

The Fine Structure of the Epithelial Cells of the Mouse Prostate*

I. Coagulating Gland Epithelium

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PLATES 275 TO 280

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ABSTRACT

The fine structure of the epithelial cells of the anterior lobe, or coagulating gland, of the mouse prostate has been investigated by electron microscopy. This organ is composed of small tubules, lined by tall, simple cuboidal epithelium surrounded by connective tissue and smooth muscle. The epithelial cells are limited by a distinct plasma membrane, which covers minute projections of the cytoplasm into the lumen. The cell membranes of adjacent cells are separated by a narrow layer of structureless material of low density. The cavities of the endoplasmic reticulum are greatly dilated, and the cytoplasmic matrix is reduced to narrow strands, in which the various organelles are visible. The content of the cavities of the endoplasmic reticulum appears as structureless material of lesser density than the cytoplasmic matrix. Material which may be interpreted as secretion products can be seen in the lumina of the tubules. The possible nature of the material inside the cisternal spaces and the secretory mechanisms in these cells is discussed.

INTRODUCTION

In the mouse, as in some other rodents, the prostatic gland is formed of separate lobes: the dorsolateral lobes, the ventral lobes, and the anterior lobes or coagulating gland; all of which have a common embryologic origin (5). The gland is dependent on androgens for the development and maintenance of its normal histological and cytological characteristics (1, 11). In other words castration, or the administration of estrogens, produce profound changes in the histological and histochemical picture of the mouse prostate (1, 11). It therefore appeared of interest to investigate the fine structure of this organ and to see whether the changes revealed by light microscopy are accompanied by corresponding changes in fine structure.

In this part of the work the fine structure of only one component of the prostate of the untreated mouse—the coagulating gland—will be described.

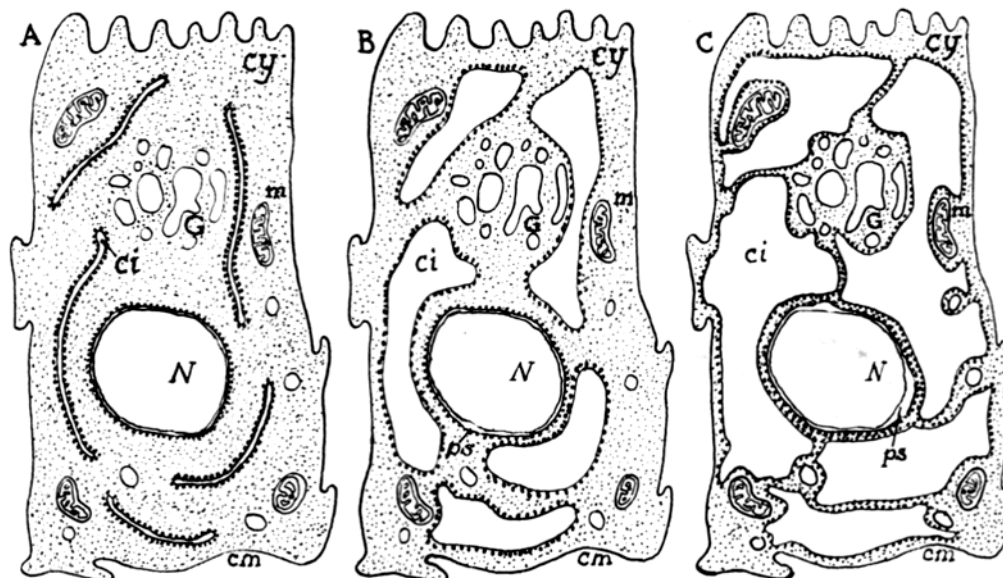
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Materials and Methods

Swiss albino male mice, 5 to 6 months old, were used for this study. The mice were anesthetized with ether and the coagulating gland was partially freed from the surrounding connective tissue and fat. Sjöstrand's (21, 25) or Caulfield's (3) modification of Palade's OsO₄ fixative (13) was dripped onto the exposed organ. After several minutes, the apparently impregnated tissue was excised, transferred to a drop of OsO₄ fixative upon dental wax, and cut into minute fragments. These were transferred to cold fixative (4°C.) for periods varying from 1 to 2 hours. After fixation the blocks were rinsed either in Tyrode's solution or 25 per cent alcohol. Dehydration was attained in graded series of alcohols. The tissue was embedded in a mixture of 95 per cent *n*-butyl and 5 per cent methyl methacrylate with 2 per cent benzoyl peroxide. Sections were cut with a Porter-Blum microtome or with a Sjöstrand ultramicrotome and observed with an RCA model EMU-2C or an RCA EMU-3D electron microscope. Da Fano's method (4) was employed to demonstrate the Golgi apparatus. Alkaline phosphatase was demonstrated by means of Gomori's method (8).

RESULTS

General Description.—Figs. 1, 5, and 10, indicate the over-all features of the tall cuboidal



TEXT-FIG. 1. Schematic diagram of the epithelial cells of the coagulating gland of the mouse. An interpretation is here provided of the mechanism whereby the prevailing aspect seen in the adult epithelium could be established. The cisternae are still flattened at *A*, and a moderate dilation is present at *B*. At *C* the dilatation of the cisternae has become very marked. It is possible to visualize through this diagram the relative distribution of cytoplasmic matrix and intracisternal space during the various stages of dilation of the cisternae, as well as the relation between organelles and cisternae.

Abbreviations

<i>cy</i> = cytoplasmic matrix	<i>m</i> = mitochondria
<i>ci</i> = cisternae of the endoplasmic reticulum	<i>cm</i> = cell membrane
<i>N</i> = nucleus	<i>ps</i> = perinuclear space
<i>G</i> = Golgi complex	

epithelium and its relation to the surrounding tissues. The cell membrane appears as a dense line. A basement membrane is visible, separating the epithelium from an interstitial space containing connective tissue elements. The nucleus occupies a central position and shows foldings and invaginations; the nuclear envelope consists of two membranes. The cavities of the endoplasmic reticulum occupy the largest part of the cytosome. The Golgi apparatus is visible in the supranuclear region. Mitochondria, small particles, and smooth surfaced profiles can be seen randomly distributed in the cell.

A. The Epithelial Cells:

The Cell Membrane.—This structure appears as a dense line limiting the cell. In the zone of contact of two adjacent cells the cell membranes are separated by an intermediary layer of lower density (Figs. 2, 4). Plications of the cell membrane

are visible at the lateral aspects and at the base of the cells (Figs. 2, 4).

Cytoplasmic Matrix.—This consists of a structureless material of medium density which shows a number of small discrete dense particles. At the free border, immediately beneath the cell membrane, a continuous band of cytoplasmic matrix devoid of granules is present (Figs. 3, 9). Due to the intense dilation of the cavities of the endoplasmic reticulum, the cytoplasmic matrix in between appears in most cases reduced to narrow strands or either oval or circular profiles (Figs. 1, 5, 7, 9, 10). The exception to this is in the region of the Golgi complex where the various components of this organelle lie in a broader zone of cytoplasmic matrix (Figs. 8, 9).

Endoplasmic Reticulum.—The most striking feature of these cells is the pronounced dilation of the various cavities (cisternae) of the endoplasmic reticulum. In the apical region (Fig. 9), the

profiles are of the elongated, oval, and circular types, and show varying degrees of dilation. The cytoplasmic matrix in between these dilated sacs appears as narrow interlacing bands. Most of the profiles of the endoplasmic reticulum appear clearly individualized and are bound by a thin membrane, covered by small particles on the surface facing the cytoplasmic matrix. In the middle portion of the cell and in the basal region (Figs. 5, 7), the disposition of the cavities of the endoplasmic reticulum is somewhat different. Here, the cavities have almost completely lost their individuality, and appear as freely communicating spaces, in between which the cytoplasmic matrix appears as either narrow strands or as oval and circular profiles (Figs. 7, 10). The limiting membranes of the profiles of the endoplasmic reticulum convey more the impression of actually limiting the strands of cytoplasmic matrix. The outside surface of these membranes is covered by small particles regularly disposed in rows (Figs. 4, 9). The various cell organelles appear embedded in a scanty amount of cytoplasmic matrix.

The content of the dilated cavities is homogeneous and less dense than the cytoplasmic matrix, and it does not appear to come into contact with the cell membrane at any point of its extension. A narrow band of cytoplasmic matrix always exists between the dilated cavities and the cell membrane. Around the nucleus, which shows the greatest part of its perimeter surrounded by dilated cavities, a narrow rim of cytoplasmic matrix is visible which is limited on both its aspects by a thin membrane, dotted with small granules (Figs. 4, 5, 7). The membrane limiting the cytoplasmic matrix towards the nucleus is to be considered as the outer membrane of the nuclear envelope (22). The extreme dilation and the free communications existing between the dilated cavities of the endoplasmic reticulum at the mid-portion and at the base of the cell convey the idea of an extensive cisternal space in which the cytoplasmic matrix with its various organelles is "suspended."

Small Particulate Elements.—Granules corresponding to those described by Palade (14), are applied to the outer surface of the limiting membranes of the endoplasmic reticulum or scattered in the cytoplasmic matrix, where they sometimes tend to be disposed in rows or in circular or rosette-like patterns (Figs. 4, 9, 10).

Mitochondria.—Their matrix shows a slightly greater density than that of the cytoplasmic

matrix. The mitochondria are bounded by a double membrane and show a system of internal folds or cristae. Those present in the mid-region and in the basal region of the cell appear as if suspended in the cavities of the endoplasmic reticulum, but invariably there is a small rim of cytoplasmic matrix around them, limited in its turn by a membrane of the endoplasmic reticulum (Figs. 5, 10).

Golgi Complex.—Its situation in the supranuclear region corresponds to that observed in light microscope preparations (Figs. 5, 6). It is composed of profiles bounded by smooth membranes, corresponding to flattened vesicles, and to various types of vacuoles and vesicles with contents of low density. In the region where the Golgi elements are situated the cytoplasmic matrix is more abundant than elsewhere in the cell, and this zone of matrix tends to be circumscribed by the dilated cavities of the endoplasmic reticulum (Figs. 5, 8, 9).

The Nucleus.—This is situated toward the center of the cell, its shape is irregularly oval and may show invaginations (Fig. 1). The content consists of aggregates of dense granules in a homogeneous matrix, the nucleolus appearing as an accumulation of more densely packed granular material (Fig. 4). The nuclear envelope consists of two membranes, the outer one of which limits the cytoplasmic rim that surrounds the nucleus (Figs. 4, 5, 7). In some portions of the periphery of the nucleus the separation between the outer and inner nuclear membranes becomes widened, and shows the presence of the perinuclear space (Figs. 4, 5, 7). In some preparations, this perinuclear space seems to be in direct communication with the system of cavities of the endoplasmic reticulum (Fig. 5, arrows), a structural feature already described for other cells (15, 22).

The Free Border of the Cell and the Lumina of the Acini.—The free border shows in favorable section, the presence of microprojections of cytoplasmic matrix, which are covered by the cell membrane (Figs. 3, 9). In many cases the lumen of the acini appears empty but occasionally it is filled with homogeneous material of moderate density, with a few formed bodies, represented by various types of profiles, some surrounded by membranes (Fig. 3).

The Basement Membrane.—This appears as a layer of amorphous substance of moderately low density, applied to the outer surface of the epi-

thelial cell membrane. Its free surface delimits on one side the interstitial space where connective tissue elements are present (Fig. 10).

B. The Tissues Surrounding the Acini:

In some locations capillaries may be seen lying in the vicinity of the epithelium. The cytoplasm of the endothelial cells of these capillaries is covered by a basement membrane which in such cases completes the delimitation of the interstitial space (Fig. 10). Smooth muscle fibers can be seen near the acini.

DISCUSSION

In relating the observations of this work to previous histochemical findings in this organ (1) it is noticeable that the presence of PAS-positive granules, Sudan black-positive material, and a positive test for acid phosphatase in the region between the nucleus and the luminal border, corresponds in the electron micrographs with the presence of the various components of the Golgi complex. Furthermore, as the secretion in the lumen is also PAS- and acid phosphatase-positive (1), one may assume that the various vacuoles and vesicles in the Golgi region might represent material of a secretory or presecretory nature. The region of the basement membrane, as shown in light microscopy with appropriate techniques—such as silver impregnation, PAS reaction, or alkaline phosphatase tests (Fig. 11)—is shown in electron micrographs to be made up of several constituents, such as the amorphous layer adjacent to the cell membrane and an interstitial space of variable width. It would appear that the silver impregnation techniques and the PAS test actually reveal the reticular fibers pertaining to connective tissue present in the interstitial space, and to the adjacent smooth muscle fibers.

The extreme dilation of the cisternae in the coagulating gland with the corresponding decrease of the area occupied by the cytoplasmic matrix, is a distinguishing characteristic of this organ (2). A certain degree of dilation also occurs in some other organs, such as the thyroid gland (24); the exocrine cells of the rat pancreas, the acinar cells of the parotid gland and plasma cells (17); the exocrine cells of guinea pig pancreas (16); the epithelium of the fallopian tubes of the rabbit (12); the endothelial cells of the maternal capillaries of the placenta of the cat (6), the albumin secreting glands of the hen's oviduct (10), and the

prostate of the normal rat (9). In none of the above mentioned cells, however, do the cisternal spaces seen to reach such an extreme degree of dilation as that encountered in the coagulating gland, in which many sections show the basal part of the cell as wholly occupied by few gigantic cisternae, with only narrow strands of cytoplasm intervening. The opacity of the material in the sacs seems to vary, not so much in the same cell as reported in the albumin-secreting cells of the hen oviduct (10) but from one cell to another. This may indicate a different functional state of the various cells of the same acinus.

As yet the nature of the amorphous material that fills the dilated cisternae of the coagulating gland has not been determined. However, Hendlar *et al.* (10) have performed a thorough study of the secreting cells of the hen's oviduct, which display enlarged cisternae containing an amorphous material of quite similar density to the one present in the coagulating gland. They consider this material to be of albuminous character, and believe it is eventually released into the lumen of the gland, either by a mechanism of merocrine or apocrine secretion. There is good reason to believe that the content of the dilated cisternae in the coagulating gland is also of albuminous character and that it constitutes a secretion product. It may be postulated also that the endoplasmic reticulum in these cells may be directly involved in protein synthesis, as has been suggested by Porter and Blum (18), Weiss (23), and Palade and Siekevitz (16, 19, 20). The mechanism of the release of this secretion product into the lumen of the coagulating gland remains uncertain. It has not been possible to detect cisternae opening into the lumen, nor the extrusion of vesicles. In some favorable sections, however, the lumen of the acinus contains circular or oval profiles (Fig. 3).

The reduction in the amount of cytoplasm present in the epithelial cells of the coagulating gland appears to be produced by compression from the accumulation of secretion in the dilated cisternae (Text-fig. 1). Consequently the suppression of secretory activity in these cells should produce a collapse of the distended cisternae, and a corresponding increase in the amount of cytoplasmic matrix. Some evidence that this occurs in the ventral prostate of the rat is already indicated by the work of Harkin (9). Experiments done in this laboratory and ready for publication tend to corroborate this conclusion.

No evidence is provided by this study that the Golgi apparatus participates in the elaboration of the material contained in the dilated cisternae of the endoplasmic reticulum. This agrees with the results of Hendler *et al.* (10), with respect to the albumen present in the cisternae of oviduct cells. However, the presence of PAS-positive granules, Sudan black-positive material, and a positive test for acid phosphatase in this region may indicate, as already suggested by other authors (7), the participation of the Golgi apparatus in the elaboration of secretory products other than the material present in the cisternae of the endoplasmic reticulum.

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REFERENCES

1. Brandes, D., and Bourne, G. H., *Brit. J. Exp. Path.*, 1954, **35**, 577.
2. Brandes, D., Belt, W. D., and Bourne, G. H., *Exp. Cell Research*, 1959, **16**, 683.
3. Caulfield, J. B., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 827.
4. Da Fano, C., in *Histological Techniques*, (H. M. Carlton, editor), London, Oxford University Press, 1957.
5. Deanesly, R., and Parkes, A. S., *J. Physiol.*, 1933, **78**, 442.
6. Dempsey, E. W., Wislocki, G. B., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 743.
7. Farquhar, M. G., and Wellings, R. S., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 319.
8. Gomori, F., *Microscopic Histochemistry*, Chicago, University of Chicago Press, 1952.
9. Harkin, J. C., *Endocrinology*, 1957, **60**, 185.
10. Hendler, R. W., Dalton, A. J., and Glenner, G. F., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 325.
11. Horning, E. S., *Quart. J. Micr. Sc.*, 1947, **88**, 45.
12. Nilsson, O., *Exp. Cell Research*, 1958, **14**, 341.
13. Palade, G. E., *J. Exp. Med.*, 1952, **95**, 285.
14. Palade, G. E., *J. Biochem. Cytol.*, 1955, **1**, 59.
15. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 567.
16. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 417.
17. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 85.
18. Porter, K. R., and Blum, J., *Anat. Rec.*, 1953, **117**, 685.
19. Siekevitz, P., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 309.
20. Siekevitz, P., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 557.
21. Sjöstrand, F. S., and Hanzon, V., *Exp. Cell Research*, 1954, **7**, 415.
22. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 257.
23. Weiss, J. M., *J. Exp. Med.*, 1953, **98**, 607.
24. Wissig, S. L., *The Anatomy of Secretion in the Follicular Cells of the Thyroid Gland*, Dissertation for Ph.D. Thesis, Yale University, New Haven, 1956.
25. Zetterqvist, H., *The Ultrastructural Organization of the Columnar Cells of the Mouse Jejunum*, Karolinska Institutet, Stockholm, Aktiebolaget Godvil, 1956.

EXPLANATION OF PLATES

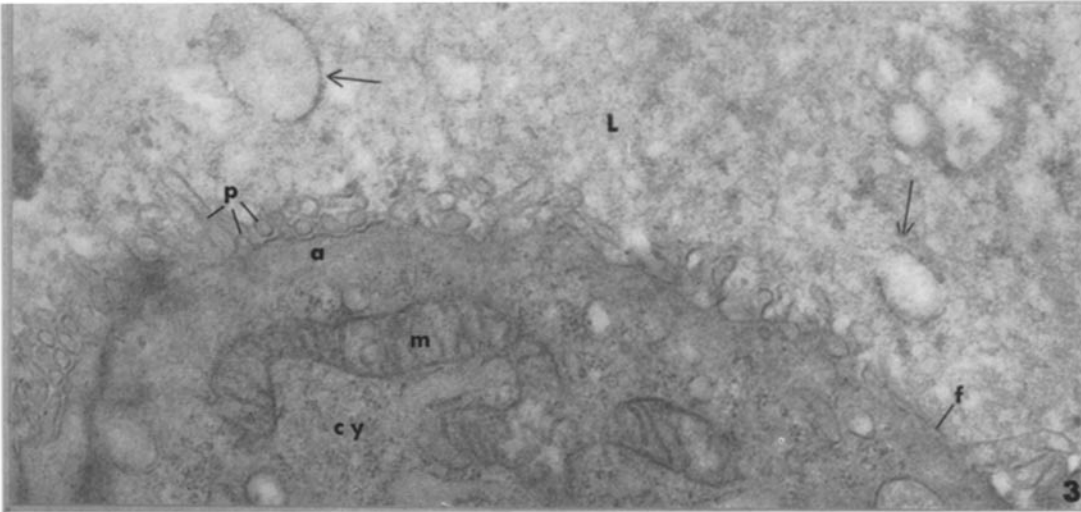
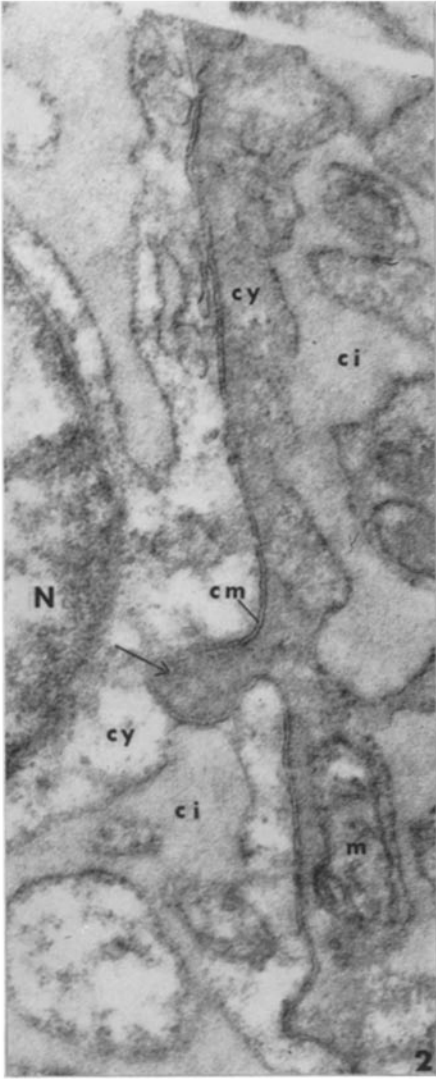
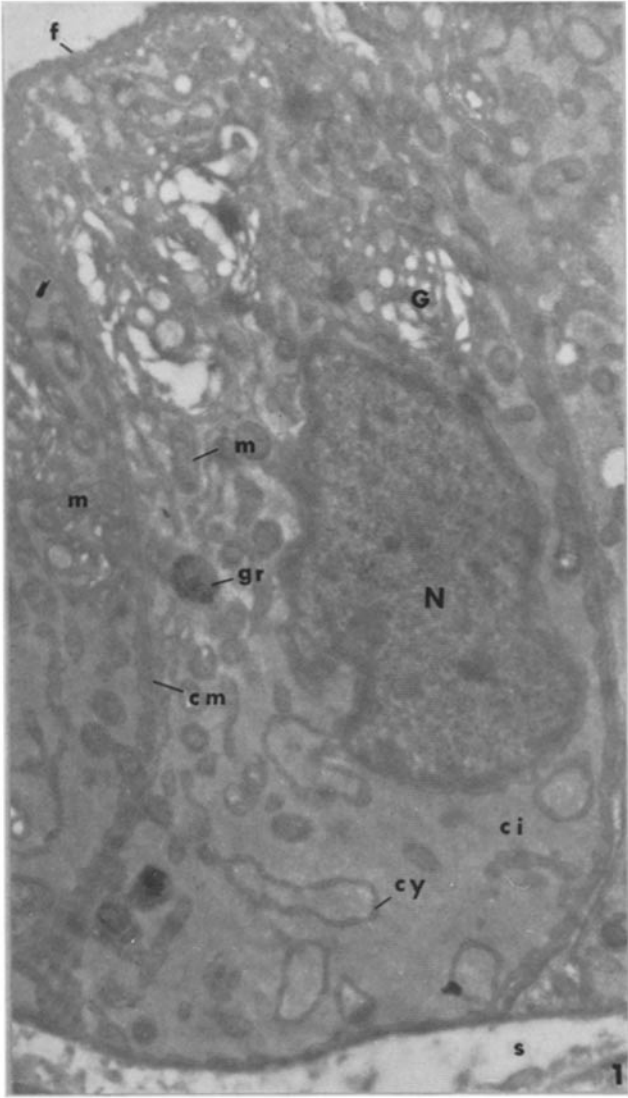
<i>a</i> = cortical layer of agranular cytoplasm	<i>m</i> = mitochondria
<i>bm</i> = basement membrane	<i>N</i> = nucleus
<i>ci</i> = cisternal of the endoplasmic reticulum	<i>n'</i> = inner nuclear membrane
<i>cm</i> = cell membrane	<i>n''</i> = outer nuclear membrane
<i>cy</i> = cytoplasmic matrix	<i>nc</i> = nucleolus
<i>ep</i> = epithelium	<i>p</i> = microprojections of the free border
<i>f</i> = free border of cells	<i>ps</i> = perinuclear space
<i>G</i> = Golgi complex	<i>s</i> = interstitial space
<i>gr</i> = granule	<i>sm</i> = smooth muscle
<i>k</i> = alkaline phosphatase test	<i>v</i> = vessel
<i>L</i> = lumen of acini	<i>va</i> = Golgi vacuole
<i>l</i> = limiting membrane of the endoplasmic reticulum	<i>ve</i> = Golgi vesicle

PLATE 275

FIG. 1. General view of an epithelial cell. The largest part of the cytosome is occupied by dilated cisternae (*ci*) of the endoplasmic reticulum. The cytoplasmic matrix (*cy*) is reduced to narrow strands. The mitochondria (*m*) and dense granules (*gr*) are embedded in the cytoplasmic matrix. A band of cytoplasmic matrix separates the cisternal spaces from the cell membrane (*cm*). The Golgi (*G*), is seen above the nucleus (*N*). $\times 11,500$.

FIG. 2. Section showing two adjacent epithelial cells. The cytoplasmic matrix (*cy*) appears to be pushed against the cell membrane (*cm*) and against the nucleus (*N*) by the expansive cisternae of the endoplasmic reticulum. The cytoplasmic matrix of one cell can be seen protruding into the neighboring one (arrow). The content of the cisternae (*ci*), is less dense than the cytoplasmic matrix. $\times 40,000$.

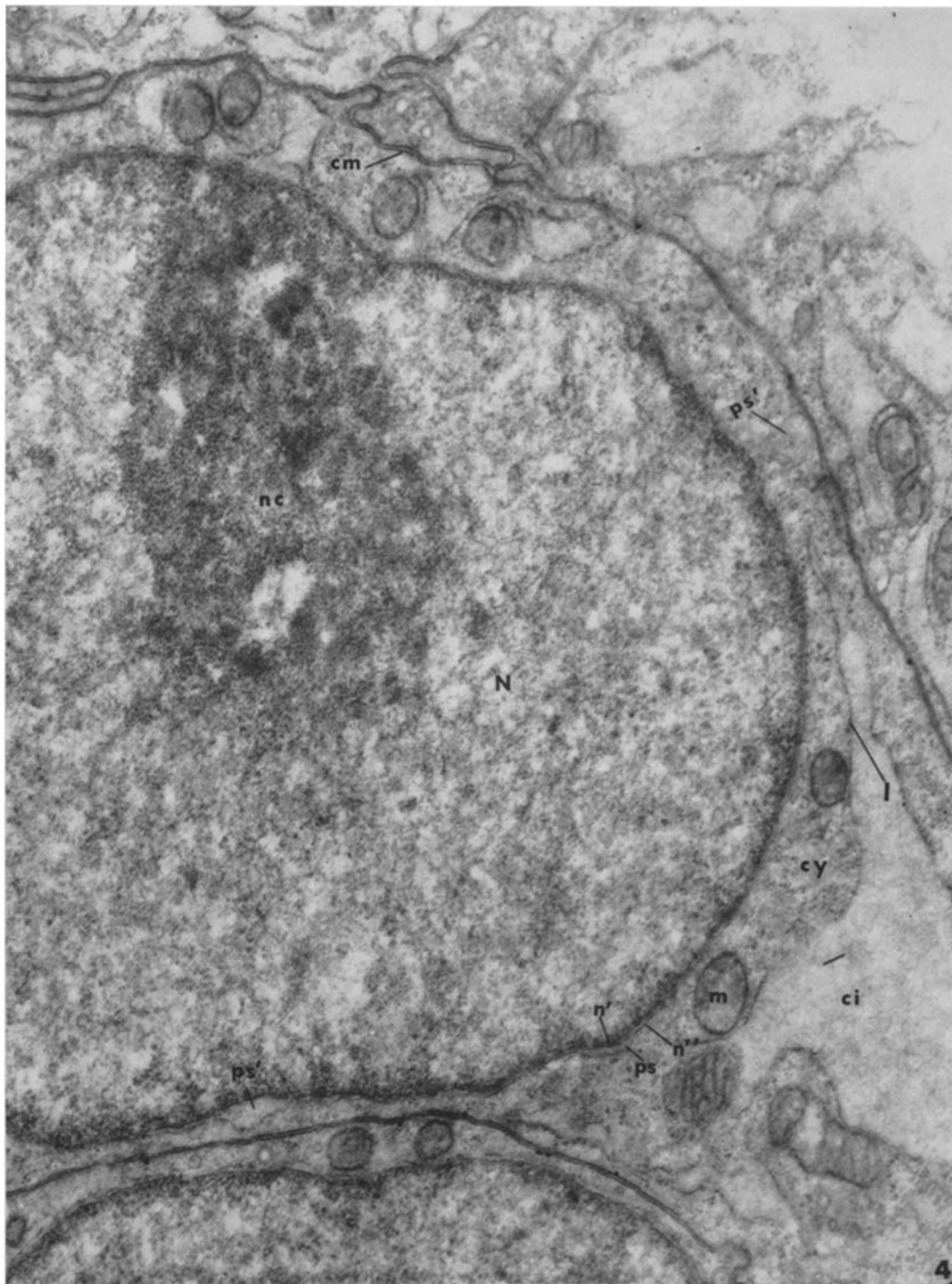
FIG. 3. A cortical layer of denser cytoplasmic matrix (*a*), devoid of Palade granules is visible. Microprojections of the cytoplasmic matrix (*p*) appear at the free border (*f*) and project into the lumen (*L*). Structureless material and various profiles (arrows) appear in the lumen. $\times 30,000$.



(Brandes and Portela: Coagulating gland eipthelium)

PLATE 276

FIG. 4. Transverse section of an epithelial cell showing the nucleus (*N*) in detail. A border of cytoplasmic matrix (*cy*) surrounds the outer nuclear membrane (*n''*). The perinuclear space (*ps*) is visible between the inner (*n'*) and the outer nuclear membranes, and may show projections into the cytoplasm (*ps'*). The nucleolus (*nc*) and plications of the cell membrane (*cm*) can be observed in this section. The content of the perinuclear space is similar in density to that of the cisternae (*ci*). Mitochondria (*m*) appear embedded in the cytoplasmic matrix. Small particles are attached to the limiting membranes (*l*) of the endoplasmic reticulum. $\times 50,000$.

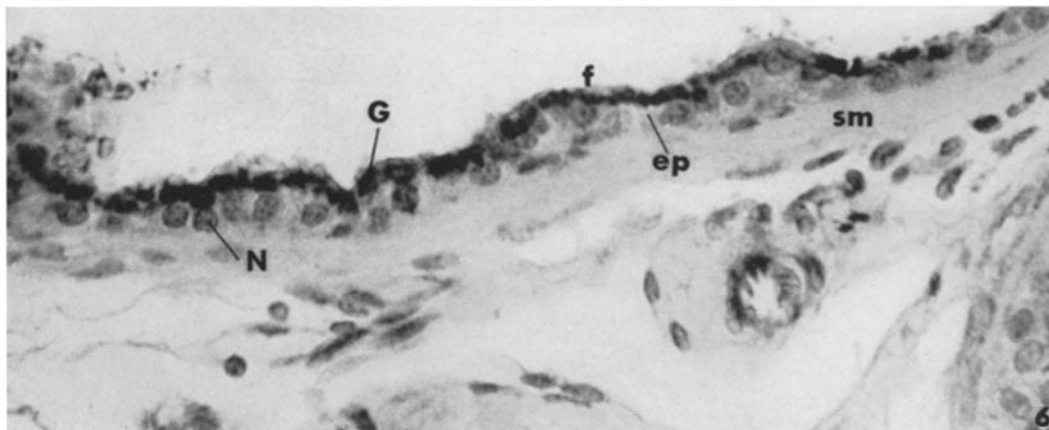
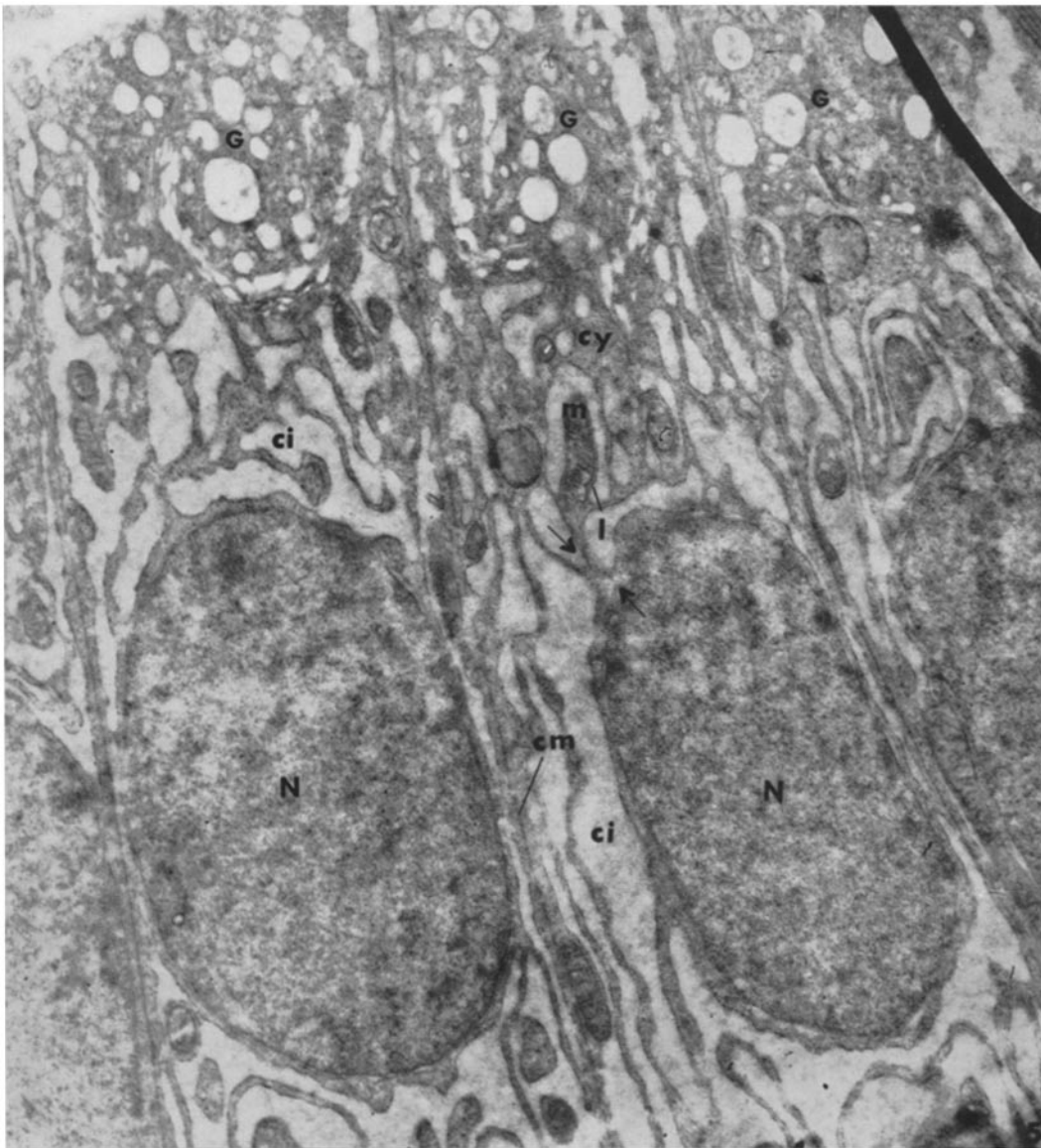


(Brandes and Portela: Coagulating gland epithelium)

PLATE 277

FIG. 5. Section showing the extent of the dilation of the cisternae (*ci*), in the mid-portion of the epithelial cells. A mitochondrion (*m*) appears protruding into a cisternal space, and is embedded in a scanty amount of cytoplasmic matrix (*cy*), bounded by a limiting membrane (*l*). The two arrows indicate what probably constitutes a communication between the perinuclear space and the general system of cisternae. The Golgi complex (*G*) shows a supra-nuclear location in agreement with light microscopy findings (compare with Fig. 6). $\times 15,000$.

FIG. 6. Photomicrograph of the epithelium (*ep*) showing the Golgi complex (*G*) located between the nucleus (*N*) and the free border (*f*). The acini appear surrounded by smooth muscle fibers (*sm*). Da Fano's method for the demonstration of the Golgi complex. $\times 1,600$.

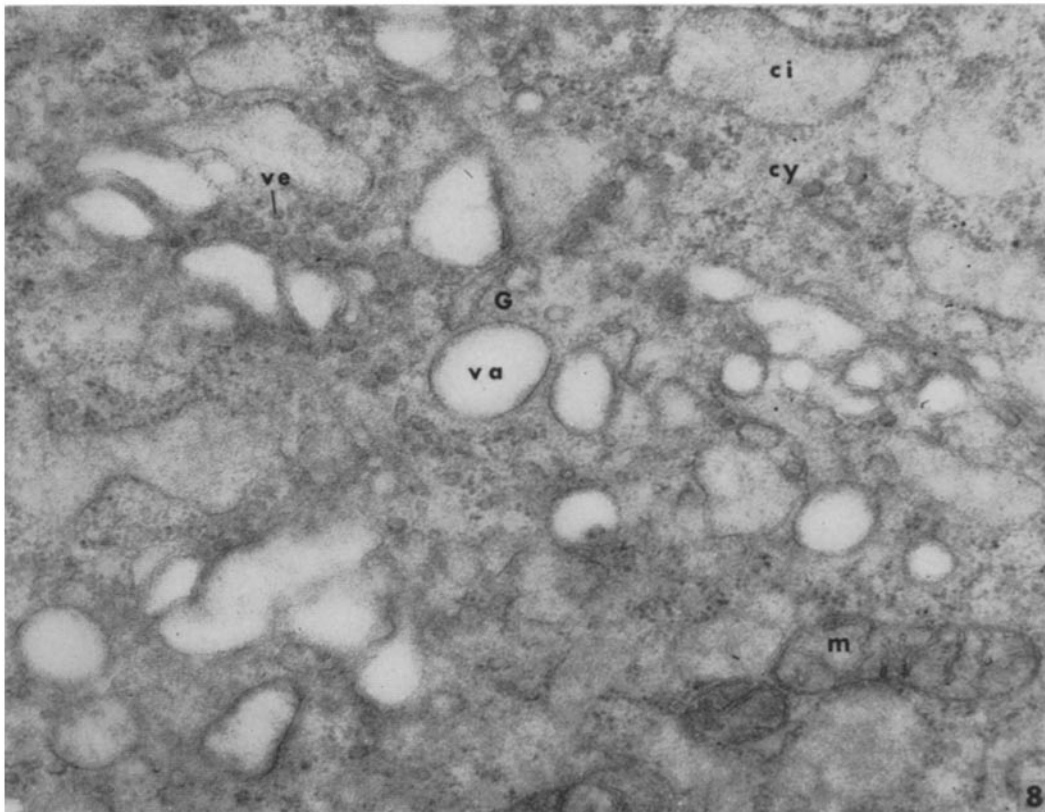
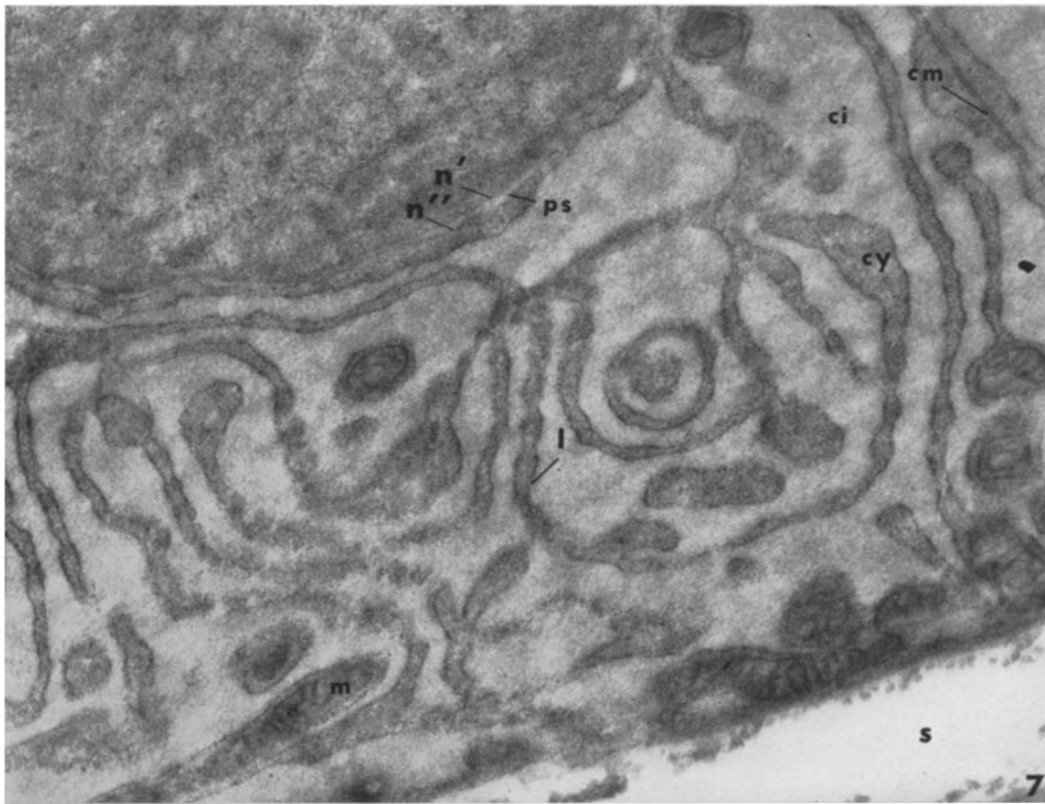


(Brandes and Portela: Coagulating gland epithelium)

PLATE 278

FIG. 7. Basal part of epithelial cell, showing a great dilation of the cisternae of the endoplasmic reticulum (*ci*), which appear to have lost their individuality. The perinuclear space (*ps*) is clearly visible. Comparison with Fig. 9 shows that the ratio of cisternal space to cytoplasmic matrix is greater in the basal half than in the upper half of the cell. $\times 39,000$.

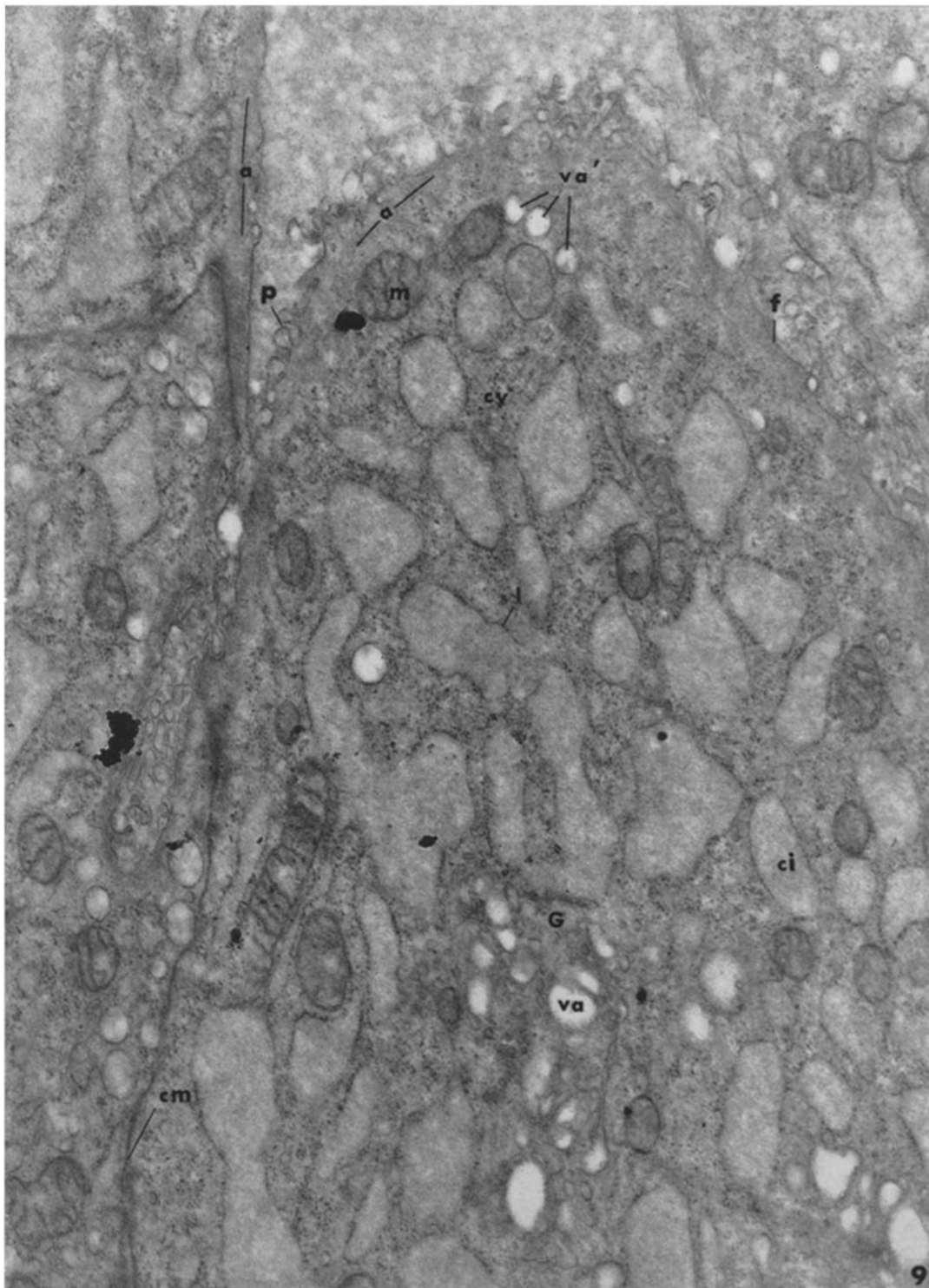
FIG. 8. Electron micrograph showing the Golgi region in greater detail. The various components of the Golgi complex (*G*), vacuoles (*va*), and small vesicles (*ve*), appear surrounded by dilated cisternae of the endoplasmic reticulum (*ci*). Palade granules are also present in the cytoplasmic matrix. $\times 60,000$.



(Brandes and Portela: Coagulating gland epithelium)

PLATE 279

FIG. 9. Apical pole of an epithelial cell, showing microprojections (*p*) at the free border (*f*). In the Golgi region (*G*), vacuoles of low density (*va*) are visible. Similar vacuoles (*va'*) are present towards the free border. The cisternae of the endoplasmic reticulum (*ci*), although dilated, preserve their individuality and their limiting membranes (*l*) appear clearly at many points. Mitochondria (*m*) and Palade granules are scattered through the cytoplasmic matrix. Immediately beneath the free border (*f*) there is a cortical layer of cytoplasmic matrix (*a*) devoid of Palade granules. $\times 39,000$.

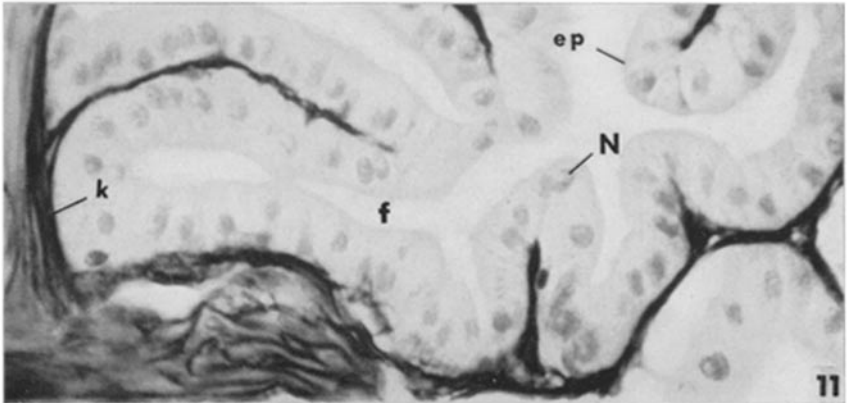
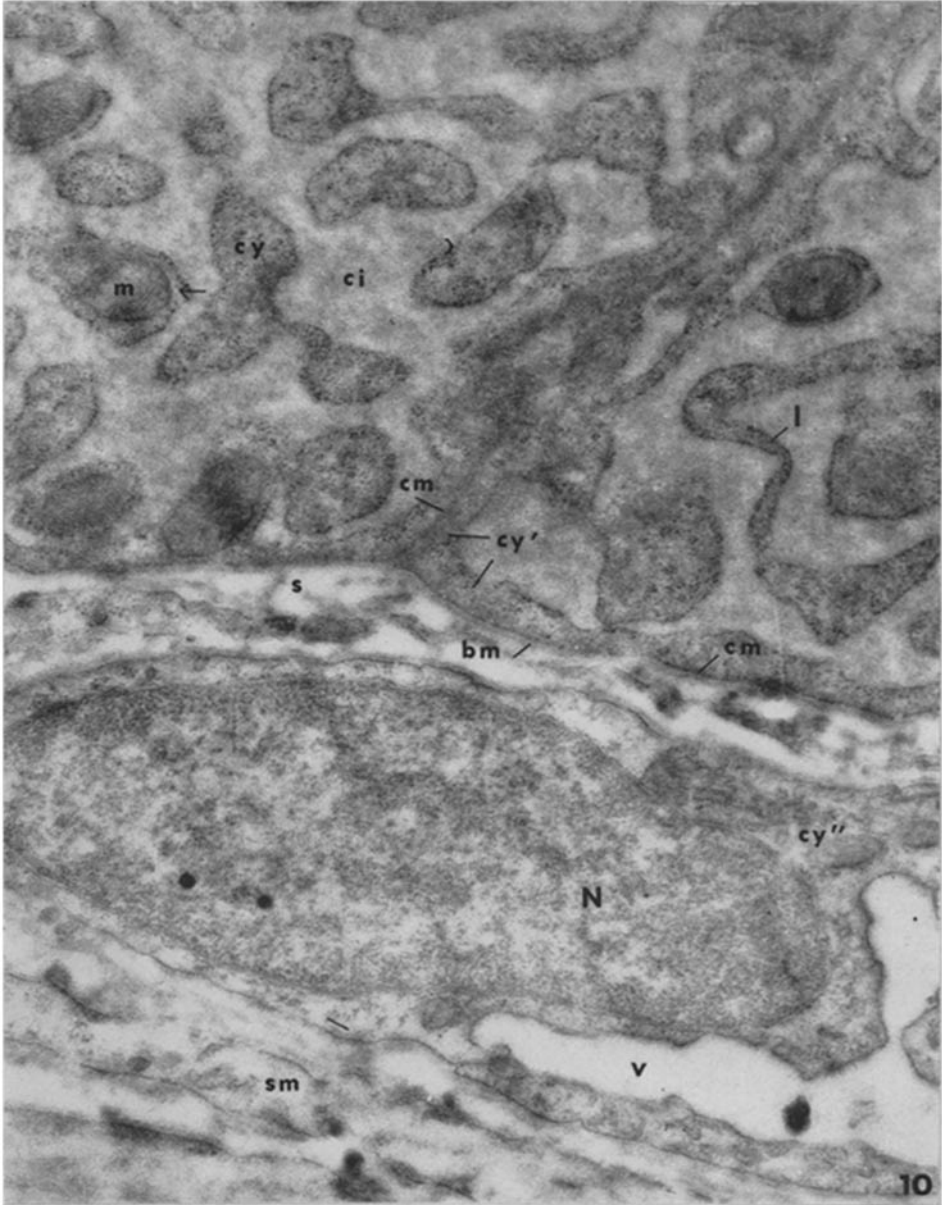


(Brandes and Portela: Coagulating gland epithelium)

PLATE 280

FIG. 10. Section of the basal part of two epithelial cells showing the basement membrane (*bm*) and the relations of the epithelium to the interstitial connective tissue. The cisternae (*ci*) are dilated and have lost their individuality. The cytoplasmic matrix appears in the form of isolated profiles (*cy*) or as a continuous band (*cy'*) separating the cisternae from the cell membrane (*cm*). Some mitochondria (*m*) appear as if suspended in the cisternae, but their outer membrane is surrounded by a scanty amount of cytoplasmic matrix (arrow). Beneath the basement membrane an interstitial space (*s*) is visible, as well as a capillary vessel (*v*), showing the lumen, and the nucleus (*N*) and cytoplasm (*cy''*) of an endothelial cell. Smooth muscle fibers (*sm*) can also be seen in this section. $\times 35,000$.

FIG. 11. Microphotograph of the coagulating gland epithelium. Gomori's technique for alkaline phosphatase. The reaction is positive (*k*) in a region which appears to correspond to some of the subepithelial structures seen in Fig. 10, and not localized to the "basement membrane" as usually described in the literature. $\times 1,600$.



(Brandes and Portela: Coagulating gland epithelium)