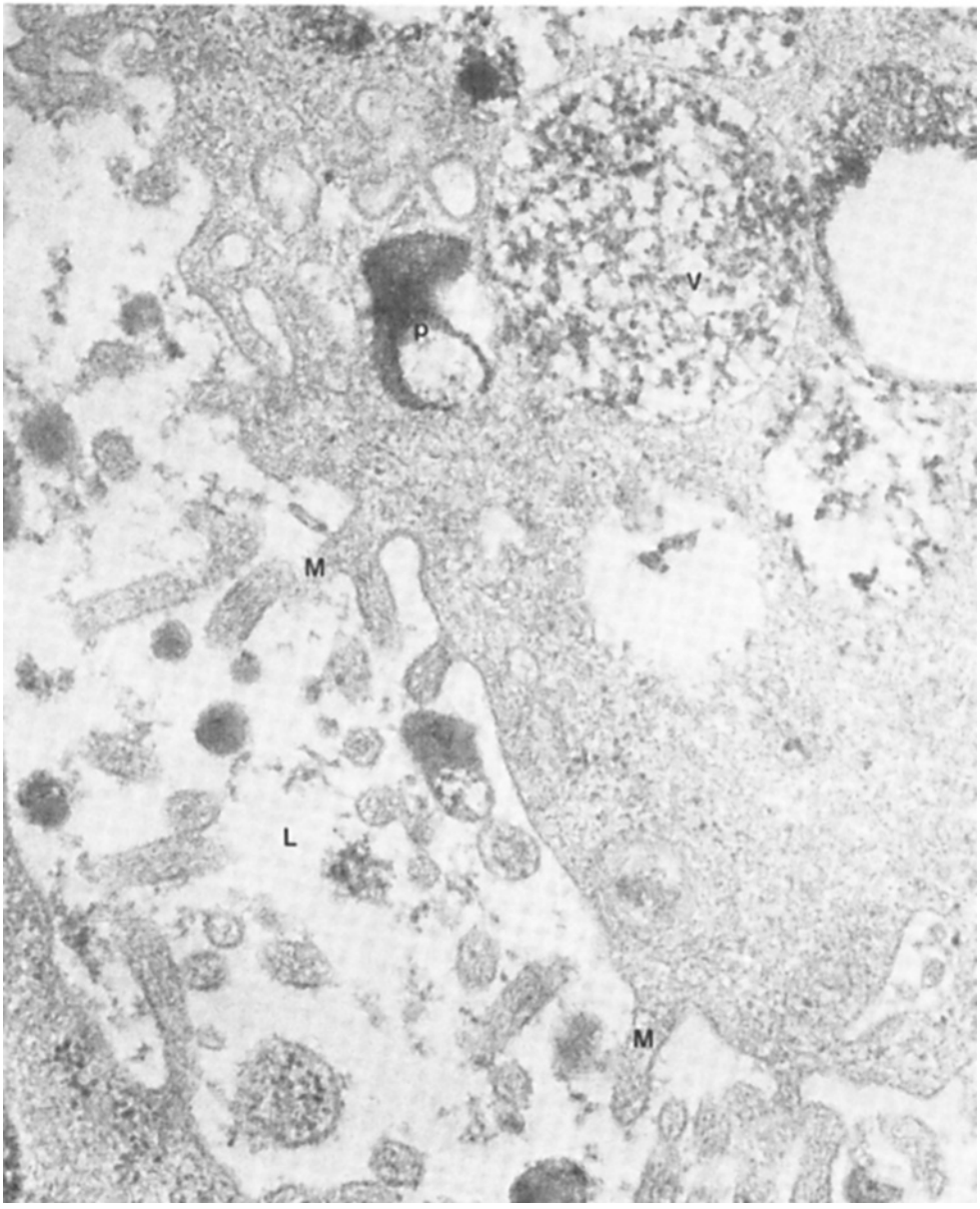


Fig 5. Prostatic secretory cell showing numerous surface microvilli (M). A prostasome (p) lies free in the cytoplasm. Storage vesicle (V) only containing electron dense substance. L, acinar lumen. (orig mag x 69 000).

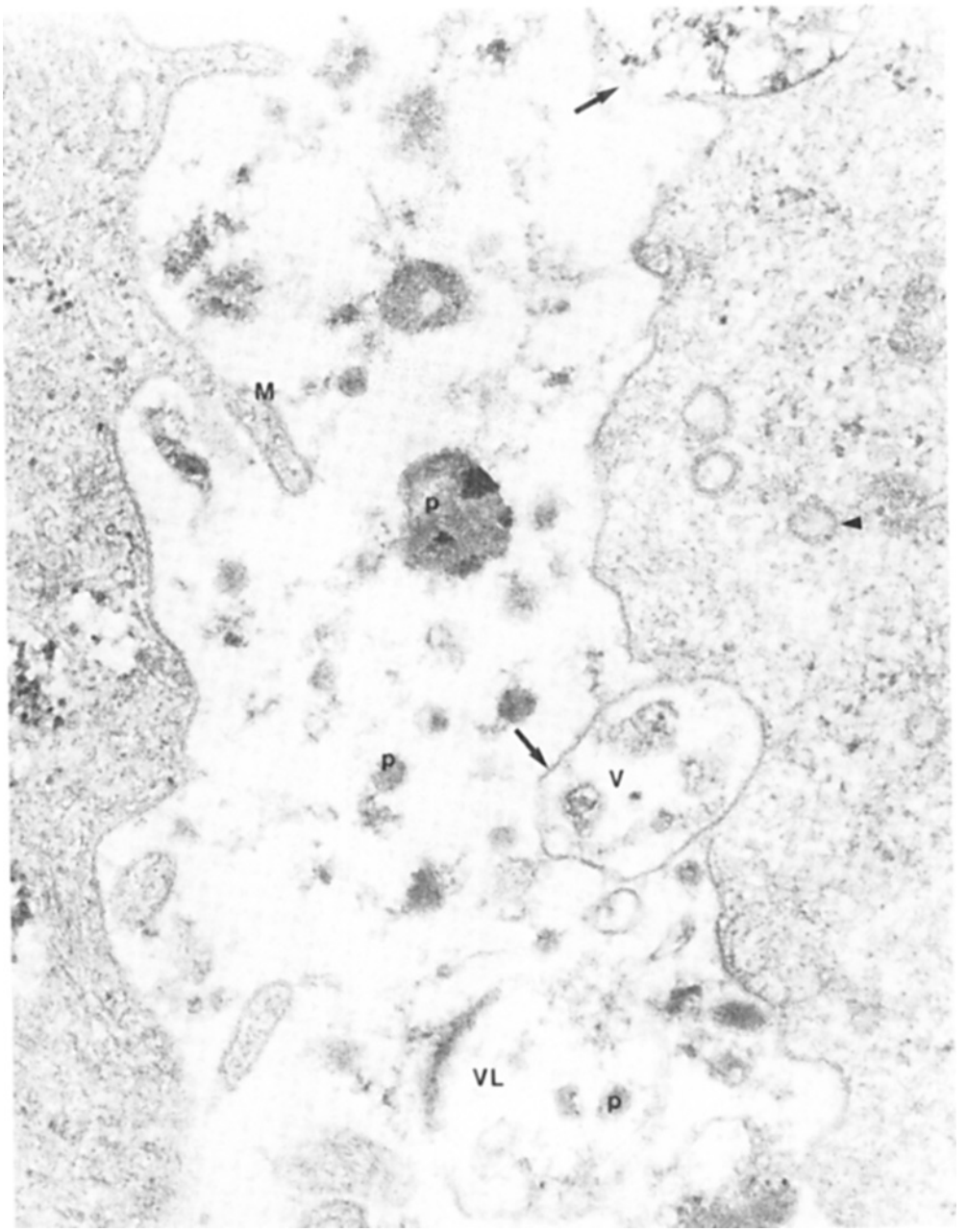


Fig 6. Diacytosis with storage vesicles (arrow) in intermediate position between the intracellular and extracellular compartments. In the acinar lumen there are prostasomes (p) of varying size and a storage vesicle (VL) containing prostasomes. M, microvillus. Coated vesicle (arrow-head). (orig mag 83 000).

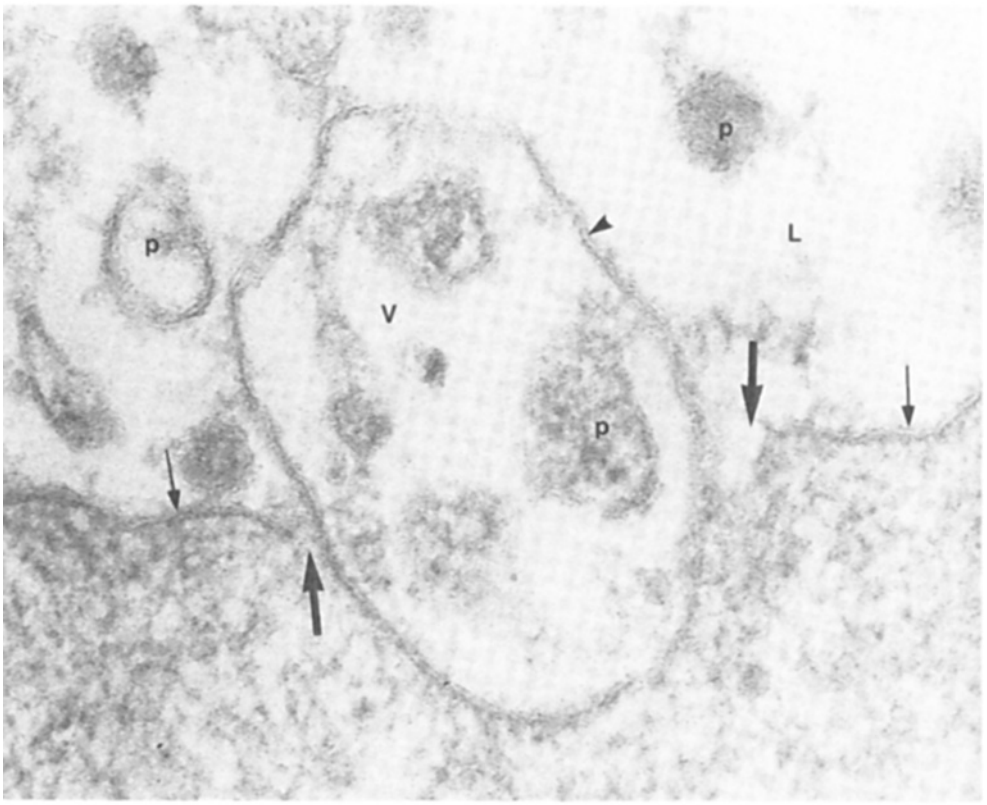
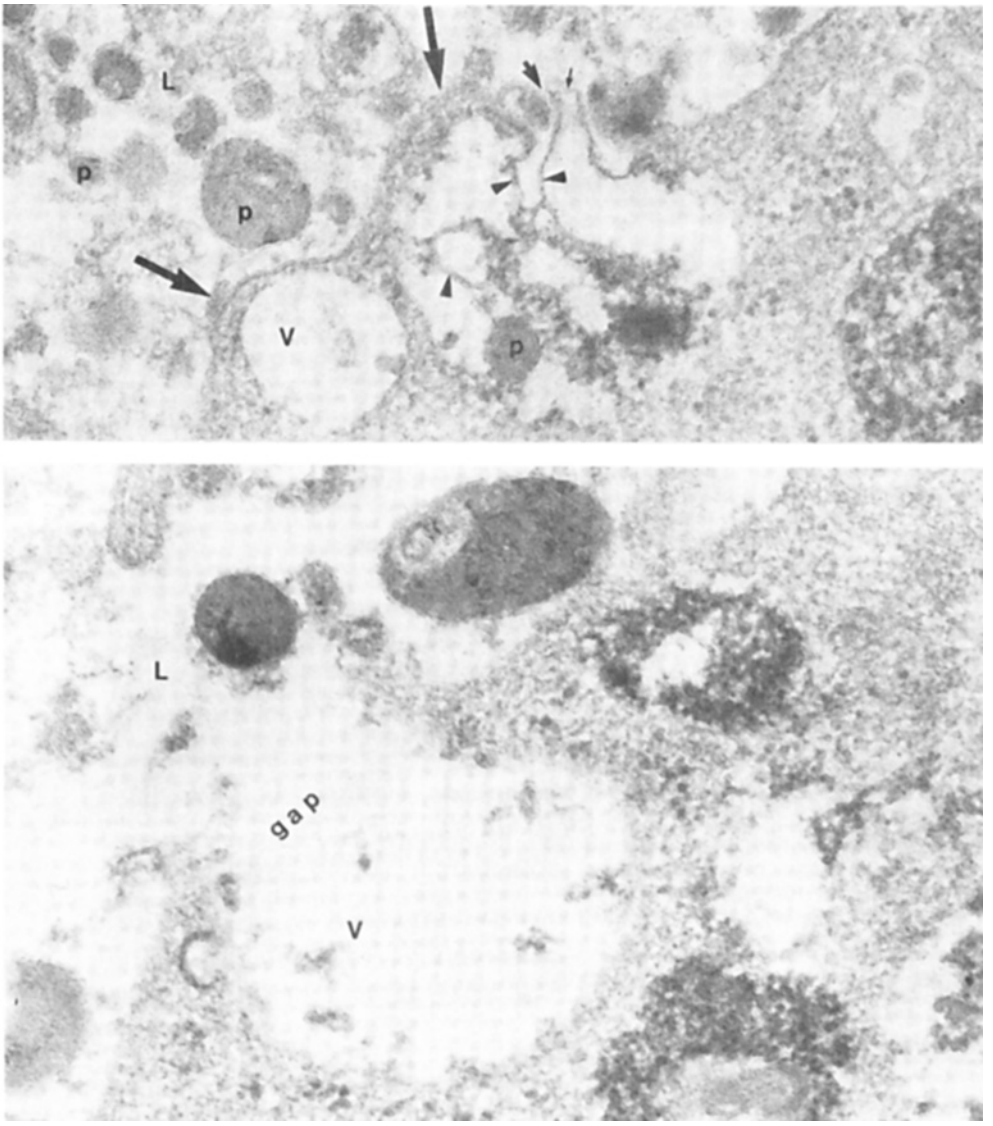


Fig 7. Higher magnification of the storage vesicle (V) during diacytosis in Fig 6. The storage vesicle is bounded in its entire circumference by a continuous unit membrane (arrow-head). On each side of the storage vesicle the plasma membrane (thin arrow) displays distinct discontinuities (thick arrow). p, prostasome. L, acinar lumen. (orig mag x 208 000).



Figs 8 (upper) and 9 (lower). Different stages of exocytosis.

Fig 8. Above the single storage vesicle (V) and the storage vesicles with fusion of their adjacent membranes (arrow-head) the plasma membrane (long thick arrow) bulges towards the acinar lumen (L). In one area there is a fusion between the membranes of the storage vesicle and the cell boundary (short thin arrow), while in an adjacent area a narrow pore (short thick arrow) connects the interior of the storage vesicle with the acinar lumen. p, prostasome. (orig mag x 100 000).

Fig 9. A large pore (gap) connects the interior of a storage vesicle (V) with the acinar lumen (L). (orig mag x 93 000).

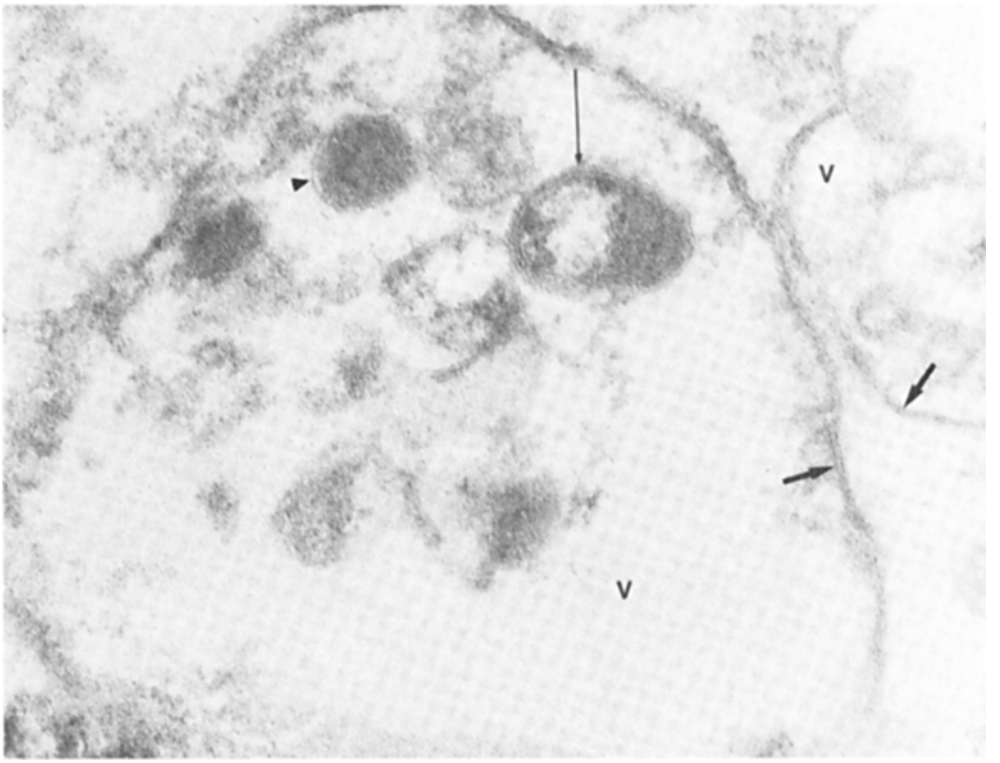


Fig 10. Storage vesicles (V) in pellet I material. The vesicles are enclosed in a unit membrane (short thick arrow) and contain prostasomes. The prostasomes are bounded by a trilaminar (arrow-head) or pentalaminar (long thin arrow) membrane. (orig mag x 190 000).

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