An Electron Microscopic Observation of the M organ in the Eyestalk from Fresh-water Crab, *Geothelphusa dehaani* (WHITE)

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Abstract

The structures of the minute gliosecretory organ (M organ) in the eyestalks from fresh-water crabs, *Geothelphusa dehaani,* were examined with light and electron microscope. The M organ was constructed of slender cells and observed as a spindle-shaped minute follicle-like organ on the ventral side of the proximal part of the medulla interna in the eyestalk. Each slender cell possessed an ellipsoidal nucleus which had some cytoplasmic invaginations. The cytoplasm of the slender cell contained many mitochondria varying in size and shape, a large number of free ribosomes, poorly developed Golgi complexes and granular endoplasmic reticula. In the inner lumen, this organ contained a colloidal mass of CH positive and AF positive stainability. The colloidal mass was composed of predominant moderate electron dense substances and some groups of electron dense particles.

A few granules of CH positive and AF positive stainability were found in the cytoplasm. These granules involved some moderate electron dense materials and some particles of the same diameter as that of the particle within inner lumen.

These present results suggest that the M organ may be a secretory organ.

Introduction

The M organ was reported by Matsumoto (1970) by the name of minute gliosecretory organ in the eyestalks of Decapod crustaceans. He reported as follows. This organ was a spindle-shaped minute organ which was constructed of the slender neuroglia cells in the ventral side of the proximal part of the medulla interna in the eyestalk of the crab, and which possessed a colloidal mass of Gomori's CH positive and AF positive stainability in the central portion of the organ. The colloidal mass seemed to be discharged into the tissue space near the organ. A few granules of the same stainability as that of the colloidal mass were found in some slender cells. The slender cells much resembled the glia cells within the neuropile in size and stainability of the nuclei. On the basis of these observations he assumed that the M organ was constructed of slender neuroglia cells and that the colloidal mass might be a secretory product of the glia cells of this organ.

However, physiological roles and fine structures of this organ are not yet clear. This present report deals with a historogical study carried out on the M organ of

fresh-water crabs, *Geothelphusa dehaani* under light and electron microscope.

Material and Method

The materials in this study were the eyestalks of the adult fresh-water crabs, *Geothelphusa dehaani* (WHITE, 1847) which were collected at Kanagawa in Okayama Prefecture.

For light microscopy, the eyestalks were cut off from the crabs and fixed with Bouin's solution. These samples were decalcified in this fixative for about 7 days and dehydrated through a series of ethanol and embedded in paraffin. The serial sections were cut on a microtome and stained with Gomori's chromehematoxylin and phloxin or aldehyde fuchsin, Ehrlich's hematoxylin and light green-orange G mixture.

For electron microscopy, the eyestalks from the crabs were immersed in a drop of phosphate buffered 2.5 % glutaraldehyde and removed its exoskeleton under a binocular microscope. The materials were pre-fixed with ice-cold phosphate buffered 2.5 % glutaraldehyde for 45 minutes. They were rinsed in phosphate buffer for 45 minutes and post-fixed with ice-cold phosphate buffered 1% OsO_4 (Millonig, 1962) for 90 minutes. After the fixation, they were dehydrated through a series of ethanol and embedded in epon (Luft, 1961). The ultrathin sections were cut and stained with aqueous saturated solution of uranyl acetate for 15 minutes and Reynolds's (1963) lead citrate for 2 minutes. The sections were examined in a JEM-7A electron microscope or an HS-9 electron microscope. Furthermore, the sections of about 2 μm in thickness were cut from the material in epon, stained with toluidine blue and observed with a light microscope.

Results

1. Light microscopic observation

This organ is a spindle-shaped organ of about 0.1 mm in long axis and about 0.05 mm in short one. It is observed on the ventral side of the proximal part of the medulla interna in the nervous tissue of eyestalks of the crabs (Fig. 1).

A colloidal mass observed in the central lumen of the organ. The mass is stained intensely with Gomori's chromehematoxylin (Fig. 2) and aldehyde fuchsin (Fig. 3).

This organ is constructed of slender cells which have chromophilic ellipsoidal nuclei. The slender cells line almost radially around the central colloidal mass (Fig. 4). On the other hand, the marginal ends of these cells contact with small nerve cells or nerve fibers in the medulla interna.

A few granules of CH positive and AF positive stainability are observed in the chromophobic cytoplasm of some slender cells.

2. Electron microscopic observation

This organ is a follicle-like organ which has an inner lumen in its center. The inner lumen is filled with abundant colloidal substances of moderate electron

density (Figs. 5, 6, 8 and 9). The colloidal mass exhibits meshwork-like structure. The central portion of the mass is more compact than the marginal part. There are some groups of electron dense particles of about 27 nm in diameter in this lumen (Fig. 9).

The slender cells contact each other with smooth surface. Beneath the inner free surface, the cell membranes have interdigitations to neighboring ones (Figs. 8 and 9) and are connected by desmosomes (Figs. 5, 6, 8 and 9).

The cells have some short microvilli on the free surface (Fig. 8). On the marginal parts of this organ, the cell membranes face to small nerve cells or nerve fibers in the medulla interna (Figs. 6 and 7). Basement membranes are not observed in the intercellular space between the slender cells and small nerve cells.

The slender cell becomes more slender toward the marginal end of this organ and finally forms a thin cytoplasmic protrusions (Fig. 7). These thin cytoplamic protrusions run together along the long axis of this organ into the nervous tissue space.

The ellipsoidal nuclei of the cells have some cytoplasmic invaginations into the nucleoplasm. They contain 1 to 3 nucleoli. The heterochromatin is irregularly distributed throughout the nucleoplasm. Many of them are associated with the nuclear membrane (Figs. 5, 6 and 10).

Many mitochondria varying in size and shape are contained within the cytoplasm. They exhibit oval, rod-shaped or elongate rod-shaped figures (Figs. 8, 9 and 11). The mitohcondrial cristae are mostly villi forms.

Poorly developed granular endoplasmic reticulum is sparsely present in the cytoplasm (Fig. 9). A large number of free ribosomes are present throughout the cytoplasm. They are often in the form of polyribosomes.

The Golgi complexes consist of lamellar membraneous cisternae and small vacuoles. They are not well developed and occasionally present (Fig. 10).

Some vesicles and small vacuoles are present within the cytoplasm. A few small vacuoles contain a bit of colloidal substances of moderate electron density and particles of electron dense. The particle has the same diameter as that of the particle in the inner lumen (Figs. 10 and 11).

Discussion

The M organ was found at the similar position i. e. in the ventral side of the medulla interna of all examined eyestalks. As previously mentioned (Matsumoto, 1970), this organ was observed as a spindle-shaped minute organ which contained a colloidal mass of Gomori's CH positive and AF positive stainability in the central portion of the organ. The organ was constructed of slender cells which had chromophobic cytoplasm and chromophilic ellipsoidal nuclei. These facts indicate that this organ is generally present in *Geothelphusa dehaani.*

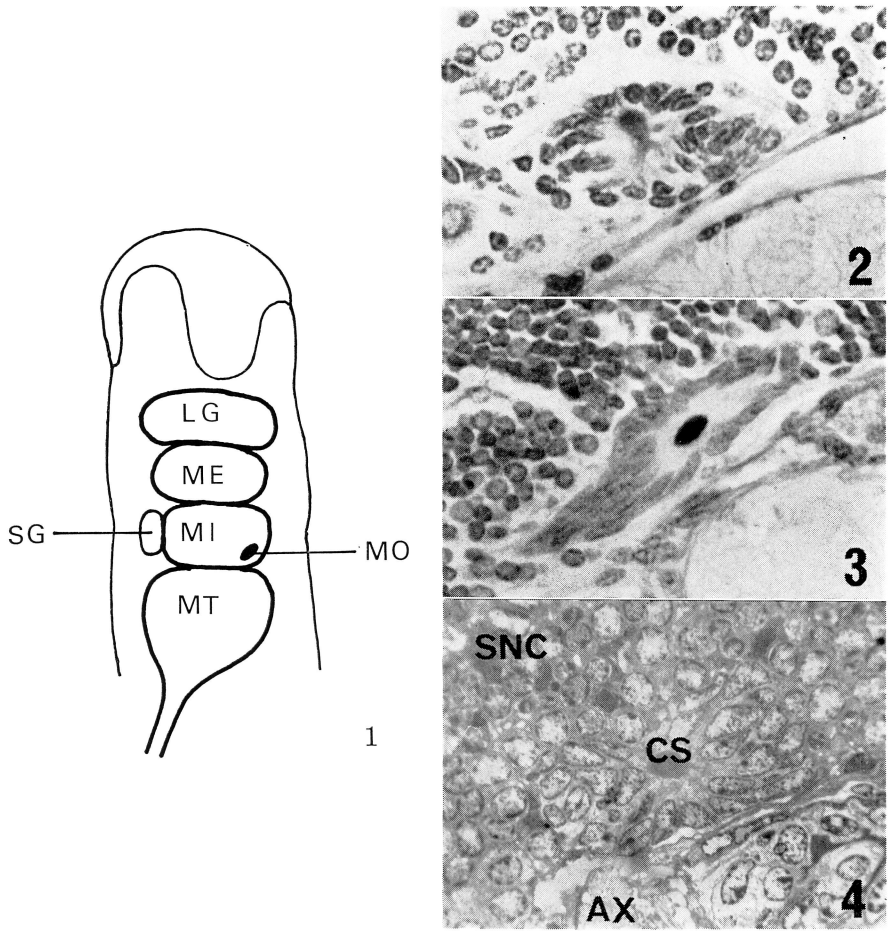

Fig. 1 Drawing of the nervous system in a eyestalk of *Geothelphusa dehaani*. LG, lamina ganglionalis. ME, medulla externa. MI, medulla interna. MO, minute gliosecretory organ. MT, medulla terminalis. SG, sinus gland.

Fig. 2 Photomicrograph of a section of an M organ, showing a central colloidal mass of Gomori's chromehematoxylin positive stainability. Gomori's chromehematoxylin and phloxin stains. ×375.

Fig. 3 Photomicrograph of a section of an M organ. This colloidal mass was stained intensely with aldehyde fuchsin. Aldehyde fuchsin, Ehrlich's hematoxylin and light green-orange G mixture stains. ×375.

Fig. 4 Photomicrograph of a part of a section through an M organ. The organ is constructed of the slender cells and possesses a colloidal mass (CS) in its center. Toluidine blue stain. SNC, small nerve cells. AX, nerve fibers. ×410.

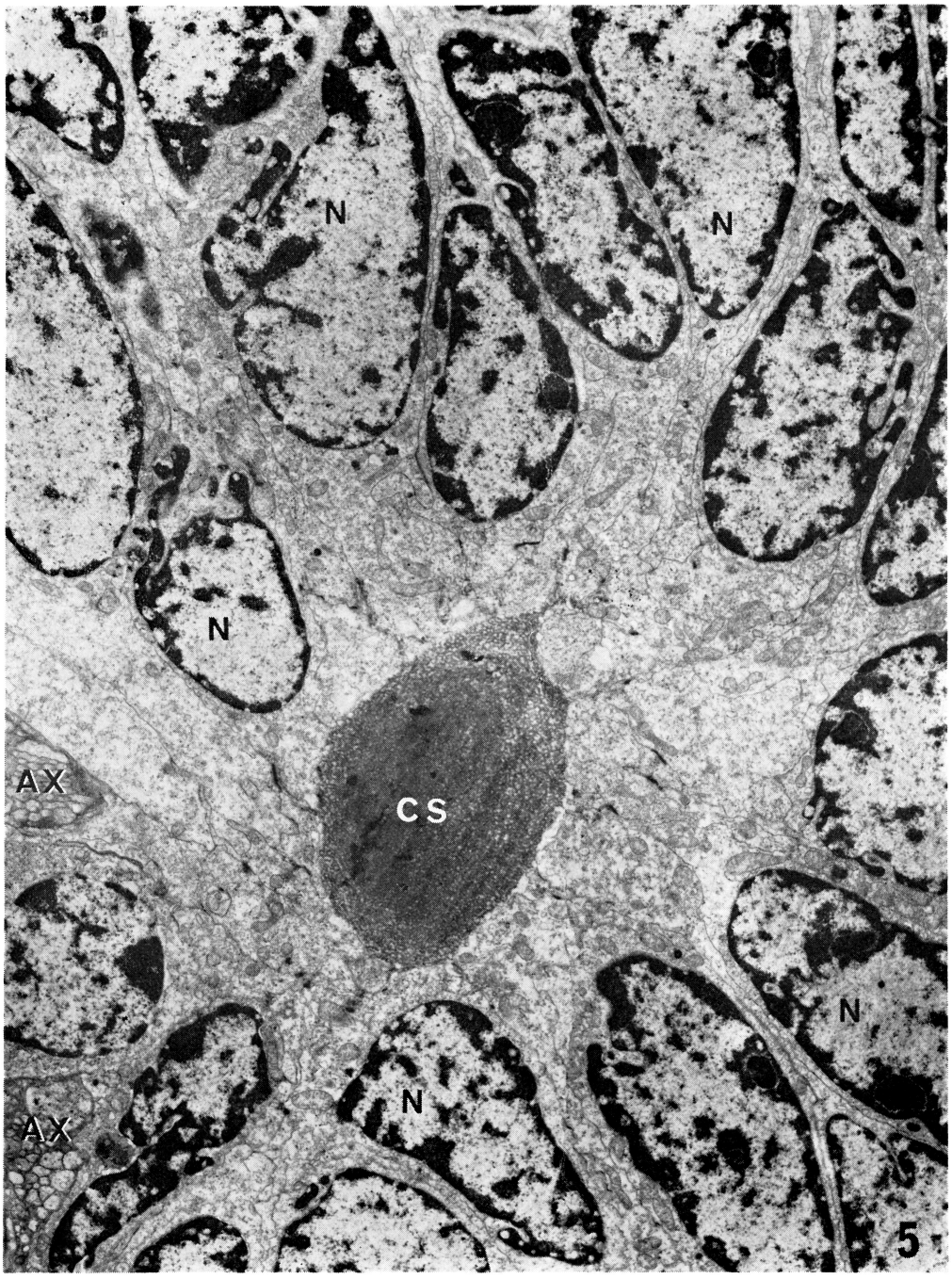

Fig. 5 Electronmicrograph of a section of an M organ from *Geothelphusa dehaani*. A mass of moderate electron dense colloidal substances (CS) is surrounded by the slender cells. The nerve fibers (AX) in the medulla interna are seen at the lower left. N, nuclei. ×3,430.

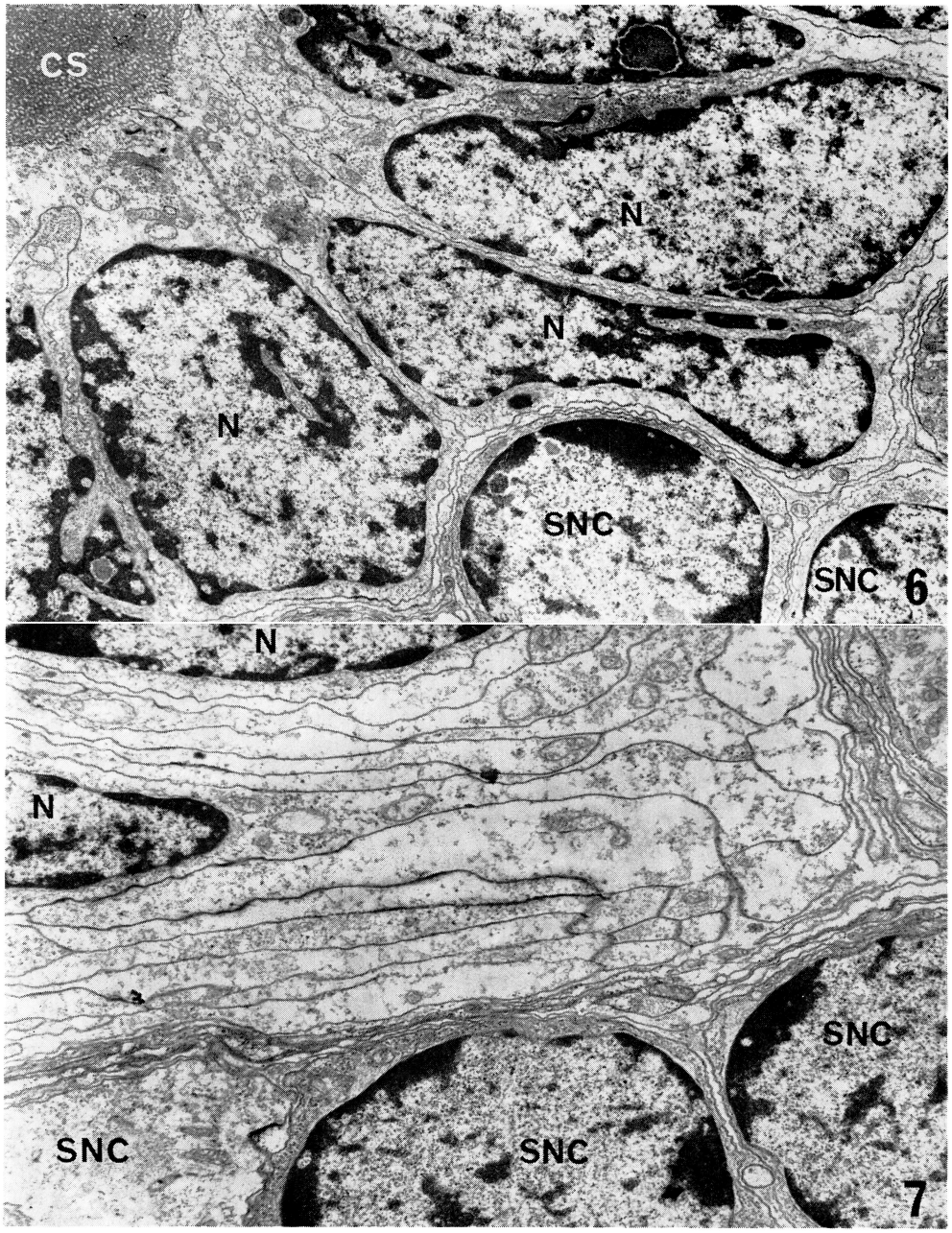

Fig. 6 Electronmicrograph of a part of an M organ and adjacent small nerve cells. The cells face with smooth surface to small nerve cells (SNC) of the medulla interna. There is no basement membrane. N, nuclei. CS, colloidal substances. ×6,200.

Fig. 7 Marginal part of an M organ and adjacent small nerve cells. The thin cytoplasmic protrusions of the cells of the organ run together in parallel. N, nuclei. SNC, small nerve cells. ×9,300.

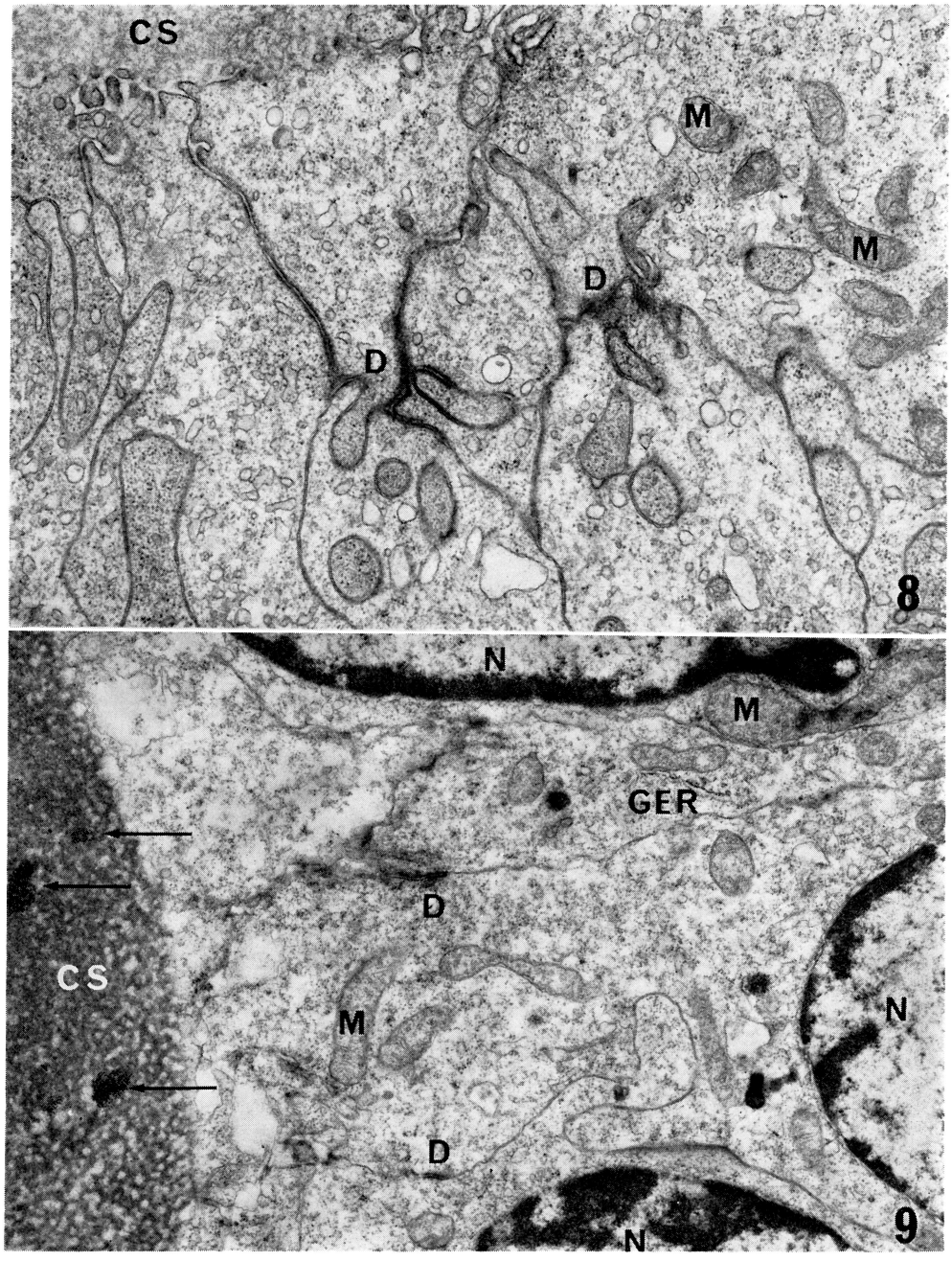

Fig. 8 Electronmicrograph of the central part of an M organ. The cells are connected by desmosmes (D) and have some short microvilli on the free surface. The lateral cell membranes show interdigitations. CS, colloidal substances. M, mitochondria. ×18,300.

Fig. 9 Part of the central region of an M organ. Three groups of electron dense particles (arrows) are seen in the meshwork-like colloidal substances (CS). A poorly developed granular endoplasmic reticulum (GER) and some mitochondria (M) are visible in the cytoplasm. N, nuclei. D, desmosomes. ×9,800.

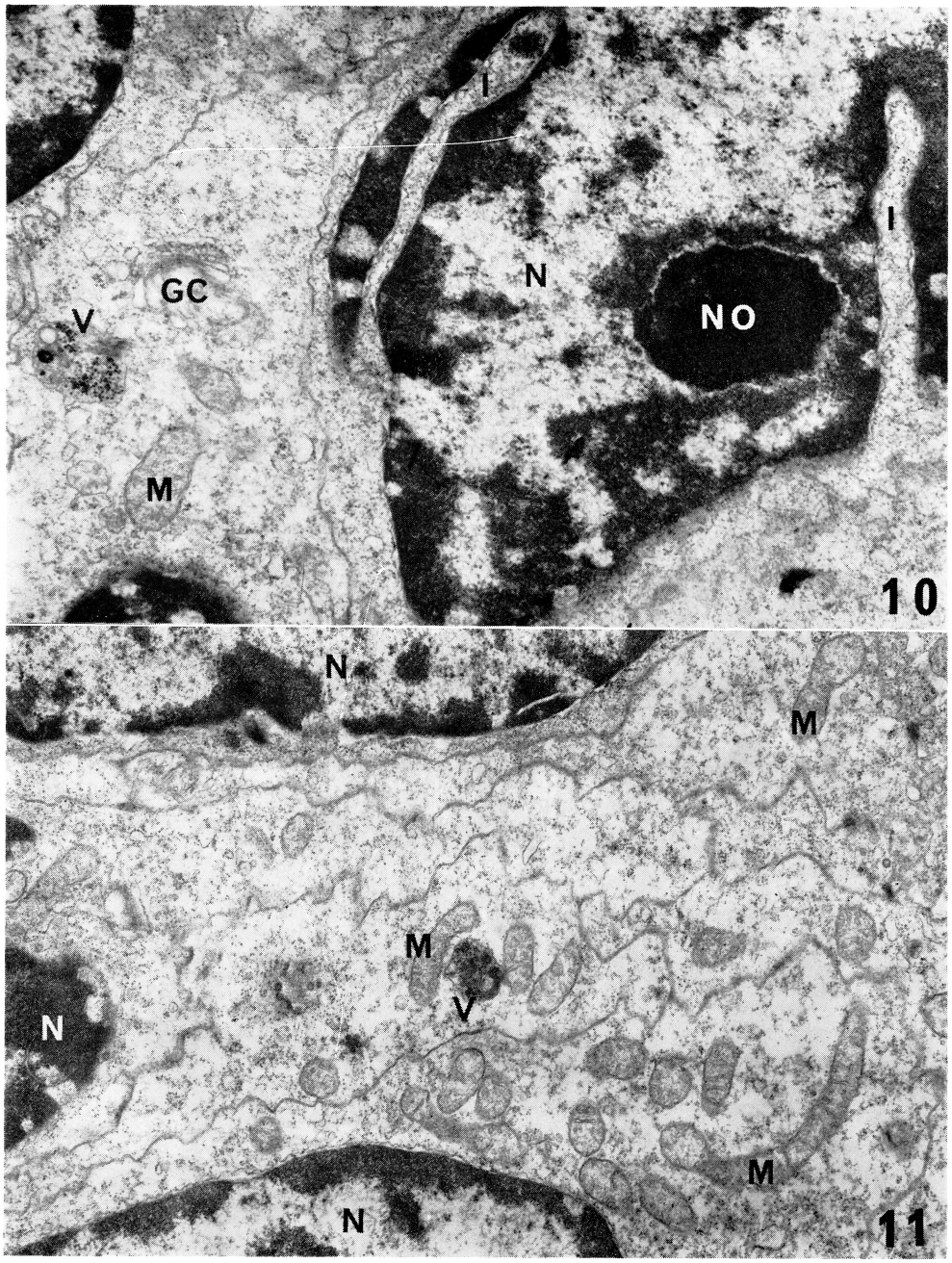

Fig. 10 Electronmicrograph of the cells of an M organ. The nucleus (N) has two cytoplasmic invaginations (I) and contains one electron dense nucleolus (NO). Most of the heterochromatin are associated with the nuclear membrane. A small vacuole (V) within the cytoplasm contains electron dense particles. GC, Golgi complex. M, mitochondrion. ×12,200.

Fig. 11 Parts of the cells of an M organ. Many mitochondria (M) varying in size and shape are seen within the cytoplasm. One small vacuole (V) contains electron dense particles and small amount of moderate electron dense substances. N, nuclei. ×10,100.

In general, it is said that the neurosecretory granules are stained with Gomori's chromehematoxylin and aldehyde fuchsin. In the present materials, a few granules within the cytoplasm of some slender cells were stained with the both stains. A colloidal mass in the lumen was also stained intensely with chromehematoxylin and aldehyde fuchsin. Electron microscopic observations reveal that the granules within the cytoplasm are small vacuoles which contain some colloidal substances and some electron dense particles, and that the colloidal mass in the lumen is composed of abundant colloidal substances of moderate electron density and some groups of electron dense particles. There is a good similarity between the contents of the lumen and that of the small vacuole. On the basis of the observations it seems that the colloidal mass might be secretory products of the slender cells of this organ.

However, the cell organelles of each slender cell are not developed well enough to produce secretory products actively. If the colloidal mass was a secretory product of the slender cell, it can be said that the cells is in resting or ending state of production of secretory materials. Our techniques could not reveal such evidences as how to form the storage of abundant colloidal substances in the lumen.

On the marginal parts, the cells formed thin cytoplasmic protrusions. Although these protrusions run into the nervous tissue space near the organ, they did not contain microtubules in their own cytoplasm such as in nerve fibers. Synapses were not observed on the cell membrane of each slender cell. As for stainability of cytoplasm and shape of nuclei, the slender cells were clearly different from the surrounding nerve cells. The stainability and size of nuclei of the cells mostly resembled to that of neuroglia cells in the eyestalk ganglia. These results indicate that the slender cells were not nerve cells but neuroglia cells.

Further studies on the M organ under the variant physiological conditions of the animals (molting, reproduction etc.) are necessary to determine the role of this organ.

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