The Electron Microscopy of the Left Colleterial Gland of the Cockroach

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PLATES 108 TO 112

(Received for publication, September 9, 1958)

ABSTRACT

A study has been made of the cells of the left colleterial gland of the cockroach, *Periplaneta americana* (L.), using the electron microscope, and the results compared with previous histological and histochemical studies.

The colleterial gland consists of an arborescent bunch of long tubules composed mainly of the cells which secrete the structural protein of the egg case ("type 4 cells"). Other types of cells: chitinogenic cells and "type 2 and 3 cells" each with a different cytology are described.

The type 4 cells, which form the structural protein, reveal a cytological pattern very similar to that described for mammalian cells in a state of active protein synthesis. There is an elaborate development of particle-studded membranes in the cytoplasm. Smaller, rounded agranular vesicles also occur.

The free secretory surface of the secreting cells forms the "end-apparatus" of the light microscopists. The invaginated surface is cast into numerous long narrow processes usually radially arranged and directed into a funnel-like formation derived from the thin intima lining the lumen of the gland (Text-fig. 2). The secretion in the form of small balls may be seen in the cavity of the end-apparatus and sometimes in the narrow processes.

The small chitinogenic cells, lying between the protein-forming cells and the thin intima which they secrete, have a different cytology perhaps related to the fact that they form a polysaccharide rather than a protein. There is a very poor development of the particle-studded membranes of the type found in protein-forming cells.

The type 2 cells, supposed to form an oxidase, have an end-apparatus that is similar to, but more complex than, those of the type 4 cells and their cytoplasm is almost completely filled with mitochondria. There is some evidence that mitochondria play a part in forming the oxidase and pass into the tubules of the end-apparatus.

Type 3 cells resemble both types 2 and 4 and are probably a transient intermediate form.

INTRODUCTION

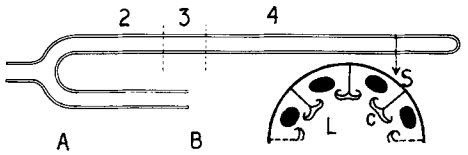
The colleterial glands of the cockroach are the sexual accessory glands which produce the tough, brown, resilient material of the egg capsule. There are two glands, a left and a right, each consisting of a mass of branched tubules. The two differ in size, appearance, and function. The left gland, which is much the larger, produces an opaque, white, voluminous secretion consisting mainly of the structural protein which later forms the bulk of the egg capsule (Pryor, 1940; Rudall, 1957), and a glucoside of an aromatic compound involved in the subsequent hardening of the protein (Brunet and Kent, 1955); the secretion also shows strong phenol oxidase activity. The right gland is known to secrete a β-glucosidase (Brunet and Kent, 1955). There are indications that the process of hardening of both egg capsule and insect cuticle takes place

in a somewhat similar way (Pryor, 1940), a circumstance that has encouraged the study of the colleterial glands.

We are concerned here only with the left gland which was chosen for detailed electron microscopic study, in the first instance, as an example of an arthropod organ exhibiting a very high rate of protein synthesis, to serve for comparison with the better studied organs of vertebrate origin. Few studies of the ultrastructure of insect tissues have appeared. Secondly, there occurs in these cells and related cells a remarkable structure concerned with secretion, known as the *end-apparatus*. It was hoped that the elucidation of its structure might add to the understanding of secretory processes in general.

A detailed description of the anatomy of the left gland has been given by Brunet (1951 and 1952). The gland opens by a single duct into the genital vestibulum; within the body this main duct divides into two, and these branches repeatedly divide to form many tubules which lie freely in the haemocoel (Text-fig. 1). Each tubule consists in the main of a single layer of glandular cells which form the walls of the tubules and enclose the lumen in which the secretion collects. The lumen itself is lined with a thin intima secreted by small "chitinogenic" cells which can be seen lying between the intima and the larger glandular cells (Text-fig. 2).

The colleterial glands are formed from an intucking of the epidermis, and on developmental grounds one would be led to deduce that the chitinogenic cells are homologous with the epidermal and the glandular cells with the dermal glands of the integument. Preliminary studies of dermal glands (Bradbury, private communication) have shown that they have many unusual features in common with the secretory cells of the colleterial glands.



TEXT-FIG. 1 *A*. Location diagram for types 2, 3, and 4 secretory cells of a tubule of the left colleterial gland. For reasons of clarity only one branch of the gland has been drawn.

B. Part of a cross-section of the tubule to show the relation of the secretory cells *S* and the chitinogenic cells *C* to the lumen *L*. The number of cells has been reduced to simplify their relations.

On cytological and histochemical grounds, Brunet (1952) had distinguished four types of glandular cells in the left gland; the location of these is indicated in Text-fig. 1.

Materials and Methods

The cockroach, *Periplaneta americana* (L.), was used throughout this work. Adult females were killed by decapitation and, in order to minimise alterations prior to fixation, portions of the colleterial glands were rapidly removed and were immediately plunged in the fixative without submerging the tissues in saline.

Fixation.—A buffered osmium tetroxide solution (1 per cent OsO_4 in veronal buffer pH 7.4 (Palade, 1952)) was used for a period of 2 hours at 0–5°C. The fixed material was then washed for 10 minutes in tap water and dehydrated in a series of ethanol solutions of increasing strength, finally being allowed to stand for at least 1 hour in pure ethanol.

Staining.—When staining was required to enhance the contrast of the section, the fixed tissue was transferred from 70 per cent ethanol to a 1 per cent solution of phosphotungstic acid in 70 per cent ethanol and left for 30 minutes, before being transferred to 90 per cent ethanol.

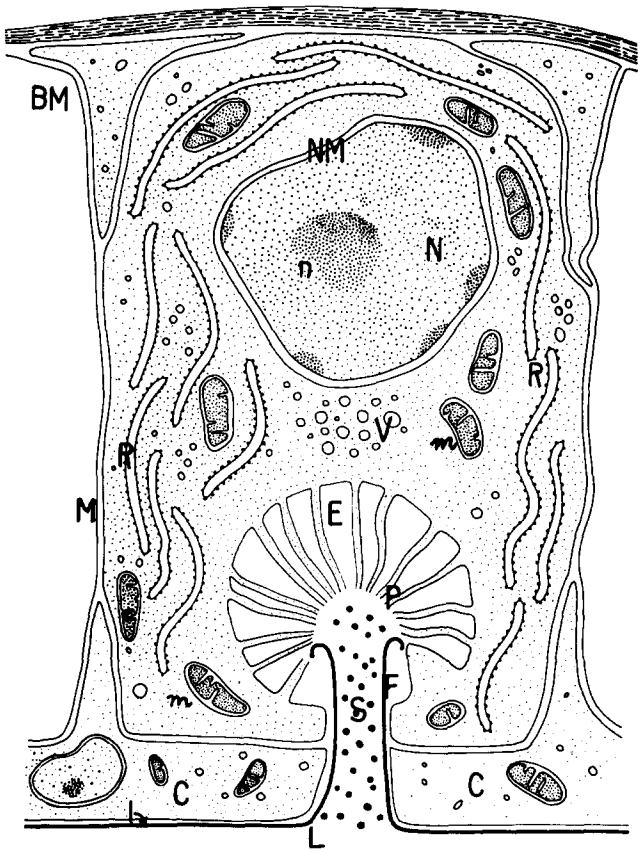
Embedding.—Methacrylates were first tried and found to lead to serious damage during polymerisation. Satisfactory results were, however, obtained with the "araldite" resin mixture described by Glauert *et al.* (1956). A description of the use of this resin has been given by Glauert and Glauert (1958) and Birbeck and Mercer (1957). The dehydrated tissue was transferred to xylene for 1 hour, then to a mixture of equal parts of araldite and xylene, and finally left for 1 hour at 60°C. in araldite. Selected portions were then removed and hardened in fresh araldite in gelatine capsules.

Microtomy and Electron Microscopy.—Sections (longitudinal and transverse to the axes of the tubules) were cut using either a modified Cambridge microtome or a Porter-Blum; these were examined in a Siemen's Elmiskop 1. Micrographs were made at instrumental magnifications of either 8,000 or 40,000.

DESCRIPTION AND DISCUSSION OF RESULTS

Low magnification electron microscopy revealed a picture of the cell relationships and cytology in general agreement with Brunet's account (1952) based on light microscopy. Reference may be made to Text-figs. 1 and 2 which combine the findings of light and electron microscopy.

It is proposed to describe the types of cell in the order: type 4, type 2, then type 3, as this appears to be a transition stage between types 2 and 4. Chitinogenic cells are described last; and type 1 cells are omitted as they are in a pre-secretory stage.



TEXT-FIG. 2. A greatly simplified reference diagram of a type 4 secretory cell embodying the combined results of light and electron microscopy. *N*, nucleus; *n*, nucleolus; *NM*, nuclear membrane; *R*, reticulum; *m*, mitochondrion; *E*, end-apparatus; *P*, processes of end-apparatus; *F*, funnel of end-apparatus; *C*, chitinogenic cell; *L*, lumen containing secretion; *I*, intima; *V*, small vesicles; *S*, secretion; *M*, cell membrane; *BM*, basement membrane.

Type 4 Cells:

These are the most numerous and the most easily found as they lie at the innermost ends of the tubules (Text-fig. 1). They are rather squat and contain a single nucleus usually located at the basal pole of the cell, the apical pole being occupied by the secretory system, the end-apparatus (Text-fig. 2). One or more large, granular nucleoli occur in the nucleus, and often deposits of dense particulate material are found adhering to the inner face of the nuclear membrane.

Particle-studded membranes are elaborately developed (*R* in Figs. 1 and 2) and form a reticulum (Porter, 1953) remarkably similar in extent and organisation to that noted in the cells of the mammalian pancreas (Palade, 1955; Sjöstrand and Hanzon, 1954). The small dense particles, probably containing RNA (Palade and Siekevitz, 1956), found both attached to the membranes and also free, seem identical with those described by Palade (1955). These findings suggest that protein synthesis in insects is effected by essentially the same cytoplasmic system as in other phyla already studied.

There are also large numbers of small rounded agranular vesicles of diameter of the order of 300 to 500 A (*V* in Fig. 1, Text-fig. 2). They are found widely distributed in the cytoplasm but numbers of them may occur together locally, giving a type of cluster sometimes referred to as the "Golgi apparatus." These vesicles do not contain secretion nor could they be seen to enter the tubules of the end-apparatus. They resemble also the vesicles found in collagen-forming cells (Jackson, 1957).

Mitochondria (*m*) are commonly present in all parts of the cytoplasm and may be closely in-

vested by the membranes of the reticulum. They are usually small and contain transverse cristae, but long forms, some with longitudinally arranged internal membranes have also been found.

The End-Apparatus.—In electron micrographs this organelle reveals itself as a remarkable specialisation of the cell membrane (Fig. 2) with which is associated a funnel-like involution of the thin cuticle lining the lumen of the tubule. The free secreting surface of each cell consists of a large number of long, radially directed, tubular processes of the plasma membrane of the cell. The most common arrangement of these processes is that depicted in Fig. 2; occasionally the contents of the cell may press forward into the funnel and penetrate almost into the lumen. The diameter of the processes (700 to 1000 A) is close to that of the balls of secretion (Fig. 5) and occasionally these may be seen apparently within the processes (Fig. 4). The membranes of the processes are about 60 A thick and often appear stratified (Fig. 4). This observation recalls those made on the microvilli of the jejunum cells of the rat (Zetterquist, 1956). The long processes sometimes appear open-ended (*O* in Fig. 4), but this observation must be accepted cautiously since we are dealing with thin sections. Droplets of secretion lie adjacent to the processes of the end-apparatus and have no enclosing membrane. Having no membrane, it appears unlikely that the secretion is simply part of a process that has broken away; the micrographs suggest that the droplets of secretion pass down the inside of the process and emerge through temporary gaps in the membrane.

Type 2 Cells:

In the posterior branches of the gland (see Text-fig. 1) are found the distinct type 2 cells. These are somewhat taller than type 4 cells, their cytoplasm is more dense, and the end-apparatus is more elaborate (Fig. 6). The end-apparatus, in addition to the relatively coarse, closely packed cell processes, has beyond the tips of these a dense feltwork of material (*H*) also found in type 3 cells (see *H* in Fig. 3).

The cytoplasm is packed with mitochondria, almost to the exclusion of all other structures. These mitochondria are usually elongate, with longitudinally disposed cristae. The conclusion that they are mitochondria is supported by the intense colouring of the cytoplasm of sections 5 μ in thickness by acid fuchsin in aniline water (the classical method for mitochondria), and by acid haematein (under conditions that demonstrate phospholipide (Baker, 1946)).

In type 2 cells there is an almost complete absence of the reticulum of particle-studded membranes characteristically present in type 4 cells. We can only conclude very tentatively that secretion of these cells is effected predominantly by mitochondria, and not by a membrane system of the type usually found in secretory cells such as type 4 cells. The mitochondria, or their decomposition products, appear to pass along the processes of the end-apparatus (see the similar situation in a type 3 cell shown in Fig. 3). The entry of mitochondria into the brush border of the Malpighian tubules of the grasshopper has already been reported by Beams, Tahmisian, and Devine (1955).

The possibility that mitochondria are directly converted into the secretion product of type 2 cells may be associated with the oxidative activity of the secretion of the gland. The secretion will oxidize 3,4-dihydroxy derivatives so long as the side-chain is short (dopa is not oxidized), *o*- and *p*-dihydroxyphenols, but not monohydroxyphenols (Brunet, unpublished).

Type 3 Cells:

These cells have characters of both types 2 and 4 cells: the cytoplasm has many mitochondria, more than type 4, but less than type 2; many, but not all the processes of the end-apparatus may contain mitochondria; the feltwork of the end-apparatus is again present (Fig. 3).

It would appear that they represent a transition stage between types 2 and 4. It is not known why the cells should become so much taller than the other types of cell at this phase.

Chitinogenic Cells:

These relatively small, flattened cells were known to line the lumen of the gland (Fig. 2), and it is thought that they secrete the thin intima (*I*, Fig. 2) and the funnel into the end-apparatus. Electron micrographs show that they also extend between the glandular cells and that they are also to be found beneath the basement membrane where they may be responsible for the secretion of part of the basement membrane that surrounds each tubule. Our observations bear out Wigglesworth's contention (1956) that basement membranes owe their origin both to the secretion of amoebocytes and to that of the tissues they surround.

The detailed cytology of these cells and the

formation of cuticular layers will be made the subject of a further paper. Here we wish only to point out the contrast between the cytology of the typical protein secreting cell (type 4) and that of cells secreting polysaccharide membranes (*C* in Fig. 2). Mitochondria and agranular vesicles are as common in chitinogenic cells as in protein-forming cells; but in the former there is a relative absence of the particle-covered membranes which form the characteristic reticulum of cells secreting protein. This observation is in accord with the biochemical opinion that ribonucleic acid is not required for polysaccharide synthesis (Kent, 1957).

The Secretion:

The structural protein which forms by far the greater part of the output of the gland, may be seen both within the end-apparatus of type 4 cells and also in the lumen. It stains strongly with phosphotungstic acid and appears as a mass of droplets of variable size (average diameter about 700 A) and occasionally as long forms or as vacuolated droplets. They are not enclosed in a membrane demonstrable with phosphotungstic acid (Fig. 5). The diameter of the droplets is similar to that of the tubules of the end-apparatus and this may be connected with the mode of secretion.

Very occasionally (*L* in Fig. 5), one notes larger lumps of a secretion having a similar texture but different staining properties to the small droplets. Similar lumps have been noted inside the cells but it is not known what these are or how, if at all, they emerge from the cells.

Crystals, known to be calcium oxalate (Parker and Rudall, 1955), are common in all parts of the lumen. They proved very difficult to section and usually damaged the glass knife. None was noted within the cells and one must assume that the salt (or its precursors) is secreted by the cells and that the crystals grow in the lumen. They appear to be enclosed by a membrane perhaps consisting of a layer of the structural protein. Sections of the hardened egg case show the crystals, apparently unchanged, embedded in a structureless matrix.

The authors would like to thank Mr. M. J. Docherty who made the photographic enlargements.

The work has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, United States Public Health Service; and a grant to Oxford University from the Agricultural Research Council.

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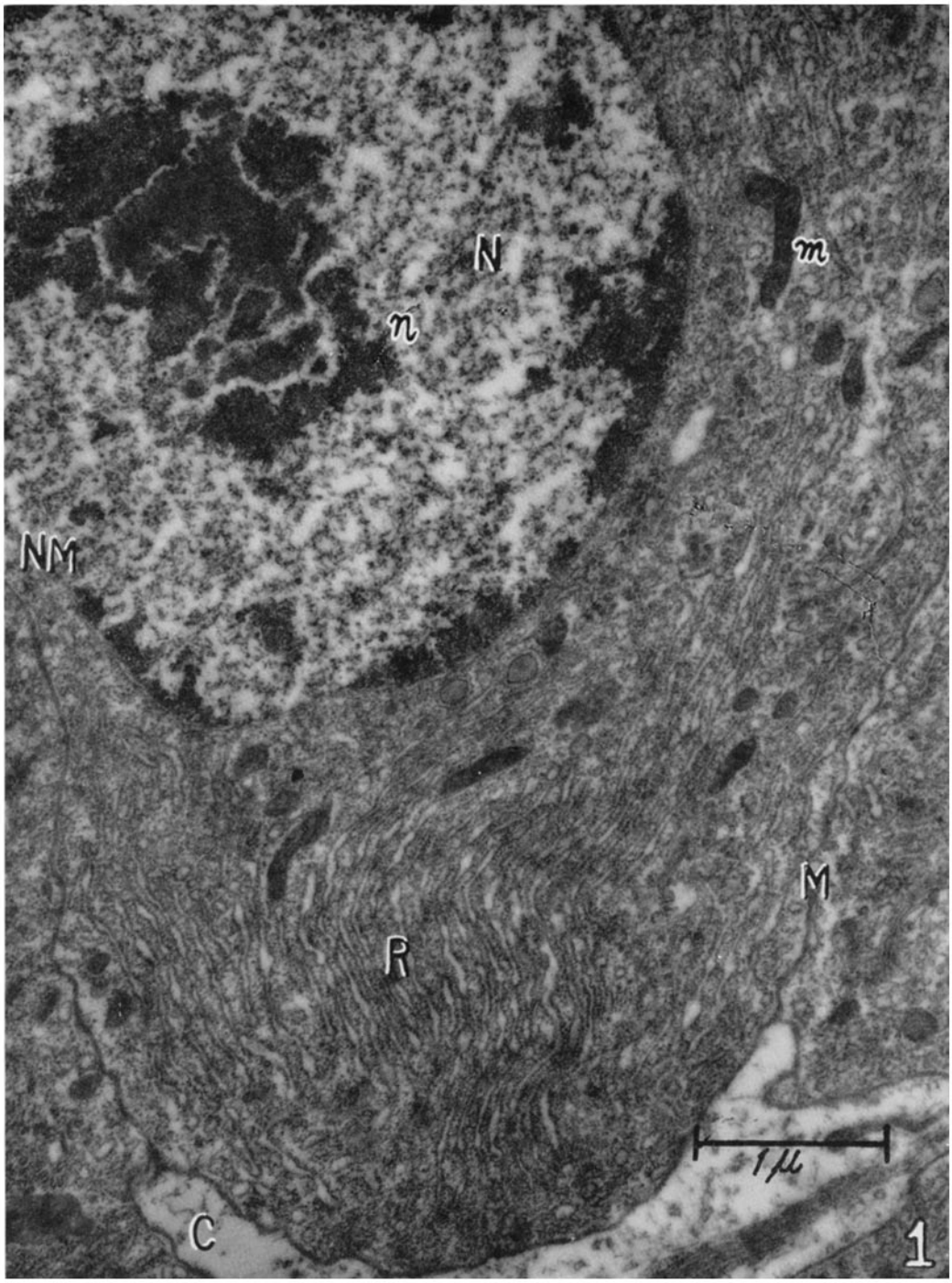
Explanation of Plates

Plate 108

Fig. 1. The basal portion of a type 4 cell complementary to the portion shown in Fig. 2. The nucleus *N* contains a large granular nucleolus *n*, and dense deposits around the nuclear membrane *NM*. The cytoplasm is largely occupied by the reticulum *R*. A portion *C* of a chitinogenic cell extends between two glandular cells. Other lettering as in Fig. 2.

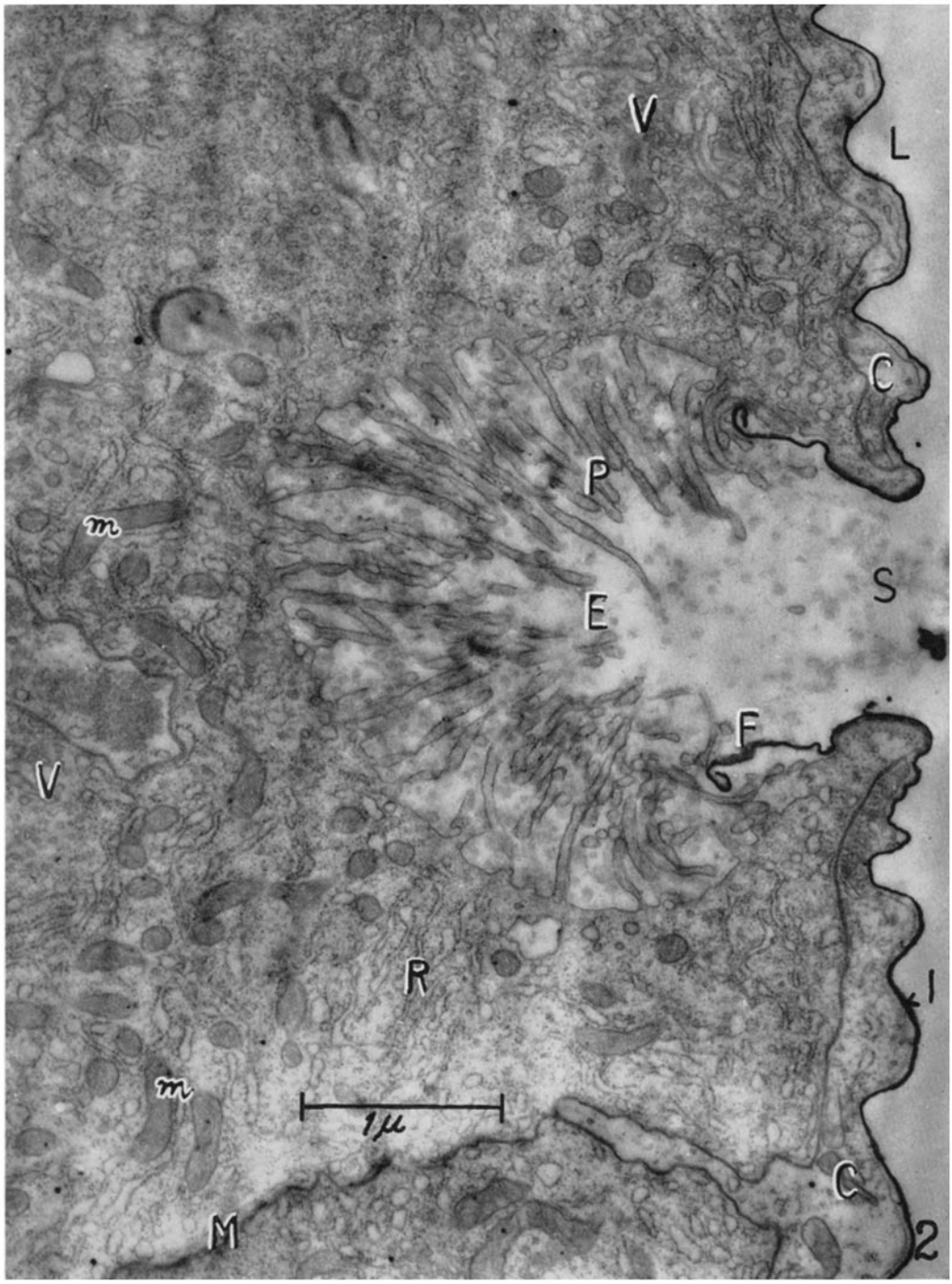


(Mercer and Brunet: Left colleterial gland of cockroach)

PLATE 109

FIG. 2. Section of a type 4 cell showing the end-apparatus *E*. The lumen *L* of the gland appears along the right hand side of the field. Portions of two chitinogenic cells *C* can be seen between the glandular cell and the intima *I*, lining the lumen. The intima turns in to form the funnel of the end-apparatus at *F*.

Particle-studded membranes of the reticulum are indicated at *R*; *m* are mitochondria, *V* small vesicles. The processes *P* of the end-apparatus seem to contain secretion which is shown outside the cell in droplet form at *S*. *M* is a cell membrane.

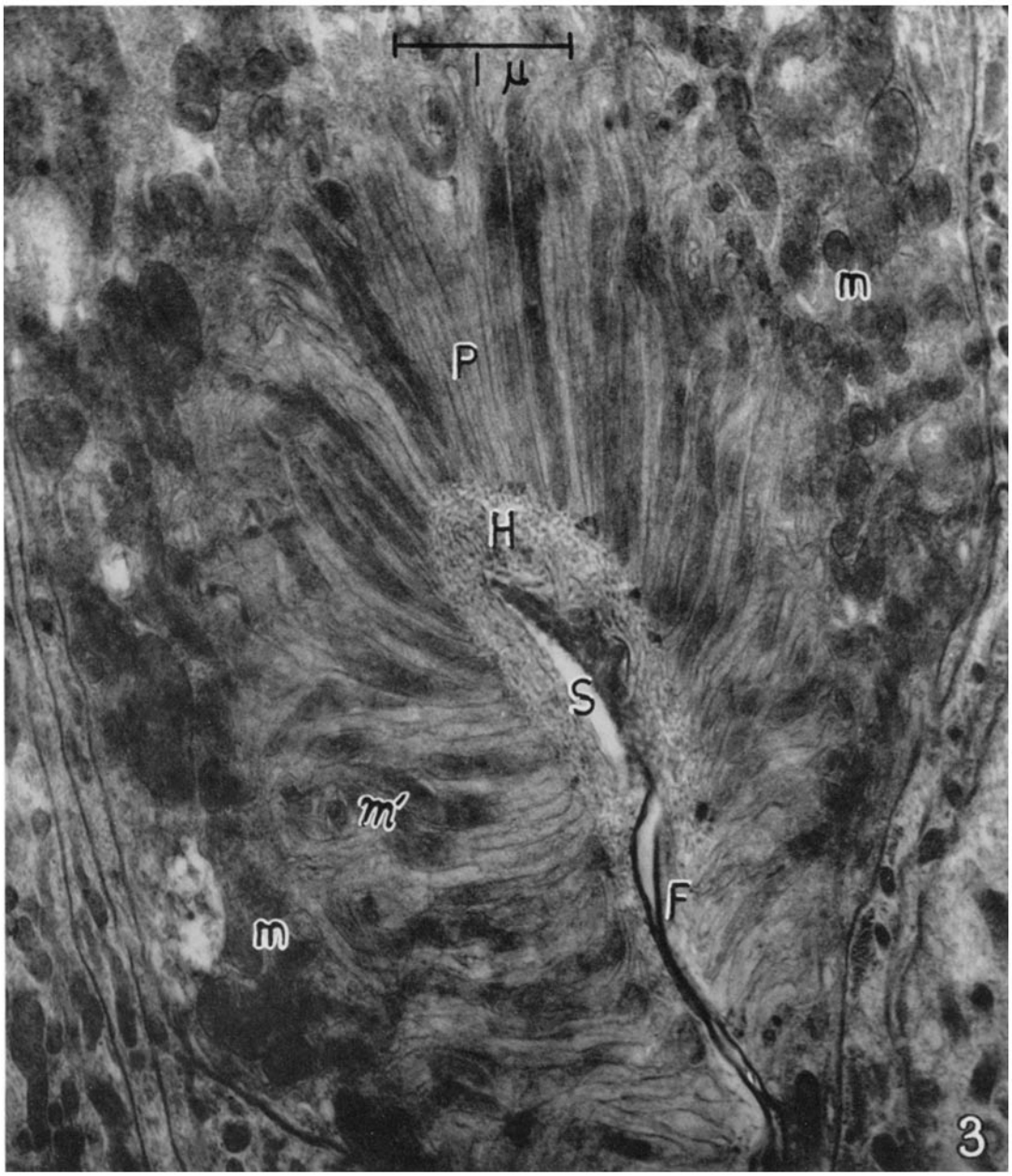


(Mercer and Brunet: Left colleterial gland of cockroach)

PLATE 110

FIG. 3. The end-apparatus of a type 3 cell which is very similar to that in a type 2 cell. Note the closely packed processes *P*, the feltwork *H* separating the ends of the processes from the secretion *S*. *F* is the long narrow funnel formed by the in-turned intima. *m* are mitochondria and *m'* are thought to be mitochondria in the processes of the end-apparatus.

Preparation stained with phosphotungstic acid.



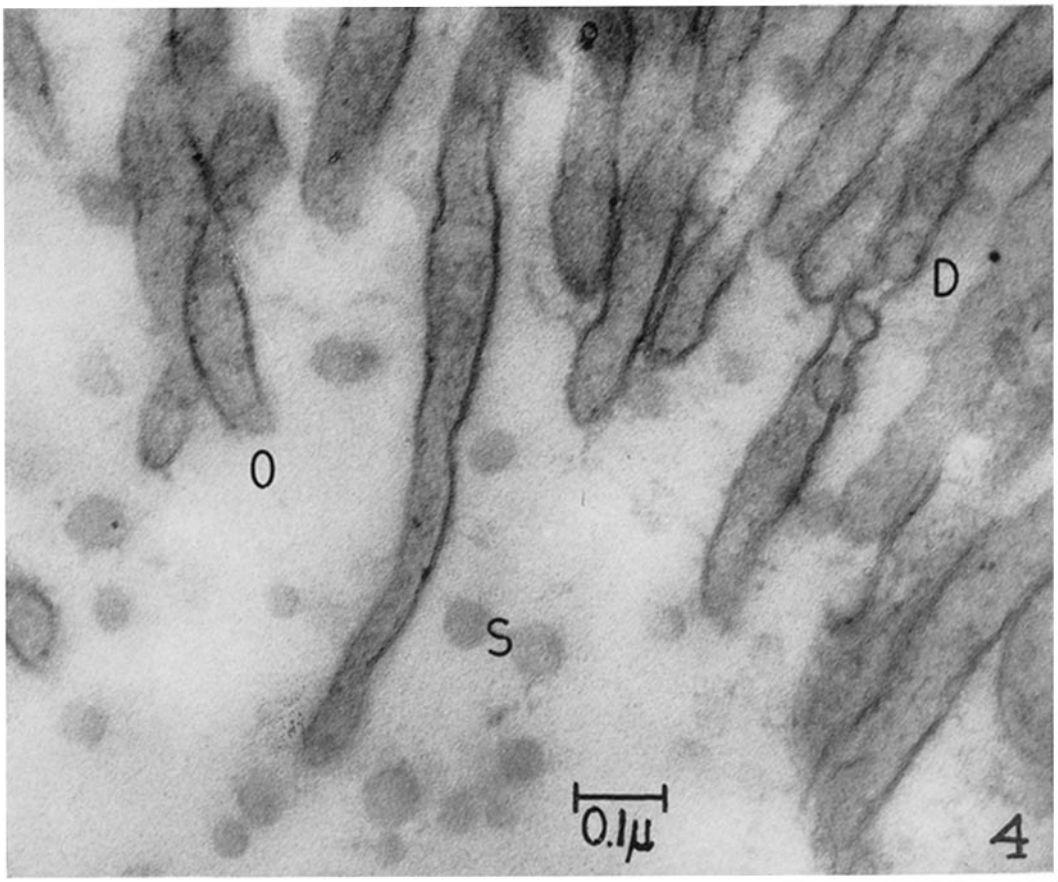
(Mercer and Brunet: Left colleterial gland of cockroach)

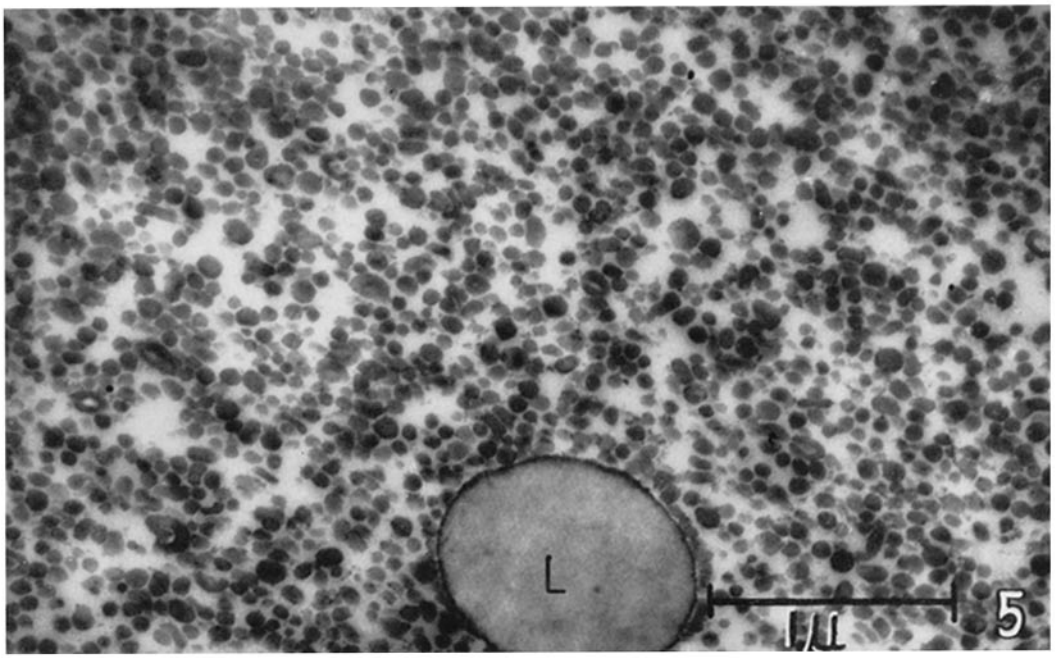
PLATE 111

FIG. 4. An enlargement of the tips of the processes of the end-apparatus of a type 4 cell. Droplets of secretion are seen at *S* and possibly within the processes *D*. At *O* is seen what may be an opening with a secretion droplet emerging. In places the membranes of the processes (extensions of the plasma membrane) appear stratified in three layers.

FIG. 5. The secretion of type 4 cells, *i.e.*, the structural protein, as seen in the lumen of the gland. The large bodies (*L*) appear rarely and seem distinct from the droplets. Large crystals (not shown) are common.

Stained with phosphotungstic acid.



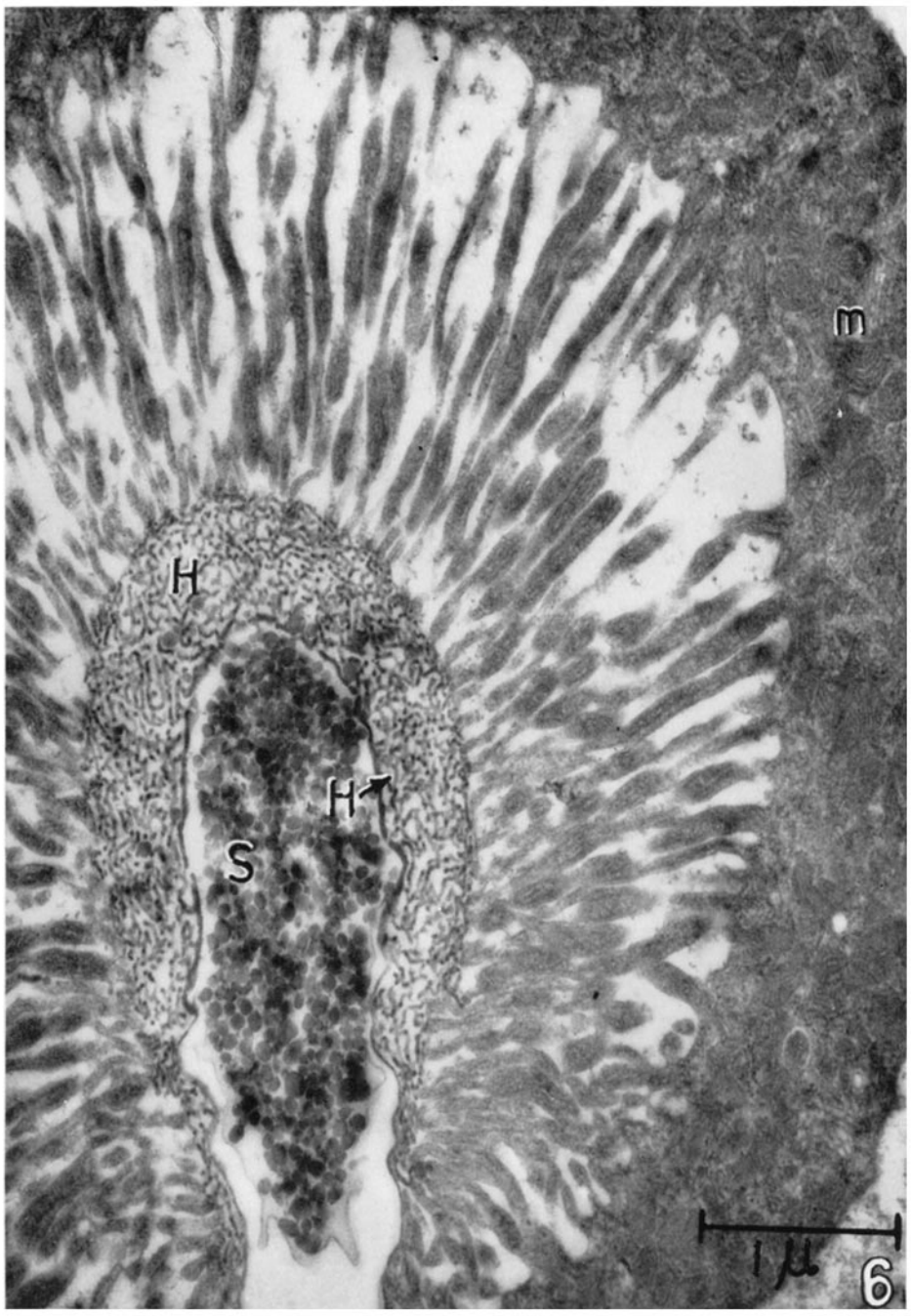


(Mercer and Brunet: Left colleterial gland of cockroach)

PLATE 112

FIG. 6. The end-apparatus and portion of the cytoplasm of a type 2 cell. The section passes to one side of the opening of the end-apparatus into the lumen. The cytoplasm is packed tightly with mitochondria (*m*), and at the ends of the processes may be seen the feltwork *H*. *S* is secretion similar to that found elsewhere in the gland (see Fig. 5). There are indications of structure within the processes of the end-apparatus.

Stained with phosphotungstic acid.



(Mercer and Brunet: Left colleterial gland of cockroach)