An Electron Microscope Study of the Cells Lining the Small Blood Vessels of *Helix pomatia* Linn.

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INTRODUCTION

In the course of another investigation narrow channels were observed in association with the esophageal ganglia of *Helix pomatia*. The channels are lined with flattened cells and are identified as small blood vessels. The Golgi complex, mitochondria, and other cell inclusions are visible in electron micrographs of these cells. Since little work has been carried out on the ultrastructure of the cells of invertebrate animals, we consider it desirable to give a brief account of our observations.

Materials and Methods

Pieces of tissue surrounding the esophageal ganglia of *Helix pomatia* were fixed in 1 per cent buffered osmium tetroxide (pH 8.0) and embedded in a mixture of *N*-butyl and methyl methacrylate.

Material for observation with the light microscope was fixed in Bouin's picroformol (picric acid, saturated aqueous solution 75 cc., formol 25 cc., acetic acid 5 cc.) and in Kolatchev solution (equal parts of 6 per cent potassium dichromate, 1 per cent chromic acid, and 2 per cent osmium tetroxide; followed by post-osmication). Sections were subsequently cut at 5 μ and 7.5 μ thickness.

OBSERVATIONS

The Golgi complex of the cells lining a small blood vessel lies some little distance from the nucleus in an area between the latter and the lumen of the vessel. In electron micrographs of relatively low resolution it is visible as three or more groups of dense structures (Fig. 1). Large dense deeply osmiophilic bodies are present in this part of the cell and in the cytoplasm between the

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‡ *Received for publication, March 3, 1958.*

Golgi complex and the lumen. A small number of bodies of similar appearance may occur in the cytoplasm close to the opposite pole of the nucleus. The nature of these bodies was not determined, but presumably they are composed of nutritive or storage material. A small number of large clear spaces, sometimes occurring singly and sometimes in contact with one another, are scattered through the cell but are not present in the vicinity of the Golgi complex. Each of these spaces appears to be enclosed by a single membrane; they possibly represent vacuoles the contents of which was dissolved out during the preparation of the tissue. When viewed with the light microscope, similar spaces are visible in cells fixed in Bouin's picroformol and in Kolatchev. In these preparations they are often larger and more numerous than in the cells shown in electron micrographs.

The examination of Kolatchev preparations with the light microscope is rendered difficult owing to the large amount of osmiophilic material present. Some of this material corresponds to the large dense bodies of electron micrographs. A number of curved rods and filaments are, however, frequently visible in the Golgi zone. In some cells they extend laterally round the anterior pole of the nucleus. We conclude that the elements of the Golgi complex revealed by the electron microscope are represented by the osmiophilic rods and filaments of cells in Kolatchev preparations.

An examination of electron micrographs of high resolution shows that the Golgi complex is composed of three components—closely applied flattened vesicles or saccules, vacuoles containing little dense material, and minute vesicles or granules (Fig. 2). The walls of the flattened vesicles are dense and their interiors comparatively clear. Frequently, a group of vesicles is intimately associated with one side of a Golgi vacuole, or almost completely surrounds a vacuole. Some of the flattened vesicles are continued from one group of vesicles to another so that, sometimes at least, neighbouring bundles are connected (Fig. 2).

The greater part of a Golgi vacuole (Fig. 2) appears in an electron micrograph as a clear space but a small amount of dense material is usually scattered through its interior. Some of this dense material seems to be membranous in form. Granules or vesicles with dense walls are scattered in the cytoplasm around the flattened Golgi vesicles and vacuoles. These, we believe, correspond to the small Golgi vesicles described by other workers.

While the present investigation was undertaken primarily with the object of determining the ultrastructure of the Golgi complex, three other components of the cytoplasm are worthy of mention. The cell or plasma membrane (Figs. 1 and 3) is clearly shown to possess intracellular folds or invaginations. Membranes composed of external dense components separated by a less dense area are present in the cytoplasm (Figs. 2 and 3). These differ in appearance from the profiles of the flattened Golgi vesicles in that they are not arranged in bundles and that the less dense inner region is considerably wider than the interior of a Golgi vesicle. We identify these structures as cytoplasmic membranes which constitute the endoplasmic reticulum. Sometimes the elements of the endoplasmic reticulum occur in the neighbourhood of the Golgi complex but do not appear to be continuous with the latter. Invaginations of the plasma membrane are frequently in close topographical relationship with the endoplasmic reticulum. It is probable that some of the latter are greatly extended invaginations of the cell membrane, but it is not possible in our material to determine with certainty whether such a connection does in fact exist.

Sections of mitochondria are shown in some of our electron micrographs (Figs. 1 and 3). Their external membranes are not well defined and their interiors are dense. In many cases the internal membranes are not visible while in others they are seen as poorly defined structures. Owing to the dense nature of the intermembranous region of a mitochondrion and the poor definition of the internal membranes, we are unable to determine the arrangement of the latter.

DISCUSSION

It is now widely accepted that the Golgi complex of the cells of vertebrate animals is composed of flattened vesicles, granules or small vesicles, and frequently large vacuoles (3, 8, 10). Further, it is claimed that the dictyosomes of germ cells and of somatic cells of invertebrates basically resemble in their fine structure the Golgi complex of vertebrate somatic cells (2–7). We believe that the Golgi complex of the cells forming the wall of the small blood vessels of *Helix* is made up of flattened vesicles, large vacuoles, and minute vesicles, and that it, therefore, closely resembles the complex of the somatic cells of vertebrates. It is of interest that in this animal neighbouring bundles of flattened Golgi vesicles are sometimes connected by

a few vesicles. A somewhat similar condition was previously observed in the neurons of *Patella vulgata* (7, 9). According to Lacy (7) the Golgi lamellae of *Patella* branch and anastomose to form a network. In *Helix* branching appears to be less marked than in *Patella* and it is doubtful whether the condition in the former could be described as a true network.

Dalton and Felix (3) believe that the structures seen in electron micrographs, and formerly described as Golgi membranes, are in reality "profiles of flattened sacs." A similar claim is made by Grassé and Carasso (6). Our conclusions support this view.

The dense nature of the mitochondria of the cells lining the small blood vessels of *Helix* is rather surprising. It is likely that the large dense bodies present in the cytoplasm arise from mitochondria, but there is no direct evidence in support of this suggestion. Reference to the relevant literature indicates that the fine structure of the mitochondria of invertebrates varies considerably according to the type of cell in which they are resident. Beams and Tahmisian (1), for example, claim that the mitochondria of the male germ cells of *Helix* differ from those of other cells examined prior to their study. They comment that "it may be that further investigation will establish the difference as due to a variation in structure within the mitochondria of certain animal species." More recently Dalton and Felix (3), with reference to the mitochondria of vertebrate animals and of some unicellular organisms, remark that "the number, spacing and orientation of the internal membranes . . . appeared to be characteristic for each cell type."

It is worthy of note that the elements of the endoplasmic reticulum shown in our electron micrographs are few in number and are not associated with granules. Beams, Tahmisian, Devine, and Anderson (2) consider that a "Golgi lamella" of the male germ cells of *Nemobius sp.* is sometimes extended as "a string of relatively small bead-like vacuoles . . . in close approximation to elements of the endoplasmic reticulum." In some of our electron micrographs the interiors of some of the flattened vesicles at the end of a bundle are wider than elsewhere, and consequently present an appearance somewhat similar to that described by Beams *et al.* There is, however, no evidence that the flattened Golgi vesicles of *Helix* are directly associated with cytoplasmic membranes.

We wish to thank Professor M. L. Barr, Department of Microscopic Anatomy, and Professor R. G. E. Murray, Department of Bacteriology and Immunology, University of Western Ontario, for permission to use an electron microscope and for other facilities. We are especially grateful to Dr. R. C. Buck, Department of Microscopic Anatomy, University of Western Ontario, for his willing help and advice. Our thanks are also due to Miss Patricia A. Hale, Research Assistant, Department of Zoology, The Queen's University of Belfast, who fixed and embedded the tissue and cut and stained material for examination with the light microscope.

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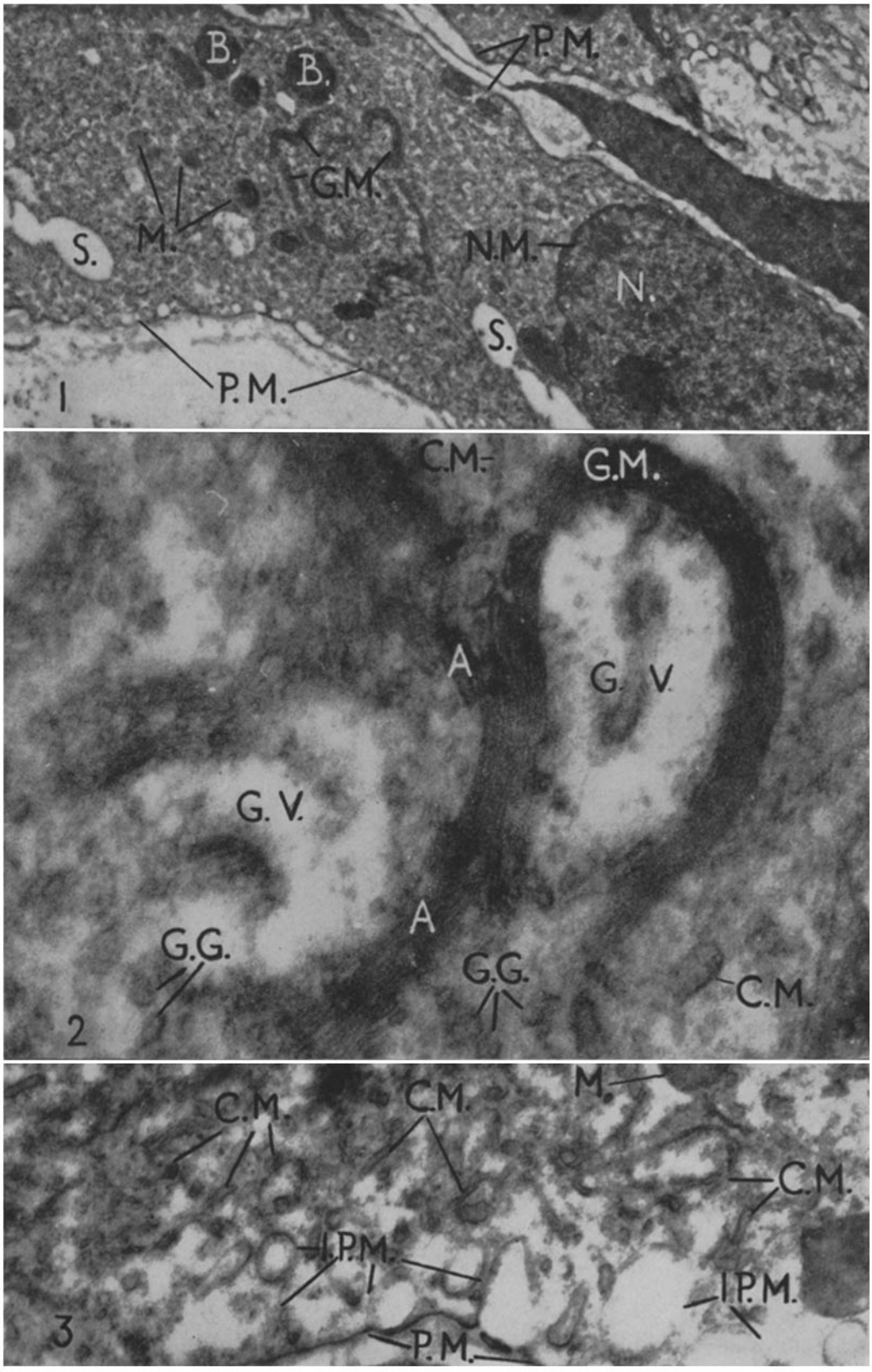
Explanation of Plate 192

Fig. 1. Electron micrograph of part of two cells from a small blood vessel of *Helix pomatia*. Large dense bodies (*B.*) Clear spaces (*S.*). Part of the nucleus (*N.*), the nuclear membrane (*N.M.*), and the cell membrane (*P.M.*) are shown. Small clear spaces at the periphery are surrounded by invaginations of the cell membrane. A group of flattened Golgi vesicles (*G.M.*) is present between the nucleus and the lumen. *M.*, mitochondria. × 9,273.

Fig. 2. Electron micrograph. Flattened Golgi vesicles (*G.M.*). Golgi vacuoles (*G.V.*). Small Golgi vesicles or granules (*G.G.*). At (*A*) the connections between adjacent bundles of flattened Golgi vesicles are shown. Cytoplasmic membranes (endoplasmic reticulum) (*C.M.*) are cut at various angles. × 85,000.

Fig. 3. Electron micrograph of small part of peripheral region of a cell. The cell membrane (*P.M.*) and intracellular invaginations of the cell membrane (*I.P.M.*) are shown. Cytoplasmic membranes (endoplasmic reticulum) (*C.M.*) are cut at various angles. *M.*, mitochondrion. × 38,636.



(Threadgold and Gresson: Cells of *Helix* small blood vessels)