CELLULAR SPECIALIZATION IN THE EXCRETORY EPITHELIA OF AN INSECT, *Macrosteles fascifrons* STÅL (HOMOPTERA)

DAVID S. SMITH, Ph.D., and VIRGINIA C. LITTAU, Ph.D.

From The Rockefeller Institute

ABSTRACT

An electron microscopic investigation of the Malpighian tubules of a leafhopper, *Macrosteles fascifrons*, shows that these organs comprise three quite distinct cell types, and the structure of these and of the mid- and hindgut epithelial cells is described. In particular, a comparison is made between the organization of the basal and apical surfaces of cells in the Malpighian tubule and in the vertebrate kidney, and it is suggested that similarities between these excretory epithelia reflect functional parallels between them. While the midgut and one region of the Malpighian tubule bear a typical microvillar brush border, elsewhere in the tubule and in the hindgut the apical surface bears cytoplasmic leaflets or lamellae. The sole solid excretory material of these insects consists of the brochosomes, secreted by cells of one region of the Malpighian tubule. The structure, geometry, and development of these unusual bodies, apparently formed within specialized Golgi regions, has been investigated, and histochemical tests indicate that they contain lipid and protein components.

INTRODUCTION

A functional analogy may be drawn between the renal excretory system of vertebrates and the Malpighian tubules of insects. The most important role played by the latter is in the separation of waste products from the haemolymph, followed by the secretion of these products or their derivatives into the lumen of the tubule to form the urine, which, together with the residue from the alimentary tract, constitutes the excreta of the insect. In addition, the tubules perform an osmoregulatory function, and their product ranges in physical appearance from a clear solution to a dry solid material, depending on the environment in which the species lives, or on the physiological condition of the individual.

As in the vertebrate renal tubule, which is composed of a number of clearly defined regions, specialization either of parts of each Malpighian tubule, or of certain entire tubules out of the full complement, is of frequent occurrence, and may be clearly associated with functional specificity. For example, Wigglesworth (1931) found that, in the bloodsucking bug *Rhodnius*, the lumen of the distal two-thirds of each tubule contained a clear alkaline solution, while granules of uric acid were restricted to the lumen of the proximal region. He suggested that sodium and potassium acid urates, in solution, secreted into the lumen of the distal part of the tubule, reacted with carbon dioxide to form the more insoluble uric acid, which, by virtue of water reabsorption taking place through the brush border of cells lining the

This work was supported in part by United States Public Health Service Training Grant 2G-70 and by United States Public Health Service Grant E-1537.
Received for publication, December 20, 1959.

lumen in the proximal region, was precipitated locally, prior to elimination *via* the hindgut and rectum, where further water resorption took place. The occurrence of such morphological specialization and division of labor within the Malpighian tubule system is perhaps not surprising in view of the diversity of food-stuffs utilized by different insects. Consequently, care must be taken in interpreting the light or electron microscopic structure of these, as other cells, as cellular fine structure may be affected, for example, by the nutritional condition of the individual, the availability of water, and possibly by secretory or other cycles.

In the present study, an attempt has been made to investigate the cytological structure of each cell type occurring in the Malpighian tubules of a leafhopper (*Macrosteles fascifrons*), an insect which feeds by inserting its stylus-like mouthparts into the vascular bundles of plants. The choice of this material was prompted by the discovery of Day and Briggs (1958) that the cells of the Malpighian tubules of these insects are the site of production of very unusual excretory bodies, which they describe as typically having the form of "regular dodecahedra with projections from each corner." They concluded that these bodies are formed intracellularly, and are voided into the tubule lumen, prior to their elimination from the gut. These structures were first discovered by Tulloch *et al.* (1952, 1953) and Wilde (1957) on the surface of the insect's body, particularly on the wing membranes, and, because of their reticulated form, were termed "brochosomes" by these workers. That these might be products of the Malpighian tubules was suggested to Day and Briggs by Storey and Nichols' observation (1937) of a behavior pattern in leafhoppers in which the insect smeared a pellet of excrement over its body and wings with its hind pair of legs. Day and Briggs state that brochosomes are present only in the "posterior" region of the tubule, and that anteriorly the cells bear microvilli on the lumen surface. A morphological account of the gut and Malpighian tubules of *M. fascifrons* is given in the next section, and it is sufficient at this point to say that the tubules are divided, not into two, but into four distinct regions, of which the third, numbering from the insertion of the tubule, contains brochosomes.

Apart from the preliminary work by Bradfield (1953) on the structure of Malpighian tubule cells in the larva of the wax moth, *Galleria*, and the study of Day and Briggs mentioned earlier, electron microscopic investigation of these organs has been restricted to members of the order Orthoptera: the locust *Melanoplus* (Beams *et al.*, 1955) and the cricket *Gryllus* (Berkaloff, 1958, 1959). Since the Malpighian tubules of *M. fascifrons* afford an excellent example of morphological specialization, and, furthermore, since each of their component cell types differs cytologically from those of the species mentioned above, the structure of each region will be described in turn.

Information derived from electron microscopy on the cytology of Malpighian tubule cells is scanty, but it is probable that examination of a wider range of species, especially those in which physiological and light microscopic studies have already been carried out, would reveal an underlying pattern in the distribution of the cell types found in these organs. Through such studies cell and tissue morphology might more precisely be related to such general functions as water resorption, ionic balance, and the production of soluble and insoluble nitrogenous and other excretory products.

MATERIALS AND METHODS

The species of leafhopper employed in this work was *Macrosteles fascifrons* Stål. This insect was available in culture on rye seedlings, and individuals were fixed shortly after they had been collected from the plants.

Since good fixation of Malpighian tubule cells is notoriously difficult to achieve even for light microscopic examination, and, since a preliminary dissection in Ringer's solution might result in postmortem changes, the gut and Malpighian tubules were freed from the rest of the insect directly in the fixative, 1 per cent osmium tetroxide buffered at pH 7.75. The material was fixed for 30 minutes, dehydrated rapidly, and embedded in a 20:80 methyl:butyl methacrylate mixture. A Porter-Blum microtome was used for sectioning, and preparations were examined in an RCA EMU-2 and a Siemens Elmiskop I. Contrast in the specimen was enhanced by "staining" with uranyl acetate (Watson, 1958*a*) or lead hydroxide (Watson, 1958*b*; Peachey, 1959), and sections were blanketed with collodion or carbon films.

Intact brochosomes for electron microscopic observation were obtained by disrupting the cells in distilled water; drops of this preparation being dried on coated grids, either unfixed, or after exposure to osmium tetroxide vapor. In addition, Anderson's

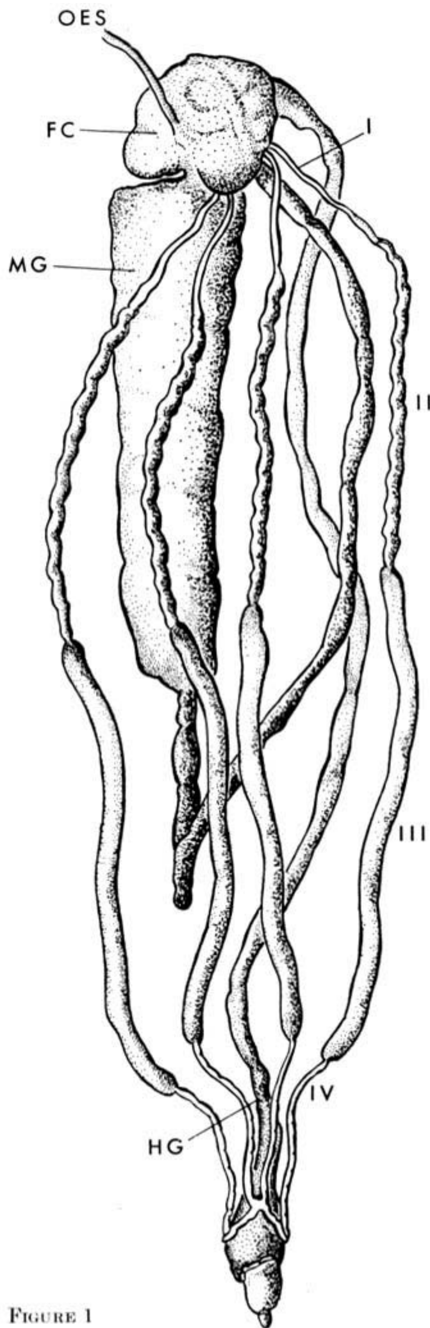


FIGURE 1

The mid- and hindgut regions of *Macrosteles fascifrons*, showing their relationship with the four Malpighian tubules. The morphologically distinct regions of the Malpighian tubules are numbered, from the anterior region I, which joins the gut within the filter chamber, to region IV which is closely applied to the rectum, or termination of the hindgut. *OES*, oesophagus; *FC*, filter chamber; *MG*, midgut; *HG*, hindgut.

"critical point" method (1951) was employed in an attempt to minimize distortion during drying.

The chemical nature of the brochosomes was investigated by the application of several color tests and extraction procedures either to thick (1 to 2 μ) sections of osmium-fixed methacrylate-embedded material, or to whole brochosomes, obtained by smearing Malpighian tubules on a slide and fixing in formaldehyde vapor. Nucleic acids were localized with the basic dye azure B (Flax and Himes, 1952) employed both on untreated sections and on sections treated with the enzyme ribonuclease (Worthington Biochemical Laboratories, Freehold, New Jersey). The ribonuclease was used, at a concentration of 0.02 per cent at 37°C. for 2 hours, on methacrylate-embedded sections from which the plastic had been removed by treatment with chloroform for 1 hour at 60°C. The periodic acid–Schiff reaction (Hotchkiss, 1948; McManus, 1946) was used as a test for 1,2 glycol groups, as in different kinds of polysaccharides. Sudan black B (a saturated solution in propylene glycol) was used for general lipid staining based on differential dye solubility, and was applied both to untreated sections and to sections extracted with hot pyridine, while unsaturated lipid was tested for by the performic acid–Schiff reaction (Pearse, 1953). Protein was demonstrated by the affinity of its basic groups with the acid dye fast green (0.1 per cent aqueous solution, pH 1.3) and by its digestibility with the enzyme trypsin (Worthington Biochemical Laboratories; 0.4 per cent aqueous solution buffered at pH 7.6 in tris buffer at 37°C. for 2 hours). A method employing ethyl gallate, devised by Wigglesworth (1957) to show the location and approximate concentration of osmium in tissue sections, was carried out as follows: after the usual fixation in osmium tetroxide, the tissues were placed for 24 hours in a saturated solution of ethyl gallate in 0.5 per cent cresol, and following this treatment were dehydrated and embedded in methacrylate in the standard fashion.

Tests for purines, urates, and other purine-like substances were performed on whole Malpighian tubules dissected from the insect. Both the murexide test and the Hollande modification of Courmont-André's method were carried out following the procedures outlined by Glick (1949).

OBSERVATIONS AND RESULTS

The Morphology of the Alimentary Tract and Malpighian Tubules

Reference to Fig. 1 will clarify the morphological relationship between the Malpighian tubules and the alimentary tract in *Macrosteles*. In the sub-order Homoptera the relations between the fore-, mid-, and hindgut are frequently complicated, due to the pres-

ence of the so-called "filter chamber." In these insects the slender oesophagus, or foregut, leads into a dilated sac, designated as the filter chamber, representing the anterior region of the midgut, and opening into the stomach. The remainder of the midgut is reflexed forward, and the transition zone between the mid- and hindgut, including the point of origin of the Malpighian tubules, is inserted into the filter chamber. A similar situation occurs in allied insects, and it is thought that the function of the filter chamber is to provide a short cut for the passage of the copious amount of water imbibed by the insect directly from the first to the last part of the midgut, thus by-passing the main absorptive epithelium of this region of the digestive tract.

As is usual in the Homoptera, four Malpighian tubules are present. They pass backward from their point of origin, lying free within the haemolymph, and not only do they join posteriorly as two pairs, but the latter are also linked by a cross-connective which is closely apposed to, although apparently not fused with, the epithelium of the dilated rectum. In Fig. 1, the regions of the Malpighian tubule have been denoted by the Roman numerals I through IV and, for convenience, this notation will be used throughout the text. The figure has been somewhat simplified: it does not show that in fact the hindgut and Malpighian tubules are asymmetrically placed in the body cavity, and are frequently contorted.

As in other insects, the Malpighian tubules of *Macrosteles* are formed from a single layer of cells, so arranged that, in transverse sections, as few as two or three cells surround the lumen.

The Malpighian tubules of *Macrosteles* lack the spirally arranged intrinsic striated muscle fibers responsible for the writhing motions of the tubules observed in a wide variety of insects, but this intrinsic musculature is found in both the mid- and hindgut.

Region I of the Malpighian Tubule

The Malpighian tubule as it leaves the filter chamber is a narrow tube between 25 and 35 μ in diameter. The wall of the tubule in this region is extremely thin, and Fig. 2 represents, in transverse section, a transect from the basement membrane at the upper left to the apical region and lumen, at the lower right, a distance of about 3μ.

The cytoplasm of the apical region of the cell, bounding the lumen, is produced into an array of microvilli or brush border elements, up to 3 μ in length, and from 0.1 to 0.15 μ in diameter. An estimated density of 30 microvilli per square micron was derived from areas where the array was sectioned transversely (Fig. 3). Here, as in the microvilli of renal cells (Sjöstrand and Rhodin, 1953), no trace of organized structure is apparent, and the cytoplasm contained within them is granular and of the same density as that found elsewhere in the cell. The relatively loosely packed nature of the brush border in these cells is apparent when it is remembered that Rhodin (1958*a*) gave a value of 215 per square micron for the concentration of the microvilli lining the collapsed lumen of the proximal convolution.

The basal region of the cell is in contact with the haemolymph of the general body cavity of the insect. The basal membrane appears, in Fig. 2, as an apparently amorphous sheath, 600 to 700 A in thickness. Beneath this is the strongly osmiophilic plasma membrane, which more closely resembles that of mammalian distal tubule cells in the depth and complexity of its involutions, and which may be regarded as defining cytoplasmic

Electron micrographs showing the structure of cells of region I of the Malpighian tubules of *Macrosteles fascifrons*.

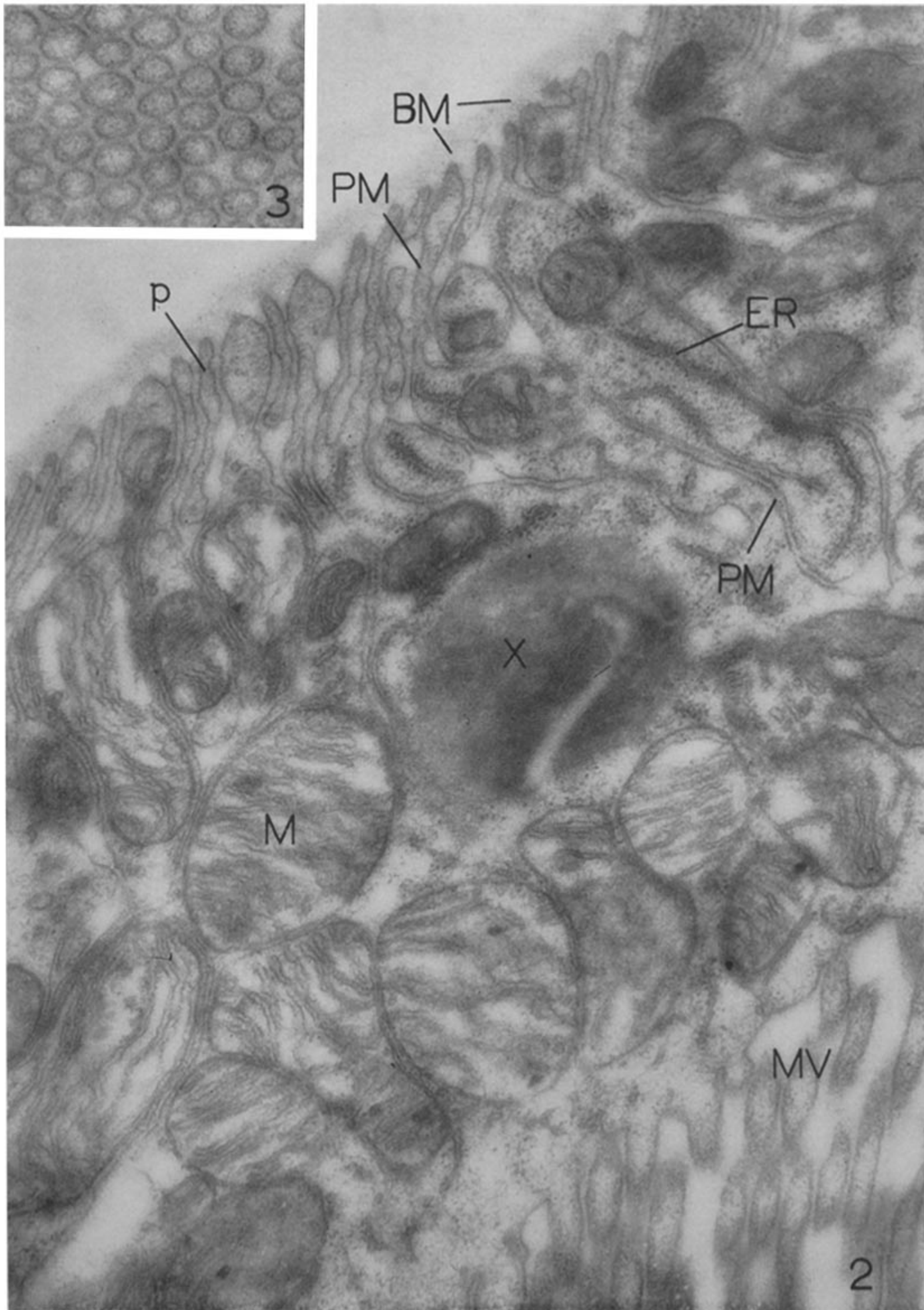
FIGURE 2

Electron micrograph showing the chief features of these cells. The plasma membrane (*PM*) of the basal surface, lying beneath the basement membrane (*BM*), is deeply infolded into the cell. Between the folds are seen (as at *P*) a number of isolated profiles of interdigitating processes from adjacent cells. Mitochondria (*M*) are abundant, especially in the apical portion of the cell, and the dense body (*X*) may represent a degenerating mitochondrion. A few elements of the granular endoplasmic reticulum (*ER*) are present in the cytoplasm. × 42,000.

FIGURE 3

Transverse section of microvilli of the brush border borne on the apical surface of these cells, adjacent to the lumen.

In Fig. 2 are seen obliquely sectioned microvilli (*MV*) at their point of origin. × 48,000.


3
BM
PM
P
ER
PM
X
M
MV
2

lamellae or processes, between which extracellular clefts deeply indent the cell body. It is suggested that they allow the haemolymph an extensive and intimate access to the cell surface.

Most of the mitochondria lie in the apical half of the cell, where they may attain a length of 1 μ. The large dense, homogeneous body at the center of the field shown in Fig. 2 is a frequently occurring cell component in this region and perhaps represents a degenerating mitochondrion. A few small mitochondria occur in the basal region, between the folds of the cell membrane.

A dense population of granules is present both attached to the scattered cisternae of the endoplasmic reticulum and also lying freely in the cytoplasm. In the pattern of their distribution within the cell, and in their estimated diameter (150 A), these granules correspond to the ribonucleoprotein particles of Palade and Siekevitz (1956).

Regions II and IV of the Malpighian Tubule

The second and fourth regions of the tubule are designated as those which lie on each side of the brochosome-secreting area, and, because of their cytological similarity, these two regions will be considered together.

Under the dissecting microscope, the tubule in region II is seen to be helical, while in the most distal region (IV) it is smooth, and the diameters of the tubule at these levels are 35 to 45 μ and 25 to 30 μ respectively. The low power electron micrograph, Fig. 4, illustrates the general cytological features of region II. The lumen is bounded by three or four large cells which differ markedly in fine structural details from those situated elsewhere in the tubule, and which exhibit certain unusual structures to be described in detail below.

A well defined basement membrane is present (Fig. 6) and beneath this, the plasma membrane is thrown into a series of infoldings which extend across approximately one-fifth of the distance between the basement membrane and tubule lumen. This situation somewhat resembles that described by Berkaloff (1958, 1959) in Malpighian tubule cells of the cricket *Gryllus*, except that in the cricket, the folds in the plasma membrane of the basal region of the cell make much greater inroads into the cell. However, these cells in *Gryllus* secrete urate granules, which, as Berkaloff (1959) showed, accumulate in the cytoplasm, prior to their synchronous emission into the tubule lumen, while the cells of regions II and IV in the Malpighian tubules of *Macrosteles* do not secrete granules or other solid excretory products, nor was uric acid found in any region of the gut or Malpighian tubules.

The mitochondria are numerous, and are distributed randomly throughout the cytoplasm. They are typically dense, and size variation is considerable.

The cytoplasm of these cells, in common with that of cells from region I, contains large numbers of particles, some of which are attached to the membranes of the endoplasmic reticulum, while others are free. The endoplasmic reticulum is moderately well developed, and in places forms

---

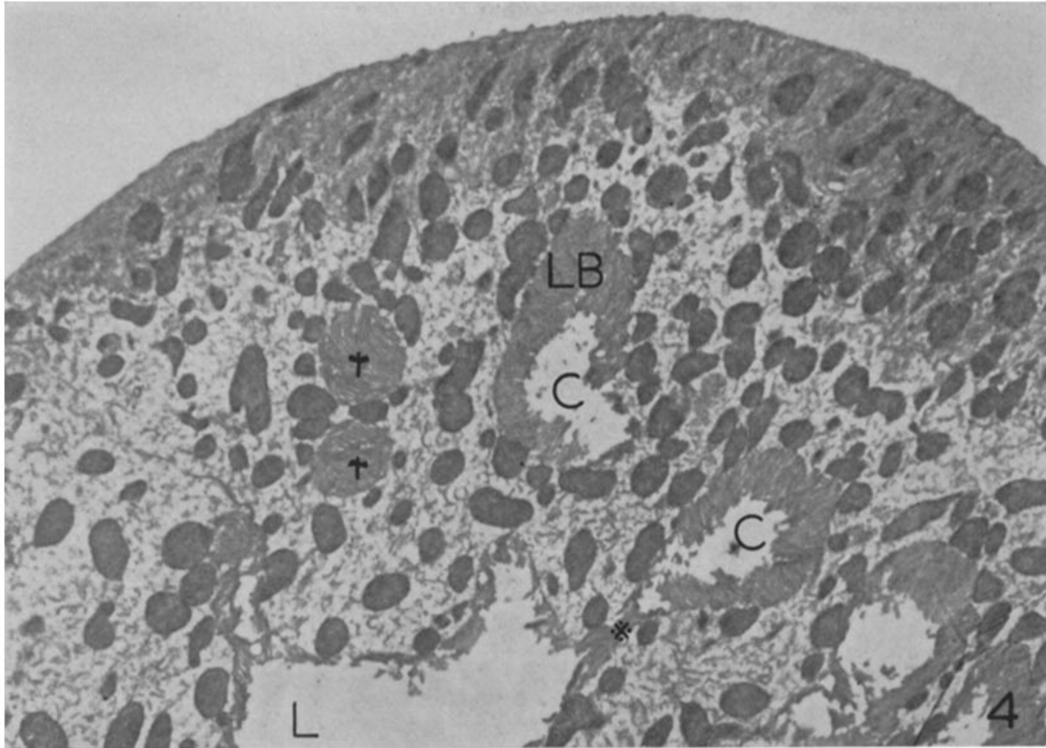
Electron micrographs illustrating the cellular structure of regions II and IV of the Malpighian tubule.

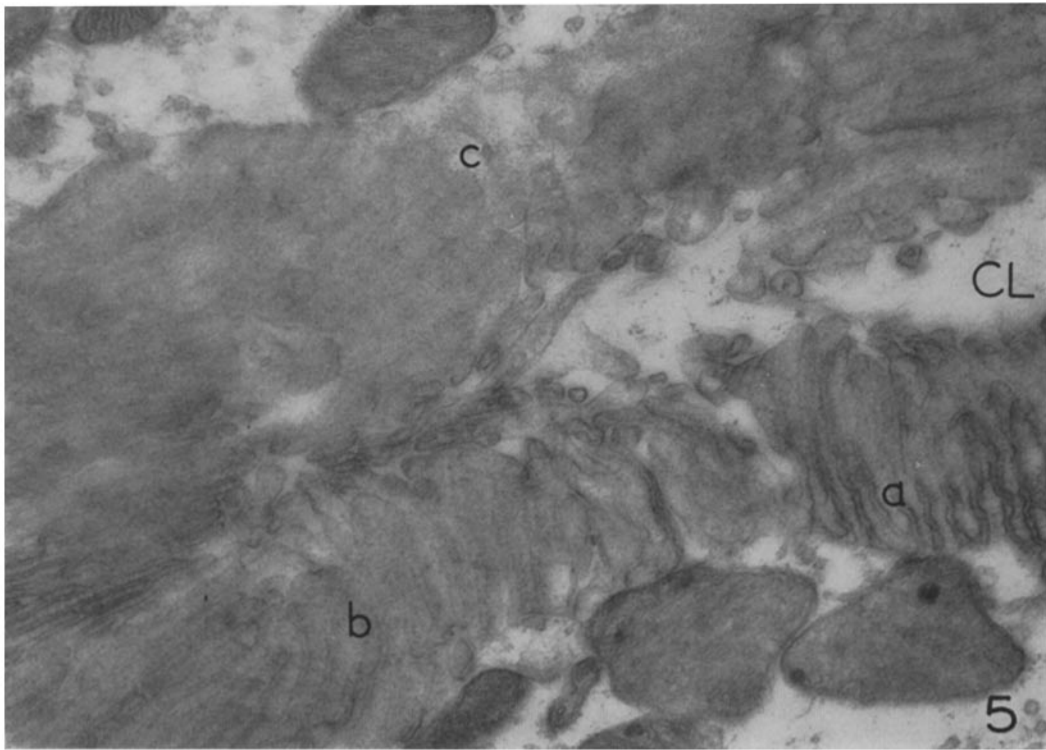
FIGURE 4

A transverse section of the tubule including the majority of one of the large cells of which it is composed. The surface bounding the lumen (*L*) is at several points invaginated within the body of the cell to form a number of flask-shaped cavities (*C*). At the position marked (*) the neck linking the cavity with the lumen may be seen, while in other cases the neck is out of the plane of section. The cavities are bounded by a cytoplasmic lamellar border (*LB*), shown at higher magnification in Figs. 5, 6, 7; at (†) only this border, and not the lumen itself, lies in the plane of section. × 4,800.

FIGURE 5

Showing in greater detail the structure of the lamellar border lining a cavity the lumen of which is seen at (*CL*). Transversely sectioned lamellae are seen at (*a*), others more obliquely sectioned at (*b*), while at (*c*) the plane of section is more nearly frontal. The lamellar nature of this modification of the cell surface is also clearly seen in Fig. 7. × 19,000.


LB
C
C
L
4


c
CL
a
b
5

loose piles of cisternae. Golgi regions, consisting of small groups of agranular vesicles, occur sparsely throughout the cytoplasm.

The unique aspect of the cytology of these cells relates to the extensive modification of the plasma membrane of the apical region. Within each cell are situated a number of flask-shaped cavities, up to 15 μ in length, each connecting with the general tubule lumen through a narrow neck. Each cavity is thus to be regarded as an intracellular involution of the apical plasma membrane, by means of which the fluid contained in the general tubule lumen is also channeled within the body of the cells bounding the lumen. In the low power survey micrograph (Fig. 4) portions of five or six such cavities are seen. In four of these, the plane of section has passed through the cavity lumen, which is bounded by a wide membranous region, while in one instance (*), where the plane of section was medial, the continuity between the general tubule lumen and its derivative *via* a narrow "neck" is established. The membrane-filled areas (†) at the left of the figure represent tangential sections of the cavity lining, at a level above or below the lumen.

Whereas in region I of the Malpighian tubule a well developed brush border is present adjoining the lumen, examination shows that a different and hitherto undescribed method of obtaining increased membrane surface area has been developed in these cavities: they are lined by large numbers of interdigitating, membrane-bound leaflets of cytoplasm. The approximate thickness of each leaflet is between 350 and 500 A, and the distance between adjacent leaflets is about 250 A. Only when the plane of section is normal to the surface of the leaflets is the clear-cut picture of the array of membranes (Fig. 6) obtained. Elsewhere, when the plane of section is more oblique, a greater amount of the cytoplasm between the membranes bounding each leaflet is included in the section, resulting in the increased spacing seen in Fig. 5. This effect increases as the plane of section becomes more nearly parallel to the leaflet surface, until a situation obtains (as in Fig. 7) in which some leaflets are seen in frontal section.

Fig. 8 is a schematic representation of the relationship between the elements in this system. It is supposed that each leaflet narrows at its base, as in many instances the plane of section does not pass through the region of attachment of the leaflet, resulting in the appearance of isolated, unattached profiles. Furthermore, the edge of the leaflet appears to be crenulated and the very small profiles observed at the edge of the lumen may represent sections through such irregularities at the border of the leaflets, the bulk of which are out of the plane of section.

From the fact that within a single cavity leaflets are sectioned in various planes, it may be inferred that their arrangement is somewhat irregular. This basic pattern of cytoplasmic leaflets is also developed, although to a limited extent, along the apical margin of the cell, adjacent to the main lumen of the tubule. Neither here, nor in the lining of the cavities themselves, is the characteristic appearance of transversely sectioned microvilli ever seen.

It is interesting to note that a rather similar lamellar border occurs elsewhere in this insect: on the apical surface of the epithelial cells of the hindgut, a description of which is given later (Figs. 22 to 25).

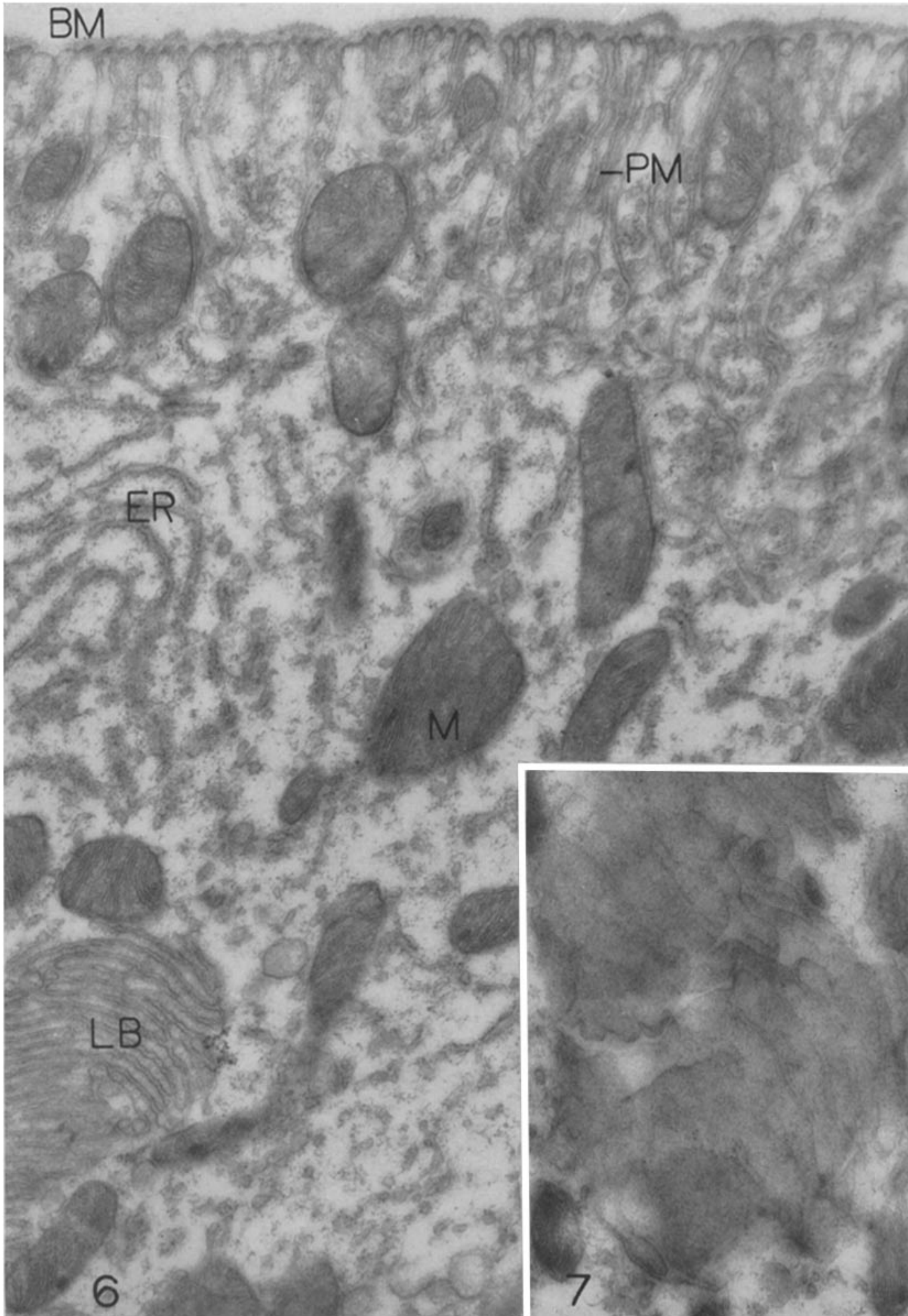
Electron micrographs illustrating the cellular structure of regions II and IV of the Malpighian tubule.

FIGURE 6

Illustrating the organization of the basal region of the cell. The plasma membrane (*PM*) lying beneath the basement membrane (*BM*) shows numerous rather shallow infoldings. Mitochondria (*M*) are scattered throughout the cytoplasm and are not preferentially distributed in any part. The granular endoplasmic reticulum (*ER*) occurs either as single isolated profiles or as rudimentary stacks of cisternae. The interdigitating cytoplasmic lamellae (*LB*) represent a section through the border of a cavity, as in the top center of Fig. 8. × 22,000.

FIGURE 7

Oblique section through lamellae bordering an intracellular cavity. × 38,000.


BM
PM
ER
M
LB
6
7

Region III of the Malpighian Tubule

This highly specialized region of the tubule is concerned with the elaboration and excretion of the brochosomes mentioned in the introduction, and is situated between the two regions just described. The tubule here is wider than elsewhere, reaching a diameter of 90 to 100 μ, although the lumen is similarly surrounded by only three or four cells, each of which is typically binucleate.

Day and Briggs (1958) conducted a preliminary examination of the mode of secretion of brochosomes, but while their finding that these bodies are intracellular products which are excreted into the tubule lumen has been confirmed, the present study has revealed additional details of the fine structure of brochosomes, and, in particular, of the steps leading to their formation.

The field covered in the low power survey electron micrograph shown in Fig. 9 represents a longitudinal section of a single cell of region III. By virtue of the lobate form of these cells, the lumen is divided into a series of narrow channels. The "foamy" or vacuolate appearance of this tissue in the light microscope (Fig. 20) may at once be related to the electron microscopic image. The dense ground cytoplasm is clearly severely restricted in extent, and occupies those areas between the vesicles containing brochosomes. Moreover, it is evident that these vesicles become more concentrated towards the lumen, finally to the virtual exclusion of other cytoplasmic components. Thus Day and Briggs' observation that "the cytoplasm is found to be reduced to interconnecting threads surrounding large cavities containing the brochosomes" is strictly applicable to the apical part of the cell only. In the basal portion of the cell a greater amount of cytoplasm is present between the vesicles, and it is here that developmental stages of the brochosomes are situated.

At higher magnification (Figs. 12 and 13) the bulk of the dense cytoplasmic matrix is resolved into an abundance of small spherical or ovoid vesicles, between 0.05 and 0.2 μ in length, to each of which are attached particles about 150 A in diameter. Similar though unattached particles occur profusely in the intervening cytoplasm. It is probable that this system of vesicles is to be regarded as a fragmented form of the granular endoplasmic reticulum, resembling that described in pancreas cells by Palade (1956). Occasionally, in the basal region of the cell, these vesicles are roughly aligned into rudimentary "stacks." The fragmented condition of the endoplasmic reticulum contrasts with the more typical form occurring elsewhere in the Malpighian tubule of *Macrosteles*, and presumably achieves a greater membrane surface area than would be provided by piles of cisternae occupying the same cell volume. The intervesicular cytoplasm was found to be strongly basophilic, when stained with azure B, and the supposition that the basis of this intense basophilia resides in ribonucleic acid was confirmed by the fact that azure B did not stain similar sections which were first treated with ribonuclease. This RNA is presumably located in the very numerous free and attached particles.

Another unusual aspect of the cytoplasm of these cells is afforded by the presence of clusters of irregular vesicles, especially in the basal region. In Fig. 9, for example, about eight such groups are visible and representative areas are shown at higher magnification in Figs. 11 through 14. These, despite their abundance and large size, have features in common with the Golgi areas of other cell types. By contrast with the endoplasmic reticulum no particles are borne on the membranes defining these vesicles, and this precise distinction is especially clear in Fig. 11 in which granular and agranular vesicles of similar size occur side by side. While the over-all length of each complex may reach 4 μ, individual vesicles within each complex or group range in size from 2 μ to minute structures, less than 0.1 μ in diameter, situated between the longer components. In addition, associated with the smaller vesicles and apparently occurring nowhere else in the cell, is found a population of large dense granules, of variable size, the diameter of which ranges from 0.04 μ to 0.10 μ (Fig. 14). Sometimes, all the vesicles in a group appear to be empty, but frequently a more or less well defined structure may be discerned, especially within the larger of them. These structures are here interpreted as early stages of the formation of brochosomes.

An early stage in production of brochosomes is shown in the Golgi region (Fig. 15) in which dense, rather ill defined foci or "nucleoids," together with an unoriented system of fibrils, are present. Elsewhere (Fig. 16) these dense "nucleoids" are seen to be replaced by empty shells of dense material. In this example, the system of

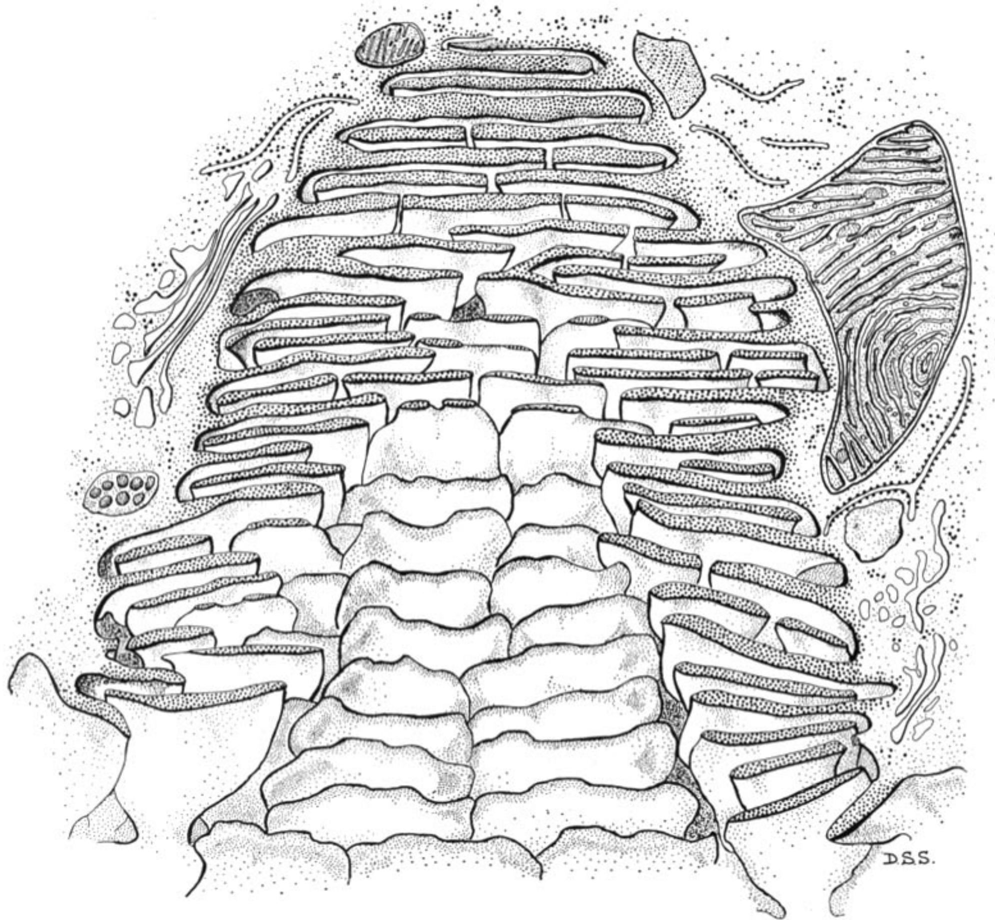


FIGURE 8

Semidiagrammatic interpretation of a section through one of the flask-shaped cavities occurring in cells of regions II and IV of the Malpighian tubules. For purposes of clarity, the space between the cytoplasmic lamellae which line the lumen of the cavity is greatly exaggerated. At the lower center, these overlapping lamellae are shown entire, since they lie below the plane of section. The surrounding cytoplasm contains such typical cell organelles as mitochondria, Golgi vesicles, and profiles of the endoplasmic reticulum.

threads defines, albeit loosely, a number of areas, each approximating to the size of a mature brochosome. The form of the mature structure is attained (Fig. 15) before the final density is reached, and at this stage the pale brochosome "ghosts" frequently retain dense material in their core, which later disappears. When cut medially, mature brochosomes appear to contain a structureless central region of low density, and around this space are arranged the spokes defining the "compartments" of the sculptured surface. Thus a remarkable structure is produced in very large numbers in what appear to be specialized Golgi regions, and furthermore, while the developmental stage of all the brochosomes within a single vesicle is similar, early and late stages may be present together within one complex of vesicles.

The role of this specialized Golgi region is more easily understood when the ultimate fate of the brochosomes is considered. As Day and Briggs observed, brochosomes formed within the cell are discharged into the tubule lumen. This is effected by the localized rupture both of the cell membrane adjoining the lumen, and of the mem-

branes of the brochosome-containing vesicles lying against it. It has been mentioned that the apical portion of the cell contains tightly packed vesicles fully charged with mature brochosomes, while the basal area includes a greater amount of the cytoplasmic matrix, including the complex Golgi regions. This distribution is compatible with the hypothesis that a migration of vesicles towards the lumen surface occurs, followed by the spilling of their contents into the lumen, an hypothesis that assumes a continuous production of vesicles. It is suggested that this is the function of the Golgi regions, which thus not only act as the sites of formation of this remarkable excretory product, but also provide the vehicle in which they are transported for removal from the cell.

Interspersed between the larger elements in each Golgi complex are many minute vesicles, which may be as small as 500 A in diameter. It is possible that the latter are to be regarded as an integral part of the complex which may undergo hypertrophy, to produce the giant vesicles in which the brochosomes are elaborated, prior to their dissociation from the parent Golgi complex.

(*a*) *Structure of Brochosomes:* As Day and Briggs mention, preparations of whole brochosomes may conveniently be obtained simply by smearing a portion of the freshly dissected tubule over the surface of a coated grid. Prepared thus, or by allowing the tubule cells to disrupt in distilled water, the individual brochosome is seen to be subspherical in form, and to include a complex electron-dense skeleton together with a less opaque constituent, interpreted by Day and Briggs as a thin outer membrane. It was hoped that examination of such intact bodies would corroborate structural inferences drawn from examination of profiles seen in section.

The average diameter of each brochosome is 0.4 to 0.5 μ, while that of the hollow center is about 0.25 to 0.3 μ (Fig. 17). The latter, as has been mentioned, appears to be bounded by a thin shell, derived, in development, from the initial dense central "nucleoid." Arranged radially around this core, as seen in thin sections, are electron-opaque spokes, usually eight or nine in number, about 1 μ in length, each of which has a "clubbed" or dilated apex. That the central region of each spoke contains material of lower density (see Fig. 21), may be observed in sections (Fig. 17) as well as in entire brochosomes "stained" with phosphotungstic acid solution. The spokes are typically linked one with another, at their bases, by sections of the edge of the central shell, and in addition, peripheral cross-connections between adjacent spokes are present.

The foregoing description of the appearance of medial sections is consistent with the model of the brochosome as a polyhedral solid upon each face of which is constructed a compartment whose radial walls, normal to the surface, correspond in section to the spokes. The suggested brochosome structure is shown in Fig. 21 in which five compartments have been drawn on the basic polyhedron.

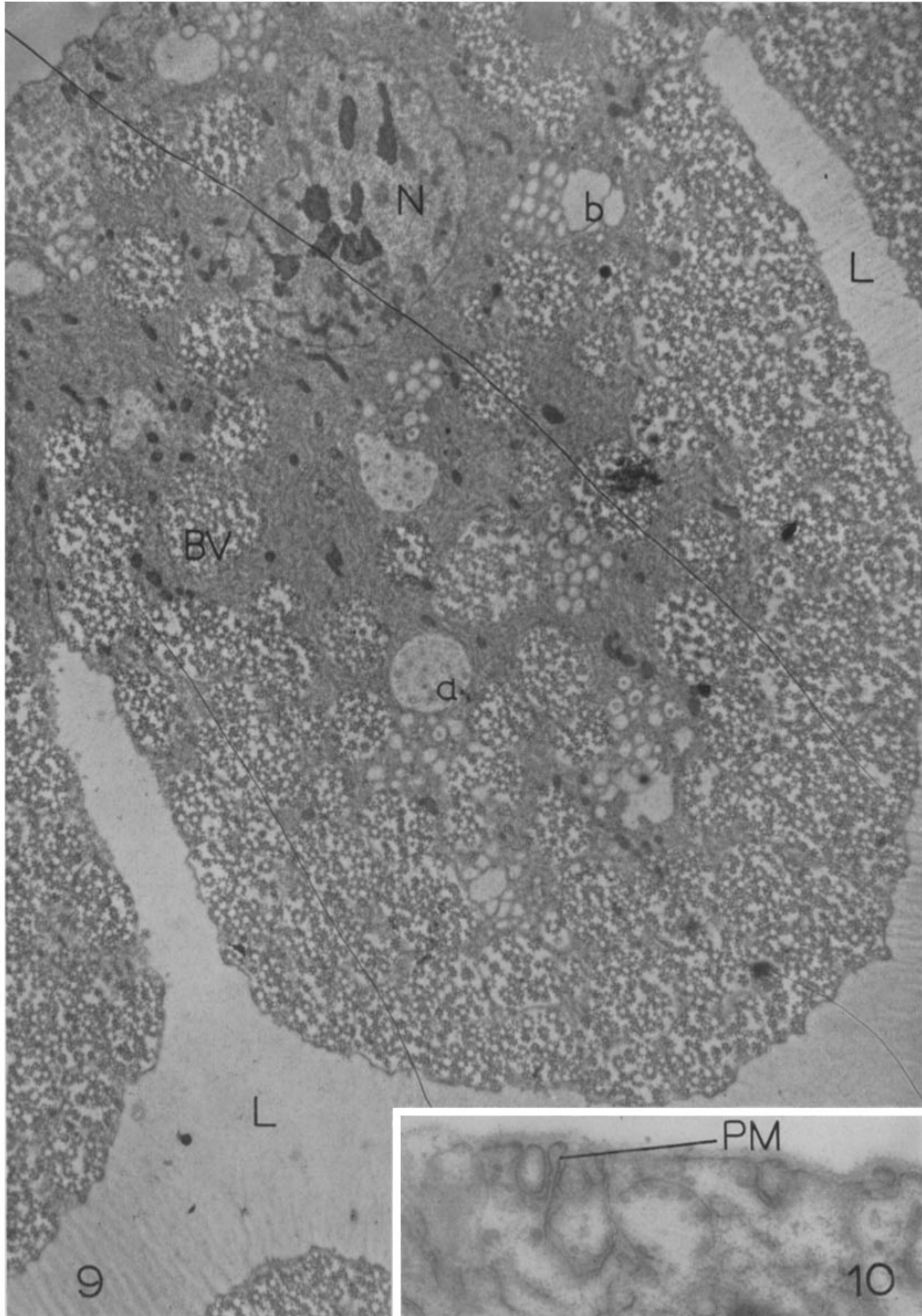
Day and Briggs proposed the regular dodecahedron of twelve pentagons as the basic model for the brochosome structure and regarded as anomalous the hexagons which they sometimes observed. However, examination of our material showed that hexagons as well as pentagons are typical or constant units of brochosome archi-

FIGURE 9

A low power electron micrograph of a cell of region III of the Malpighian tubule, the region in which brochosomes are secreted. The cells are lobate, and the apical region of the cell projects into the lumen of the tubule (*L*). The bulk of the cytoplasm consists of brochosome-containing vesicles (*BV*). A few vesicles, as at *a*, have less well defined contents while elsewhere, as at *b*, occur groups of variously sized apparently empty vesicles. The detailed structure of these cell components is shown at higher magnification in Figs. 11 through 17. The cells in this region are typically binucleate, and one of these nuclei appears in the section at *N*. × 4,800.

FIGURE 10

Showing, at high magnification, the topography of the plasma membrane (*PM*) in the basal region of the cell. In contrast with the situation in regions I, II, and IV, this here forms only a few extremely shallow and irregular infoldings. × 41,000.


N
b
L
BV
d
L
PM
9
10

tecture; a situation that, for the present purpose, rules out the twelve-sided solid. Electron micrographs published by Day and Briggs, and also those obtained during the present work, show that these bodies typically have more than the originally suggested twelve facets and compartments. However, individual brochosomes vary somewhat in size, and such variation appears to be more marked in the species employed by Day and Briggs (*Austroagallia torrida*) than in *Macrosteles fascifrons*, although in each case size increase is effected by increase in the number of unit compartments rather than in their dimensions.

In addition to the methods of preparing specimens of intact brochosomes for electron microscopy mentioned previously, the "critical point" method of Anderson (1951) following osmium tetroxide vapor fixation was also employed, in an attempt to study the shape and structure of brochosomes in minimally distorted condition, and chromium shadowing was also used on occasions. While some improvement was obtained when phase-boundary stresses were obviated through the use of Anderson's technique, flattening and distortion were not serious in brochosomes prepared simply by drying a crushed and fixed suspension of Malpighian tubule cells upon a coated grid, attesting to the strength and rigidity of the brochosome skeleton.

Examination of numbers of intact brochosomes led to the conclusion that their most frequent configuration corresponds to a polyhedron bounded by twenty facets, which consists of twelve pentagons and eight hexagons. While no analogy exists between this irregular polyhedron and regular crystalline forms, an interesting biological parallel was found in Thompson's description and interpretation (1945) of the siliceous skeleton of the Acanthometrid Radiolarian, *Dorataspis cristata*. This protozoan is encased in a shell bearing twenty radial spines which spring, not from the corners of a dodecahedron, but from the centers of the facets of a type of icosahedron. Thompson concluded that this polyhedron, enclosing the body of the animal, is a truncated hexagonal prism in which pentagons and hexagons are present in the proportions found in the case of the brochosome model, namely, twelve to eight. Thompson continues: ". . . but if we try to construct a plane sided polyhedron of this kind, we find it to be impossible; for into the angles between the six equatorial hexagons six regular pentagons will not fit. The figure, however, can be easily constructed if we replace the straight edges (or some of them) by curves, the plane facets by slightly curved surfaces, or the regular by non-equilateral polygons." In the case of the brochosome, all these requirements are met: median sections passing through the polyhedral shell show that the facets are indeed curved, moreover, the polygons constructed upon its surface are not always precisely equilateral. Fig. 21 depicts one aspect of the basic icosahedron, upon which five compartments have been completed.

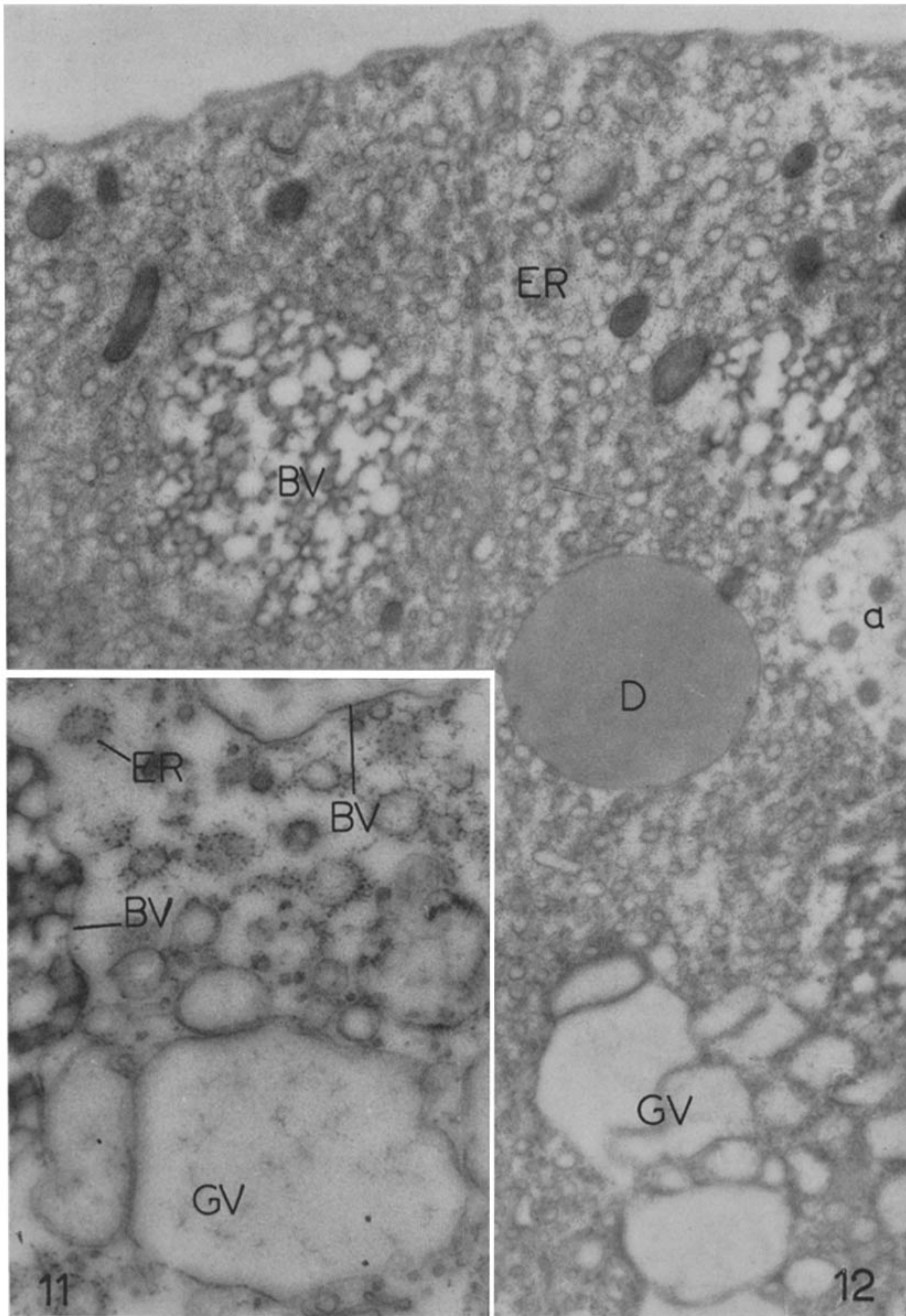
A further analogy exists between brochosomes and the above mentioned and similar Protozoa, beyond the correspondence shown by the basic polyhedral shells. In the first, as we have seen, the trihedral corners each bear a radial projection, each linked

FIGURE 11

The granular endoplasmic reticulum (*ER*) of cells in region III is in the form of small discrete vesicles which show little tendency to alignment. In the lower half of this micrograph is seen a group of vesicles (*GV*) of various sizes, defined by membranes free of particles. Similarly, no particles are borne on the membranes surrounding the brochosome-containing vesicles (*BV*). × 40,000.

FIGURE 12

A lower power survey electron micrograph of the basal portion of a cell in which the cytoplasm is less restricted than elsewhere. It consists mainly of granular vesicles (*ER*) which are considered to represent the RNP-bearing endoplasmic reticulum. Unattached particles presumed to be of the same chemical nature are also abundant. A vesicle containing fully formed brochosomes is present (*BV*), while *GV* represents a group of smooth membraned vesicles which are thought to correspond to Golgi vesicles of other cells. In the vesicle at *a* are found structures shown at higher magnification in Figs. 15 and 16, representing stages in the synthesis of brochosomes. Circular profiles containing a homogeneous substance (*D*) of unknown nature occur not infrequently, and are possibly droplets of lipid or protein. × 24,000.


ER
BV
a
D
ER
BV
BV
GV
GV
11
12

with its neighbors by distally thickened walls or vanes, defining the pentagonal and hexagonal compartments. Whereas in *Dorataspis* only the center of each facet is radially produced, in allied forms projections may be borne at each corner of the figure (*Aulastrum triceros*), or, as in the brochosome, the projections may be connected by walls normal to the polyhedron surface (*Cenosphaera favosa*).

In most Radiolaria the inorganic salts silica or strontium sulfate are employed as skeletal building materials, but fresh water Radiolaria such as *Clathrulina* produce a chitinous latticework (Thompson, 1945). This so called "chitin," according to Hyman (1940), is a mucus-like glycoprotein containing protein and carbohydrate. In each case, the characteristic skeletal form is produced by the deposition of material, inorganic or organic, along the interstices or planes of apposition of the vesicles present at the periphery of the Radiolarian cell, the form of the skeleton being determined primarily by these physical factors rather than by the chemical nature of the building material.

It has been mentioned that in electron micrographs of sections of developing brochosomes, the skeleton is at first faint and ill defined; then the walls of the compartments become more distinct and thereafter increase in density and thickness prior to the excretion of the body, by which time they have become rigid and resistant to physical deformation (Fig. 18). Developmental stages of intact brochosomes have been obtained, sometimes still included within the large intracellular vesicles, by allowing the cells to become disrupted in distilled water, prior to fixation with osmium vapor. Here, the polyhedral arrangement of facets is distinct, while the system of vanes is as yet incompletely developed. At this time, the final structural rigidity has not been reached, as evidenced by the flattening of the brochosomes and the extent of deformation of the surface facets (Fig. 19).

Although the diameter of a Radiolarian cell exceeds that of a brochosome, an intracellular product, perhaps one hundredfold, the similarity between the unusual geometrical configurations found in each is too striking to be overlooked, and it may well be that this similarity reflects the effect of comparable formative mechanisms acting upon dissimilar building materials.

(*b*) *Chemical Tests on Brochosomes and the Brochosome-Secreting Cells:* The very small size of the brochosomes, which are just resolvable in the light microscope, created problems in the histochemical part of this investigation and confirmation of these results was sought by the use of extraction and enzymatic procedures coupled with electron microscopic observation. The best interpretation of the results appears to be that the brochosomes are dense structures composed of a rigid skeleton consisting wholly or partly of protein and that this skeleton is coated with a sheath of saturated lipid. They are not osmiophilic, and do not contain nucleic acids or substances having 1,2 glycol groups. The results of the histochemical and other procedures are summarized in Table I.

When tubule cells were dissected out on a slide, lysed with distilled water, and fixed in formaldehyde vapor, it was seen that the brochosomes took up Sudan black B from a saturated solution of the dye in propylene glycol. In methacrylate sections, both brochosomes and cytoplasm were stained intensely with Sudan black B (Fig. 20). This suggested that there was lipid present, but it is interesting to note that extraction of such sections with pyridine at 60°C. for 48 hours failed to abolish their stainability with Sudan black B. While its extractability with

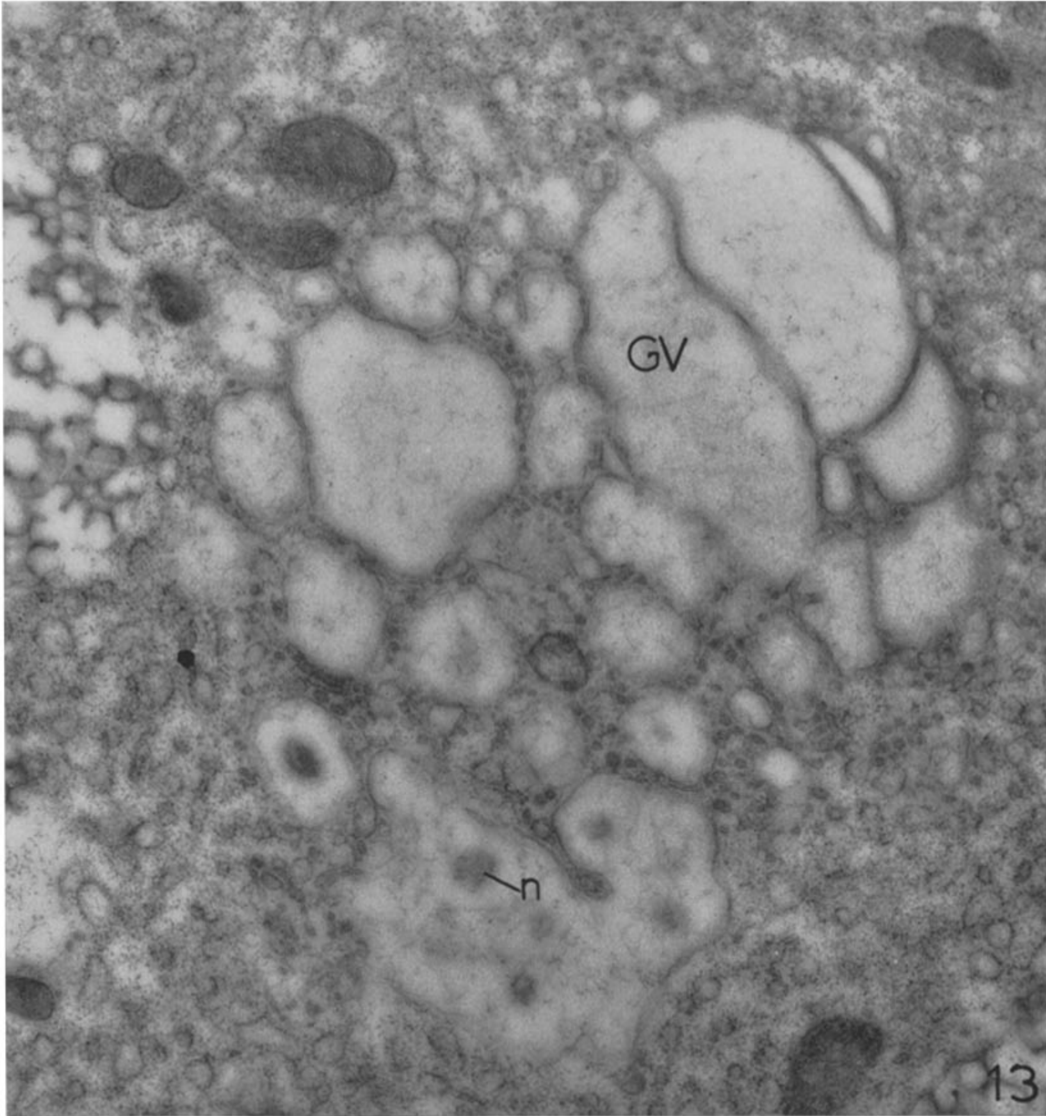
Electron micrographs illustrating the structure of the Golgi vesicle complexes within which the brochosomes are formed. Each such group of vesicles includes a wide size-spectrum, the lower limits of which are seen to better advantage in Fig. 14.

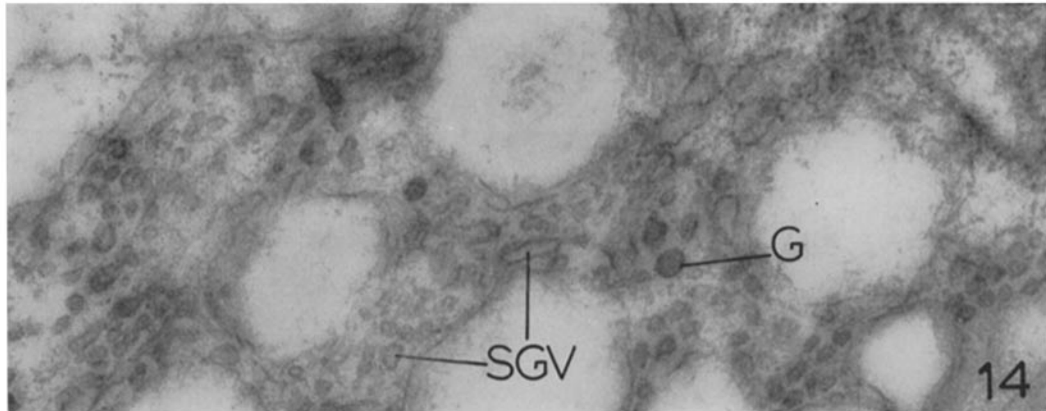
FIGURE 13

A group of smooth membraned vesicles (*GV*), the largest of which contain a very faint fibrillar material. That at the bottom of the figure contains a number of dense amorphous "nucleoids" (*n*) each of which is the center of an area demarcated by somewhat more clearly defined fibrils or filaments. × 32,000.

FIGURE 14

High magnification electron micrograph showing the mixed population of very small smooth membraned vesicles (*SGV*) and large granules (*G*), or small vesicles with a dense content, lying between the larger vesicles of the complex. × 60,000.


GV
n
13


G
SGV
14

hot pyridine tends to confirm the idea that a substance stainable by Sudan black B is lipid in nature, the failure to demonstrate this property does not conclusively disprove that it is lipid (Chayen, Gahan, and La Cour, 1959). Furthermore, when unfixed whole brochosomes, obtained by crushing dissected-out Malpighian tubules, were extracted for 3 days with pyridine at 60°C. and examined with the electron microscope, they appeared to be much damaged and distorted, leaving a collapsed skeleton of spicules, the original smooth outlines of which had been lost. Thus it is probable that the brochosomes contain a lipid extractable by hot pyridine but not stained by Sudan black B, in addition to stainable lipid which is not, however, extractable.

The type of lipid present in brochosomes is probably saturated, as suggested by the negative performic acid–Schiff test for unsaturated lipids. Since this test was employed on osmium-fixed material, however, there was the possibility that the osmium had already blocked any unsaturated sites which might have been present. To check this, the ethyl gallate method was used. This treatment, which results in a gray-blue coloration in regions of the cell which have combined with osmium during fixation, failed to reveal an appreciable amount of osmium in the brochosomes, although the cytoplasm gave a positive reaction. These results not only tend to support the hypothesis that the brochosome lipid is mostly of a saturated type, but also agree with the observation that brochosomes are electron-dense bodies even in the unfixed state.

Attempts to stain protein basic groups with an acid dye, fast green, failed to stain brochosomes in untreated sections, but in sections which had been held in pyridine at 60°C. for 2 days individual brochosomes were visualized as heavily stained bodies. Freshly dissected brochosome-containing regions of Malpighian tubules were subjected to trypsin digestion as a further test for protein. In the electron microscope they were seen to be much less severely damaged than after pyridine extraction. Although their outlines became somewhat blurred, they retained their individual structure without collapse, and this has been interpreted as again confirming the idea that the protein of the skeleton is masked by a coating of lipid.

The brochosomes do not appear to contain ribonucleic acid, although, as was mentioned above, the cytoplasm surrounding the regions where brochosomes are formed is rich in this substance. The periodic acid–Schiff reaction also was negative for brochosomes and positive in the cytoplasm, suggesting the absence, in brochosomes, of polysaccharides, mucopolysaccharides, mucoproteins, and mucins (Pearse, 1953). The nature of the periodic acid–Schiff-positive material in the cytoplasm was not investigated further.

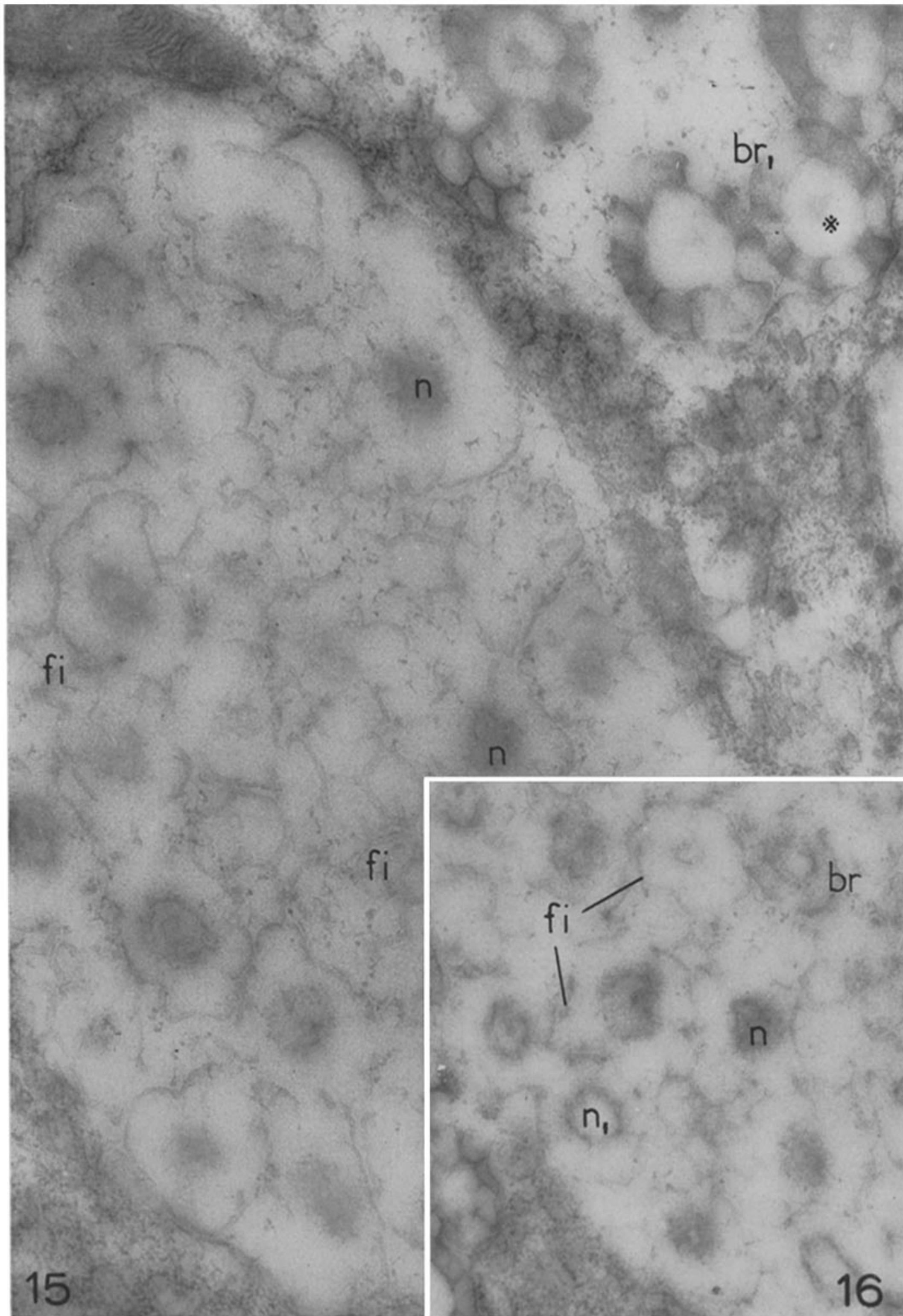
No evidence for the presence of uric acid, urates, or other purines was found when whole Malpighian tubules were subjected either to the murexide test or to Hollande's modification of Courmont-André's method.

The Mid- and Hindgut Epithelia

The precise embryological relationship between the Malpighian tubules and the mid- and hindgut is not entirely clear. The tubules appear to be derived from the undifferentiated cells at the anterior end of the proctodeal invagination, and are thus not strictly referable to either of these regions of the gut (Henson, 1932 etc., references in Wigglesworth 1950, p. 368). Whatever the phylogenetic and developmental history of these systems, it is clear that while the principal role of the Malpighian tubules lies in the excretion of nitrogen and other waste materials, the bulk of digestion and absorption of nutrients occurs in the midgut, while water reabsorption is frequently effected by the cells of the hindgut and rectum. These two regions of the gut of insects are separated by the pyloric sphincter by means of which the hindgut

FIGURE 15 and 16

Electron micrographs further illustrating brochosome development. The "nucleoids" (*n*) and their associated fibrils (*fi*), or lamellae are clearly seen, while at n_1 the "nucleoid" is represented by a profile of a hollow sphere of dense material. The association between the central hollow core and the peripheral elements, characteristic of the final product of synthesis, is shown at *br*. In the completed brochosome the core is empty (see Fig. 17) but even at a late stage in production (br_1) a remnant of the original "nucleoid" (*) may persist. (15) × 58,000. (16) × 47,000.


br₁
*
n
fi
n
fi
15
fi
br
n
n₁
16

may receive material either from the midgut or from the Malpighian tubules alone. In the case of the leafhoppers, this mechanism may enable the insect to excrete clear fluid, and at other times the dry pellets of brochosomes from the tubules, pellets which are produced by the action of water absorption in the hindgut upon the excretory material from the Malpighian tubules.

Investigations with the light microscope show that in some insects the midgut epithelium includes more than one cell type. However, in the present preliminary examination only a single cell type has been found in *Macrosteles*, despite the morphological division of the midgut into a wide chamber followed by a narrow posterior tube (Fig. 1). As in the case of the columnar absorbing cells of the mouse intestine (Zetterqvist, 1956) and in the insects, *Malacosoma* sp. and *Melanoplus differentialis* (Beams and Anderson, 1957), these cells in *Macrosteles* bear a well developed brush border on their apical surface. The individual microvilli are about 0.1 μ in diameter, and are from 1.0 to 1.2 μ in length, forming an array of 50 per square micron.

It is interesting that the dimensions of the microvilli of the cells of the midgut of *Macrosteles*, of the mouse jejunum and of the mammalian proximal convoluted tubule are similar, whereas the microvilli of Malpighian tubules so far examined are unusually long. In *Macrosteles* the cytoplasm adjacent to the brush border of the midgut contains a higher concentration of mitochondria than elsewhere in the cell, and these are typically elongate, and tend to be oriented parallel with the long axis of the microvilli. No such concentration occurs in the basal region, where the infoldings of the cell membrane are present though not deep, and lack the orientation radial to the lumen described in *Malacosoma* by Beams and Anderson (1957). The nuclei are subspherical, and the cytoplasm contains parallel arrays of profiles of cisternae of the endoplasmic reticulum, which is here more highly developed than in the cells of either the Malpighian tubule or hindgut epithelia.

The hindgut also appears to consist of but one cell type. The apical surface of the cells adjoining the lumen does not bear a brush border of the

FIGURE 17

High magnification electron micrograph of brochosome profiles. Note the hollow central cavity, and the radial knobbed spokes. At x, for example, the plane of section passes through the wall linking adjacent spokes, while at y this plane traverses the middle of a "cell" of the surface sculpturing, and the linking walls are not seen. At * is visible the less dense core of the skeletal elements. The tangential section z includes both pentagonal and hexagonal compartment profiles, and may be compared with Fig. 21. × 100,000.

FIGURE 18

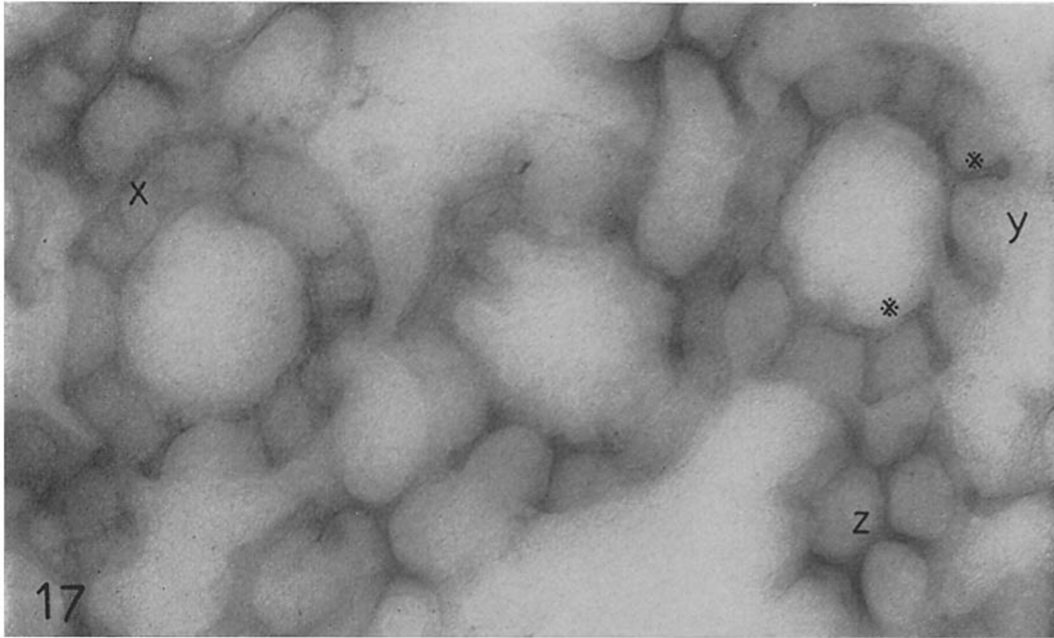
A group of whole fully formed brochosomes fixed in osmium tetroxide vapor after having been expressed from the Malpighian tubule cells. × 65,000.

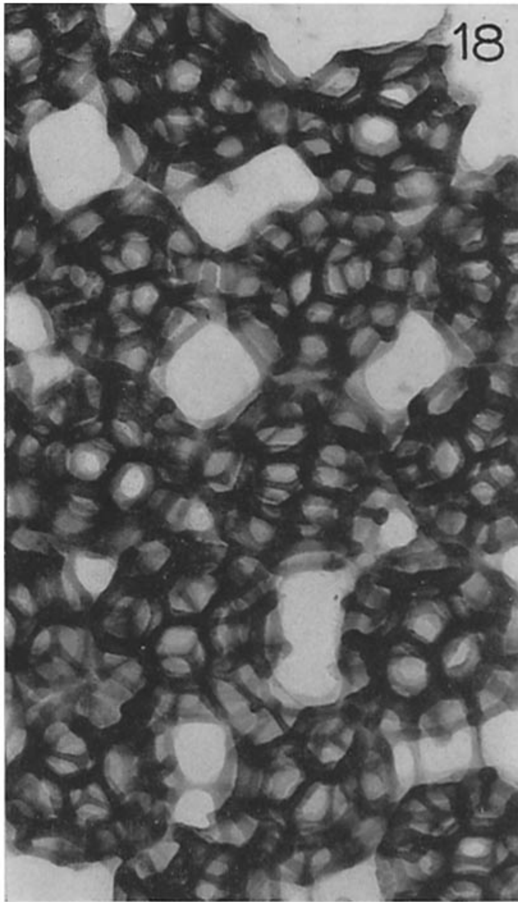
FIGURE 19

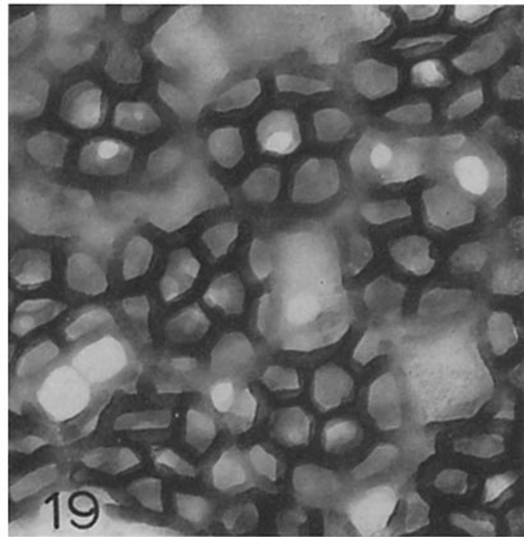
To be compared with Fig. 18; illustrating a group of brochosomes at an intermediate though late stage of formation. Here, the surface sculpturing is incomplete. It should be noted that considerable flattening and distortion occurred during preparation, while this is almost absent in the case of the very rigid completed bodies shown in Fig. 18. × 65,000.

FIGURE 20

A light micrograph of region III of a Malpighian tubule showing, at lower center, a small portion of the tubule lumen, and the vesicular appearance of the cytoplasm. Within the vesicles are resolved the individual brochosomes, in this case stained with Sudan black B as described in the text. This is a micrograph of a thick section (about 2 μ) of osmium-fixed, methacrylate-embedded material. × 1,000.


x
y
z
17


18


19

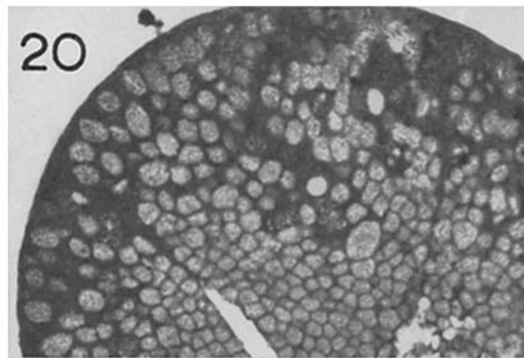

20

TABLE I

Histochemistry of Brochosomes and Brochosome-Secreting Cells

Stain or test	Substance tested for	Fixation	Preliminary treatment	Result		Conclusion
				Cytoplasm	Brochosomes	
Sudan black B	Lipid	Formaldehyde	None	+	+	Lipid present in cytoplasm and brochosomes
Sudan black B	Lipid	Formaldehyde	Hot pyridine	+	+	The lipid is not removed by hot pyridine
Observation in electron microscope	Lipid	Unfixed	Hot pyridine		Only spicules remain	Hot pyridine does extract some lipid from brochosomes
Performic acid–Schiff	Unsaturated lipids	OsO_4	90 min. hydrolysis in performic acid	+	–	Unsaturated lipid is present in cytoplasm but not in brochosomes
Ethyl gallate	Osmium	OsO_4	None	+	–	Unsaturated lipid is present in cytoplasm but not in brochosomes
Fast green	Protein basic groups	OsO_4	None	+	Weak +	Free protein basic groups found mainly in cytoplasm
Fast green	Protein basic groups	OsO_4	Hot pyridine	+	Strong +	Protein basic groups of brochosomes masked by lipid
Observation in electron microscope	Protein	Unfixed	Trypsin		Little change seen	Protein protected from action of trypsin by lipid
Azure B	Nucleic acids	OsO_4	None	+	–	Brochosomes contain no nucleic acids
Azure B	Nucleic acids	OsO_4	Ribonuclease	–	–	Ribonucleic acid is present in cytoplasm
Periodic acid–Schiff	1,2 glycol groups	OsO_4	30 min. hydrolysis in periodic acid	+	–	1,2 glycol groups are present in cytoplasm but not in brochosomes
Murexide	Uric acid, guanine, xanthine	None	None	–	–	Brochosomes do not contain uric acid or purine-like substances
Hollande modification of Courmont-André's method	Urates	Silver-formalin	None	–	–	Brochosomes do not contain urates

usual type; instead the device used for achieving surface area increase is similar to that described here in cells of regions II and IV of the Malpighian tubule. The plasma membrane is thrown into a series of folds, defining cytoplasmic lamellae arranged approximately perpendicular to the long axis of the lumen, and occupying the apical 2 μ of the cell. The thickness of each lamella is from 450 to 600 A, and the spacing between adjacent lamellae is about 200 A. Figs. 22, 24, 25 represent a transverse section of this lamellar border, while in the somewhat oblique section in Fig. 23 the apices of the lamellae are seen to bear small projections or papillae as previously described in cells of the Malpighian tubule. These cells differ from those occurring in the Malpighian tubules, however, both in the size of the cytoplasmic lamellae and in the absence of flask-shaped cavities characteristic of the cells in regions II and IV. Moreover in each cell the lamellae are richly supplied with mitochondria which are inserted within the lamellae (Figs. 22, 24). The cell membrane in the basal region, as in the midgut epithelium, forms irregular infoldings, projecting a short distance into the body of the cell.

This elaboration of the apical plasma membrane of the hindgut cells greatly increases the area of contact with the fluid of the lumen. Possibly the role of these cells is to complete the absorption of valuable food constituents remaining in the fluid passed on from the midgut. In many insects, the main function of the hindgut is the absorption of water from the lumen, with consequent concentration of the urine or feces, and the observation that leafhoppers excrete dry pellets from the Malpighian tubules in addition to clear droplets suggests that the product of the tubules is here subject to the same mechanism.

The chief cytological features of the cell types described here, namely those of the four regions of the Malpighian tubules, and of the mid- and hindgut epithelia, are shown in diagram in Fig. 26 (*a* through *e*), in which the distribution of each with respect to the gross morphology of the urinary and digestive systems is indicated.

DISCUSSION

The original aim of this work was to examine further the structure of those Malpighian tubule cells engaged in the secretion of brochosomes. It soon became apparent, however, that the Malpighian tubule of these insects is a remarkably complex organ, embodying other cytological features of great interest; indeed no cell type examined here closely resembles those previously described in the tubules of other insect groups.

At the gross anatomical level, there is perhaps no internal organ system in insects which shows more diversity than the Malpighian tubules, but only in relatively few instances is corresponding histological and physiological information available. In view of this situation, to make a cytological analysis of the urinary excretory system of an insect in the absence of such knowledge may seem to be placing the cart before the horse. However, it may be supposed that the more complete the understanding of comparative cellular structure throughout the insect orders, the easier it will be to correlate function and form in species whose size or other characteristics facilitate physiological experiment and then, by analogy, in those in which such an experimental approach is less feasible.

The Nature of the Excretory Products: Many Homoptera excrete clear droplets containing dissolved sugars and devoid of suspended material, the "honeydew," and the additional production by leafhoppers of dry pellets containing material derived from the Malpighian tubules is unusual. No analysis of fluid in the Malpighian tubules of these insects is available, and any such fluid is extracted before the urine is eliminated from the body.

On excretion in insects, Wigglesworth (1950) writes (p. 363): "The chemical composition of the urine depends upon what substances are present in the diet in excess of the needs of the body, and upon the production of waste products in metabolism . . . the most important end product, always present in excess in the proteins of food, is nitrogen; the elimination of nitrogen is the most important function of the excretory system." Evidence has been presented here suggesting that the brochosome component of the urine contains both protein and lipid, and at first sight the excretion of nitrogen in this form instead of as uric acid or its salts seems surprising. However, the excretion of proteins and amino nitrogen is by no means unknown in insects; indeed, it may be widespread, and the interest afforded by *Macrosteles* and other leafhoppers perhaps lies not so much in the alternative path

of nitrogen excretion they have adopted in place of the more usual production of uric acid, but in the cytological novelty of the means by which they effect it.

It has been suggested that the apparently wasteful excretion of large amounts of dissolved carbohydrates by aphids and coccids is related to protein deficiency in the fluid ingested from the host plant: that to achieve an adequate protein supply necessitates taking into the body an enormous excess of sugars (Büsgen, 1891). However, that protein is actually excreted by some of these insects has been demonstrated by Tóth (1933, 1937), who found that the aphid *Pemphigus* sp., feeding on phloem fluid the dry weight composition of which was 90 per cent carbohydrate and 5 per cent protein, nevertheless produced excreta containing 85 per cent carbohydrate and 3 per cent protein. In the grasshopper *Melanoplus bivittatus*, from 6 to 9 dry weight per cent of the excreted nitrogen is in the form of amino compounds (Brown, 1937), while the corresponding figure is 10 per cent in the case of the dried meconium of the silk-moth, *Antherea pernyi* (Courtois, 1929). A further instance of protein secretion, though one of quite different biological significance, occurs in those species in which the Malpighian tubules are modified for the production of silk; in the larvae of *Chrysopa* (McDunnough, 1909), in ant-lion (Myrmeleonid) larvae (Lozinski, 1911), and in several beetles, for example in the larvae of the weevil *Phytonomus* (Lebenden, 1914).

No data are available on the fat content of the phloem sap ingested by these insects. However, in other species, for example in larvae of the silk-moth *Bombyx mori* (Uvarov, 1928; and Manunta, 1935), muscid fly larvae (Mishikata, 1922), and *Aëdes* mosquito larvae (Wigglesworth, 1942), fats are formed from carbohydrates and proteins in the food. It is possible that the lipid present on the brochosomes is metabolically derived in a similar way, although the significance of its excretion is unknown.

Structure and Function in Excretory Cells: Many good electron micrographs have been published displaying the various regions of the vertebrate kidney, and it is evident that this organ has attained a form which varies little between the animal groups. Rhodin (1958*b*) writes: "From a functional point of view, it seems that the architecture of all the cells of the nephron is especially well adapted for passage of fluid, a passage that may be directed in two directions." It will be recalled that the proximal convoluted tubule cells bear a brush border adjoining the lumen, while the basal plasma membrane is produced into a series of infoldings, penetrating into the cell body, parallel with which lie many elongated mitochondria (Sjöstrand and Rhodin, 1953; Rhodin, 1958*a*, *b*; and others). In the distal tubule the incursions of basal membrane are even more highly developed, while the brush border is absent. The bulk of reabsorption of valuable constituents of the glomerular filtrate, glucose, protein, and amino acids, occurs in the proximal tubule, while the urine is concentrated by water absorption taking place primarily in the proximal but also in the distal convolution (Smith 1956). Rhodin (1958*b*) suggested that the above cytological features of the proximal convoluted tubule cells may be correlated with their role in fluid reabsorption, while in the distal segment, the main function of which is probably secretory rather than absorptive, the brush border is absent.

Pease (1956) showed that the inflection of the basal plasma membrane found in proximal and distal kidney tubules is also characteristic of secretory duct and serous cells of the submaxillary gland, of cells of the ependymal epithelium of the choroid plexus, and of the epithelium of the ciliary body of the eye. He pointed out the physiological link between these examples and the cells of the kidney tubule: that extensive water transport takes place through all these epithelia. This list of similarly modified cells may be extended by citing the Malpighian tubule cells of the grasshopper *Melanoplus d. differentialis* (Beams *et al.*, 1955) and of the cricket *Gryllus domesticus* (Berkaloff 1958, 1959). In the foregoing account of the Malpighian tubules of the leafhopper *Macrosteles fascifrons*, it has been shown that the basal plasma membrane of the brochosome-secreting cells (region III) shows little trace of inflection, while this modification is present in cells of the two adjacent regions (II and IV) and appears particularly well developed in the first or proximal region. In the latter region, the only one provided with a brush border in this species, incursions of the basal cell membrane penetrate almost to the distal surface.

The relatively constant internal environment upon which the vertebrate kidney operates is

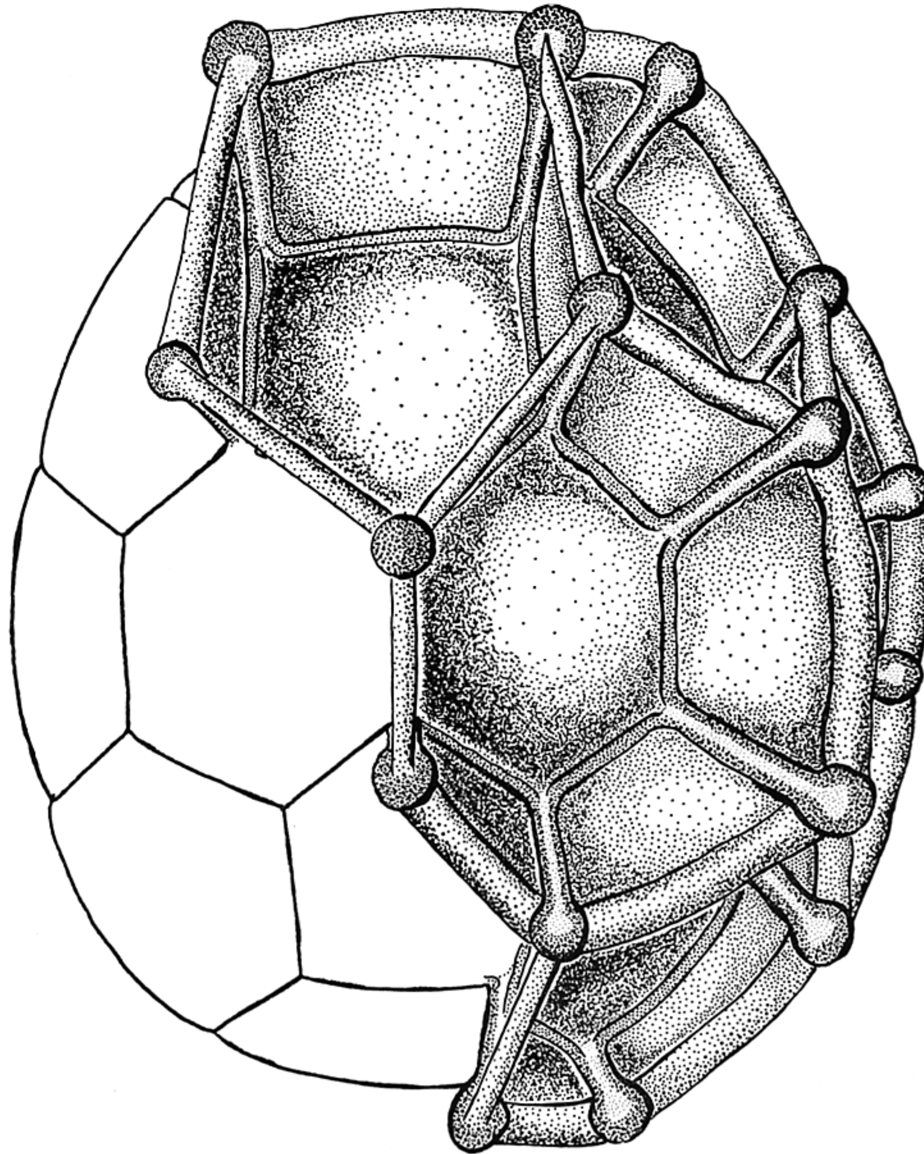


FIGURE 21

Semidiagrammatic interpretation of the surface structure and geometry of a brochosome having twenty faces, upon five of which the compartments are constructed. The twenty sided configuration of eight hexagons and twelve pentagons, as shown here, is considered to be prevalent in *Macrosteles fascifrons*. The dense periphery and less dense center of the radial spokes correspond to the appearance of material stained with phosphotungstic acid.

not paralleled in the case of insects and their Malpighian tubules. It is not surprising that the variation in physiological conditions in the latter instance, such as the nature of the ingested food, the availability of water, and the composition of the waste products, should be accompanied by corresponding variation both in the gross anatomy of the Malpighian tubules and in the structure of their cells. Nevertheless, at the fine structural level, similarities exist between the cells of the vertebrate and insect urinary systems, suggesting that certain basic physiological processes are common to both.

By analogy with the cells of the nephron, region I of the Malpighian tubule of *Macrosteles* exhibits all the structural features that have been attributed to water and solute reabsorption. The inflection of the basal plasma membrane, relative to the width of the tubule wall, is deeper and more highly developed than in either proximal or distal tubule cells of the kidney or in the other epithelia mentioned by Pease (1956). In common with the proximal tubule, these cells are richly supplied with ribonucleoprotein particles, and in addition to the basal inflections, they bear a well developed brush border. It is suggested that the role of this region of the Malpighian tubule is to effect the transfer of fluid from the lumen to the haemolymph contained between invaginations of the basal region of the cell. Moreover, the high concentration of large mitochondria in the apical half of the cell possibly has the function of supplying the energy needed for the reabsorption of water and perhaps valuable dissolved constituents from the lumen.

The cytoplasm of the cells of the widest region (III) of the tubule is concerned with the formation and discharge of brochosomes. They contain no cytological features that would implicate them in transport of fluid either into or away from the lumen. On the apical surface, through which the brochosomes are voided, only a few small and irregularly spaced papillae are present, possibly the vestigial analogues of the microvilli of a brush border. The plasma membrane of the basal surface shows only occasional infoldings, which are very shallow (Fig. 10), and in addition the mitochondria do not show the preferential concentration found elsewhere in this organ, and in the kidney.

On either side of this last region are found cells of unusual structure, whose function is more problematical (Figs. 1 and 26, regions II and IV). Here the basal plasma membrane is again extensively inflected, though, in relation to the cell height, much less deeply than in region I. The apical surface of the cell is reflexed inwards to produce a number of flask-shaped cavities. The extra membrane area thus provided is enormously supplemented by the pleating of the membrane and the adjacent cytoplasm into leaflets, which line the neck and walls of the cavities and extend over the entire surface bounding the tubule lumen. It is not known how this surface area increase compares with that obtained by means of a brush border of similar extent, but the two devices are analogous, and this modification, especially in view of the plication of the basal cell membrane, suggests that these cells in common with those of region I take part in the movement of fluid. However, while this is a reasonable inference drawn from the cytological architecture, this in itself offers no indication of the direction of transfer of water and any solutes

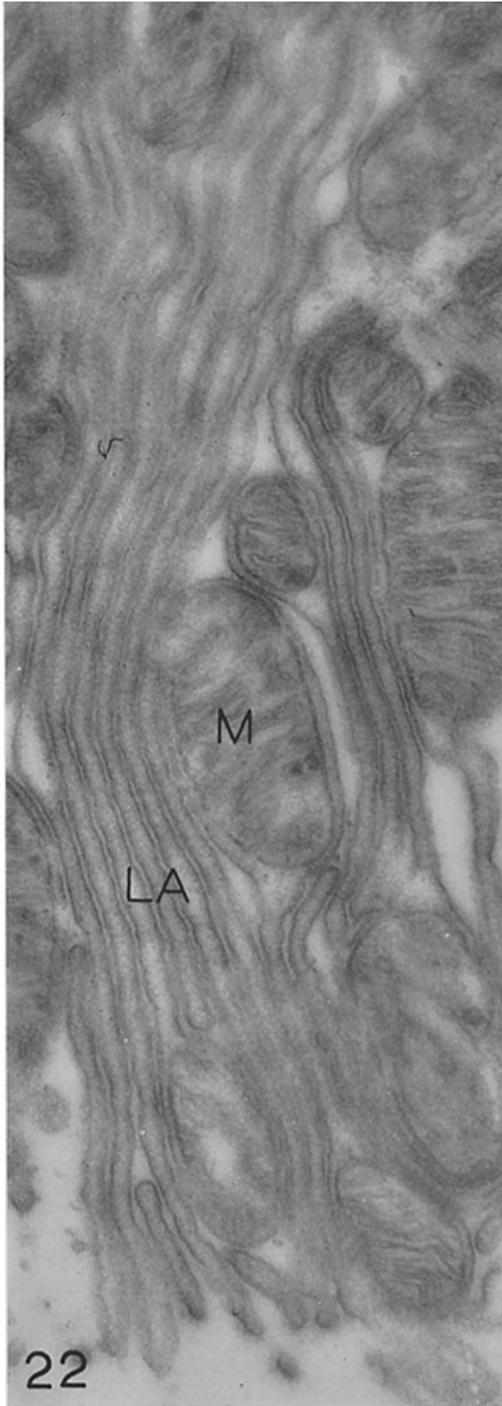
Electron micrographs of cells of the hindgut of *Macrosteles fascifrons*. In each case, the gut lumen is situated at the bottom of the micrograph.

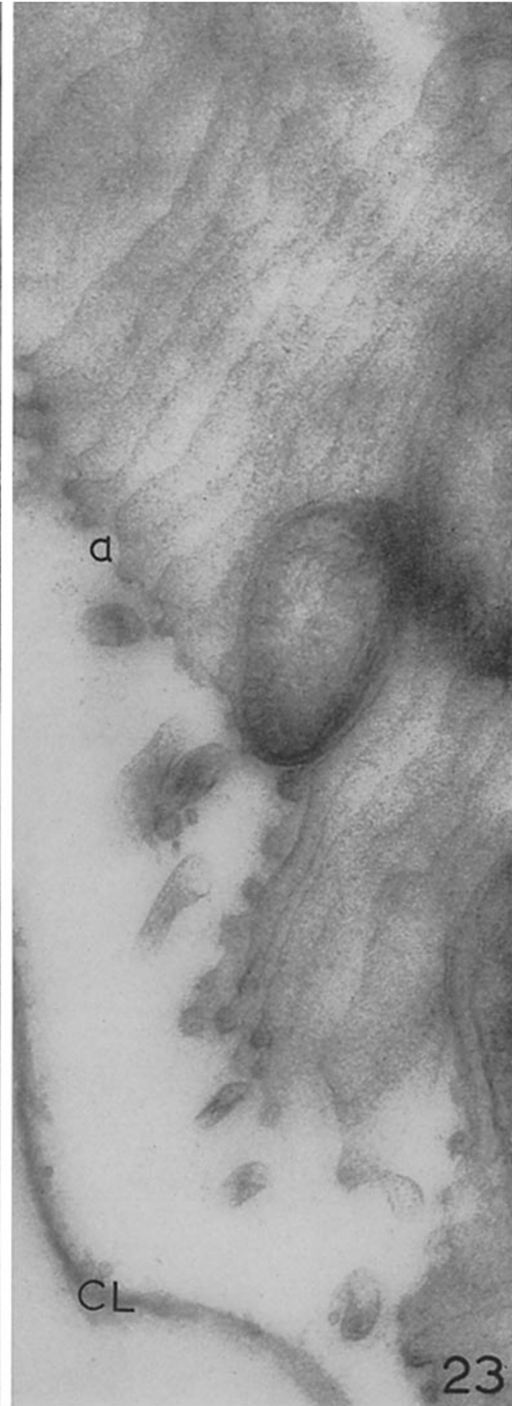
FIGURE 22

Illustrating the elaboration of the apical portion of the cell. In contrast with the brush border of the cells of the midgut (see Fig. 26 *b*), this region here bears narrow lamellae (*LA*), seen in this case in transverse section, in several of which mitochondria (*M*) are inserted. A rather similar lamellar modification is found in the cells of regions II and IV of the Malpighian tubules, as is illustrated in Figs. 5, 6, and 7. × 35,000.

FIGURE 23

A section of the same region, but one in which the lamellae are cut more obliquely showing, at *a*, their irregular apices. *CL* represents the cuticular lining of the hindgut. × 48,000.


M
LA
22


a
CL
23

it contains; either secretion or reabsorption, or possibly both processes, may take place through the membranes of the intracellular cavities. In many insects the solid waste products of the Malpighian tubules are spherites of uric acid formed either intracellularly (*e.g. Gryllus*, Berkaloff, 1958) or within the tubule lumen (*e.g. Rhodnius*, *Wigglesworth*, 1931). In *Macrosteles*, however, histochemical tests for uric acid were negative, and such spherites have not been found in any region of the Malpighian tubule; from the evidence of electron microscopy, the brochosomes are the sole solid constituents of the urine. Thus, in the absence of further physiological evidence, one may only hypothesize about the role played by the "flask cells" of the tubule. Two alternative though not mutually exclusive possibilities are that they are concerned with the secretion into the lumen of water-soluble waste products, or that they regulate the ionic balance between the haemolymph and the content of the tubule.

When the Malpighian tubules of *Macrosteles* are compared with those of *Melanoplus* and *Gryllus*, the only other species so far examined in the electron microscope, it is clear that important functional and structural differences exist between them, though at the present time it is difficult to assess their significance. Beams and his coworkers (1955) described cells in *Melanoplus* which, as in kidney tubule cells, showed pronounced invagination or inflection of the basal cell membrane, while the apical surface bore a brush border the microvilli of which frequently contained filamentous and distally clubbed mitochondria. However, there is no trace of uric acid spherites either within these cells or in the lumen, though Brown (1937) found that in the allied species *M. bivittatus* the bulk of the excreted nitrogen is in the form of uric acid. This suggests that either the production of this material is a phasic process, or else it is formed by cells elsewhere in the tubule, which were not studied. A parallel difficulty exists in the interpretation of the work of Berkaloff (1958, 1959) on *Gryllus*. In the first paper he described the intracellular formation of granules or spherites of uric acid or a derivative within cells whose basal plasma membrane is deeply inflected and which bear a brush border adjoining the lumen. Later, he described another cell type, devoid of granules, in which the basal membrane inflection is very limited. This, he suggested, represents the form taken by the secretory cell after the synchronous discharge of the granules. The possibility remains that these two cell types represent a permanent structural and functional specialization, especially in view of the observation of Kölliker (1858) that while some Malpighian tubules in *Gryllidae* are yellow, others are white and are filled with uratic granules.[1] In any event, none of the three cell types described from *Macrosteles* closely resembles those of the Orthopteran species, and it is probable that examination of the Malpighian tubules of other insects will reveal further instances of cellular specialization in these organs.

From the foregoing account of work carried out on a single species emerges a picture of considerable biochemical and cytological interest and complexity, albeit one in which at the present time more questions are asked than are answered. Nevertheless, such a picture, with all its problems, serves to stress the importance of comparative cytology in any attempt to relate fine structure, revealed by the electron microscope, to cellular function.

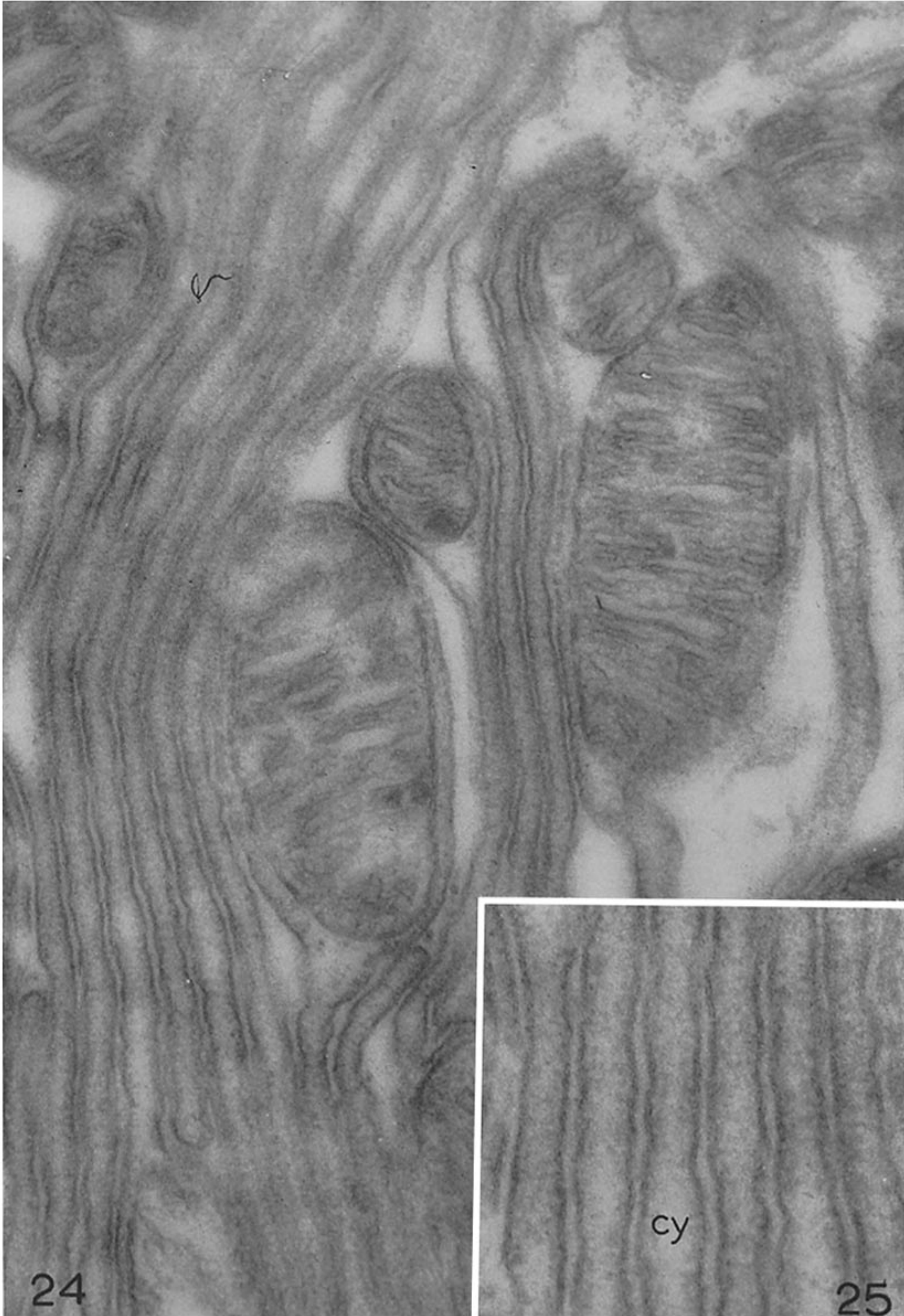
[1] In these Orthoptera additional Malpighian tubules are formed in successive instars, and Berkaloff has recently found (personal communication) that the white tubules are immature, and become pigmented in due course. There appears to be no evidence, therefore, that this color variation reflects a permanent structural or functional differentiation.

FIGURE 24

Enlarged portion of Fig. 22 showing, more clearly, the insertion of the mitochondria within dilatations of the lamellae. × 64,000.

FIGURE 25

Highly enlarged region of Fig. 22 showing the granular cytoplasm (*cy*) bounded by the plasma membrane which shows the characteristic triple-layered organization. × 100,000.


cy
24
25

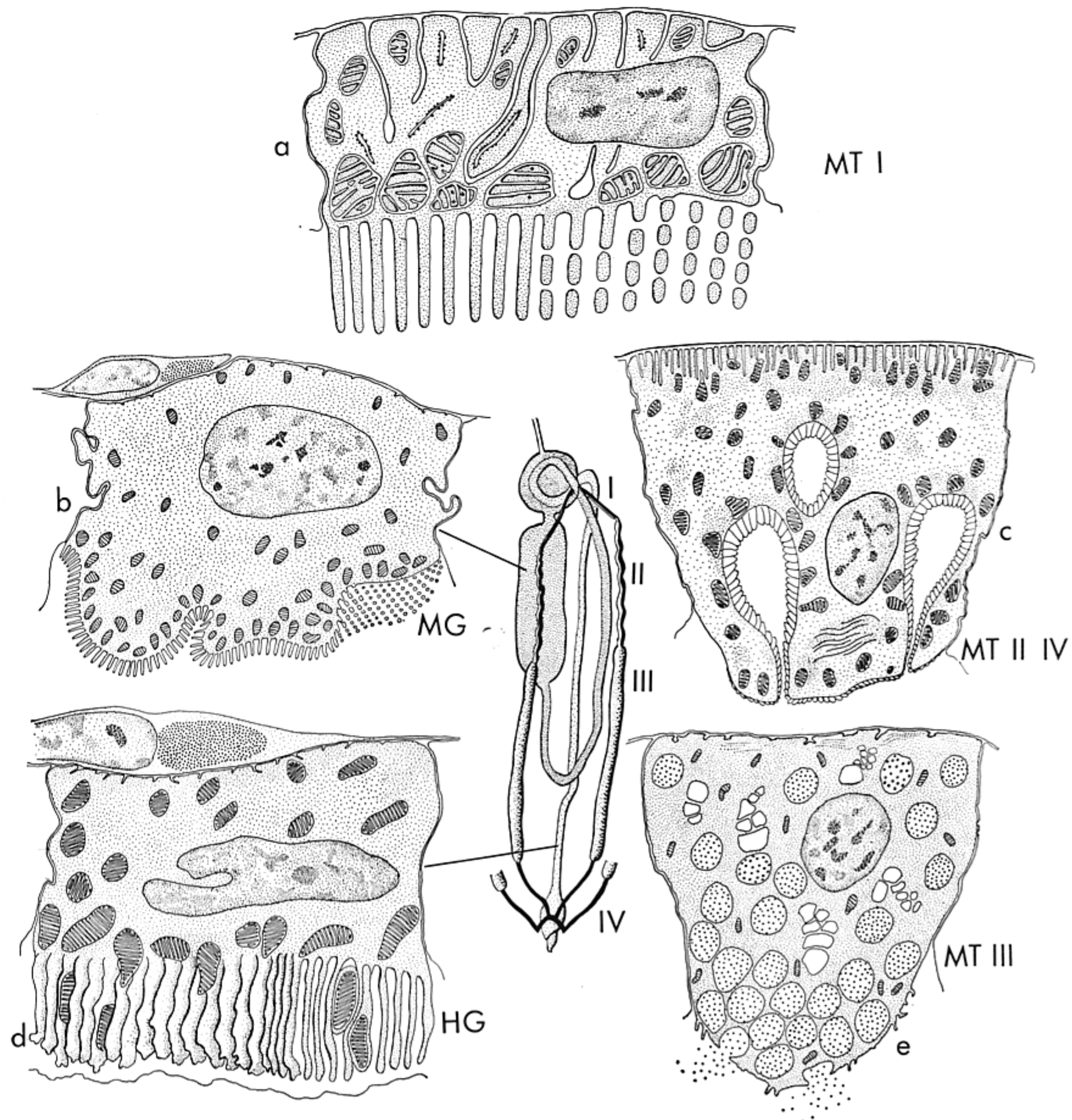


FIGURE 26

Diagram summarizing the distribution of cell types in the mid- and hindgut and Malpighian tubules of *Macrosteles fascifrons*. *MG*, midgut; *HG*, hindgut; *MT*, I to IV, divisions of the Malpighian tubules. Note that these diagrams are drawn representing different magnifications.

LITERATURE CITED

ANDERSON, T. F., *Tr. New York Acad. Sc.*, series 2, 1951, **13,** 130.

BEAMS, H. W., and ANDERSON, E., *J. Morphol.*, 1957, **100,** 601.

BEAMS, H. W., TAHMISIAN, T. N., and DEVINE, R. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 197.

BERKALOFF, A., *Compt. rend. Acad. sc.*, 1958, **246,** 2807.

BERKALOFF, A., *Compt. rend. Acad. sc.*, 1959, **248,** 466.

BRADFIELD, J. R. G., *Quart. J. Micr. Sc.*, 1953, **94,** 351.

BROWN, A. W. A., *J. Exp. Biol.*, 1937, **14,** 87.

BÜSGEN, M., *Jena, Z. Naturwissensch.*, 1891, **25,** 339.

CHAYEN, J., GAHAN, P. B., and LA COUR, L. F., *Quart. J. Micr. Sc.*, 1959, **100,** 325.

COURTOIS, A., *Compt. rend. Soc. biol.*, 1929, **101,** 365.

DAY, M. F., and BRIGGS, M., *J. Ultrastruct. Research*, 1958, **2,** 239.

FLAX, H. M., and HIMES, M. H., *Physiol. Zool.*, 1952, **52,** 297.

GLICK, D., Techniques of Histo- and Cytochemistry, New York, Interscience Publishers, Inc., 1949.

HENSON, H., *Quart. J. Micr. Sc.*, 1932, **75,** 283.

HOTCHKISS, R. D., *Arch. Biochem.*, 1948, **16,** 131.

HYMAN, L. H., The Invertebrates: Protozoa through Ctenophora, New York, McGraw-Hill Book Company, 1940, 55.

KÖLLIKER, A., *Verhandl. phys.-med. Ges. Würzburg*, 1858, **8,** 225.

LEBENDEN, A., *Zool. Anz.*, 1914, **44,** 49.

LOZINSKI, P., *Zool. Anz.*, 1911, **38,** 401.

MANUNTA, C., *Accad. Naz. Lincei, Mem.*, series 6, 1935, **6,** 75.

MCDUNNOUGH, J., *Arch. Naturgesch.*, 1909, **55,** 313.

MCMANUS, J. F. A., *Nature*, 1946, **158,** 202.

MISHIKATA, T., *J. Biochem.* (Tokyo), 1922, **1,** 261.

PALADE, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 85.

PALADE, G. E., and SIEKEVITZ, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 171.

PEACHEY, L. D., *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 511.

PEARSE, A. G. E., Histochemistry, Boston, Little, Brown and Co., 1953.

PEASE, D. C., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 203.

RHODIN, J., *Am. J. Med.*, 1958*a*, **24,** 661.

RHODIN, J., *Internat. Rev. Cytol.*, 1958*b*, **7,** 485.

SJÖSTRAND, F. S., and RHODIN, J., *Exp. Cell Research*, 1953, **4,** 426.

SMITH, H. W., Principles of Renal Physiology, New York, Oxford University Press, 1956.

STOREY, H. H., and NICHOLS, R. F. W., *Proc. Roy. Entomol. Soc.*, London, 1937, **12,** 149.

THOMPSON, D. W., On Growth and Form, New York, Macmillan, 2nd edition, 1945, 725–732.

TÓTH, L., *Z. Morphol. Okol. Tiere*, 1933, **27,** 692.

TÓTH, L., *Z. Morphol. Okol. Tiere*, 1937, **33,** 412.

TULLOCH, G. S., and SHAPIRO, J. E., *Bull. Brooklyn Entomol. Soc.*, 1953, **48,** 57.

TULLOCH, G. S., SHAPIRO, J. E., and COCHRANE, G. W., *Bull. Brooklyn Entomol. Soc.*, 1952, **47,** 41.

UVAROV, B. P., *Tr. Entomol. Soc. London*, 1928, 655.

WATSON, M. L., *J. Biophysic. and Biochem. Cytol.*, 1958*a*, **4,** 475.

WATSON, M. L., *J. Biophysic. and Biochem. Cytol.*, 1958*b*, **4,** 727.

WIGGLESWORTH, V. B., *J. Exp. Biol.*, 1931, **8,** 451.

WIGGLESWORTH, V. B., *J. Exp. Biol.*, 1942, **19,** 56.

WIGGLESWORTH, V. B., The Principles of Insect Physiology, London, Methuen, 4th edition, 1950.

WIGGLESWORTH, V. B., *Proc. Roy. Soc. London, Series B*, 1957, **147,** 185.

WILDE, W. H. A., and COCHRANE, G. W., *Proc. Entomol. Soc. Brit. Columbia*, 1957, **53,** 19.

ZETTERQVIST, H., The Ultrastructural Organization of the Columnar Absorbing Cells of the Mouse Jejunum, Thesis, Karolinska Institutet, Stockholm, 1956.