Electron Microscopic Studies on Phagocyte.
Observations on Macrophage in Traumatic Necrotic Tissue in Cerebrum of Rat. Report 1

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The macrophages in the various tissues, except for the nervous tissue, are originated from the mesenchyme.

In the nervous tissue, since there are glial cells, the origin of macrophages is complicated. Since DEL RIO-HORTEGA's report, the authors described that the macrophages are originated from the microglial cell and mesenchymal cell in light microscopically. Moreover, there are reports that the microglial cell is originated from the mesenchyme.

In recent studies, the gliogenic and mesenchymal macrophages are reported electron microscopically.[1] Namely SCHULTZ and co-workers,[2] GONATAS and co-workers,[3] and COLONNIER[4] suggested that all types of neuroglial cells seem to be involved in phagocytic activity. Thus, the cerebral macrophages are regarded to be plural origins.

In experimentally induced traumatic necrosis of cerebral tissue, we observed electron microsopically the macrophages which are named granular cells or gitter cells. As the result, the macrophages are divided into two types according to the difference of density of the cells. These are provisionary named "dark macrophage" and "clear macrophage".

Present report is described with the dark macrophage which is originated from the microglial cell.

MATERIALS AND METHODS

Adult albino rats, about 250 gm. in weight, were examined. Anesthesia was performed with ether by inhalation. A longitudinal incision was made in the scalp. A hole was drilled in the skull about 3 mm. anterior to the bregma and about 2 mm. to the right of the midline, and the length of about 3 mm. of the lumpwick was introduced into the right frontal lobe.

The rats were killed at intervals of 1 to 10 days after implantation of the wick.

The specimens for electron microscopy were taken from the surrounding tis-

sue of the implantated wick. The blocks of fresh tissue (1 mm^3) were fixed in cold 1 % OsO_4 buffered with CAULFIELD's method.[5] The blocks were immersed about 1 hour. After dehydration in alcohol, the specimens were embedded in EPON 812 according to the method of LUFT.[6] The blocks were cut with glass knifes on the PORTER-BLUM ultra-microtome, and the sections were stained with uranyl acetate. JAPAN ELECTRON OPTICS LABORATORY JEM-5HS electron microscope was used.

OBSERVATIONS

The following observations were made in the animals killed 24 hours to 3 days after the implantation of the wick.

Resting and preactivating form of the microglial cells.

These cells were observed in brain tissue which was apart from implantated wick.

The microglial nuclei are ovoid or irregularly shaped, and large in comparison with the size of the cells, and have a uniformly dense chromatin. Occasionally nuceoli can be distinguished by their great density (Fig. 1 and 3). Their cytoplasm is scant, quite dense to such a degree that the density corresponds closely with nuclei. Thus, it often becomes rather difficult to distinguish the nuclear membrane and delimit the cytoplasm from the nucleus (Fig. 1). In the high power view, the cytoplasm of the microglial cells is stuffed with dense particulate material (Fig. 2). These are randomly scattered in hyaloplasm or attached to rough surfaced endoplasmic reticulum. The mitochondria are few and seem to be small. The Golgi complex is located near the nucleus. Osminophilic inclusion, presumably may be phagocytized debris, are sometimes contained in cytoplasm (Fig. 1 and 3). The microglial cells are closely surrounded on all sides by neural and other glial cytoplasmic processes.

Activated form of microglial cell.

The cytoplasm of activated microglial cells has always various forms of pseudopods prosesses. The cytoplasm is electron-dense as a result of the dense particulate material and dense hyaloplasm, but the density is slightly decreased comparing to that of the resting form of microglial cell. The activated microglial cells are characterized by the presence of phagocytized material scattered throughout the cytoplasm. These materials are enclosed with vacuoles lined by single cytoplsmic membrane, but sometimes are in direct continuity with cytoplasm, without, the intervened limiting membrane.

The majority of phagocytized material have a lamellar pattern with a 130 Å periodicity and distinct interperiod lines similar to myelinated axon. The other materials are consisted of the lipid and cell debris. In the activated microglial cells, the Golgi complex is located just distal to the nucleus and it occupies a

large portion of the cytoplasm. The organization of the Golgi complex of these cells is basically similar to that of the resting form of microglial cells. All three components of the Golgi complex are more developed, and evidence of the dilatation of vacuoles is frequently found (Fig. 8, 9, 10 and 11). There are no phagocytized material within the Golgi area (Fig. 12).

The mitochondria are not numerous and seem to be as small as the resting form.

Since the cytoplasm is stuffed with the phagocytized material, the nucleus is frequently elongated and is located in an eccentric position of the cell (Fig. 4, 5, 7 and 8).

DISCUSSION

The identification of microglial cell has been based on the electron microscopical descriptions reported by LUSE[7], SCHULTZ and co-workers[2], FARQUHAR and HARTMANN[8], and BLINZINGER and HAGER[9].

The microglial cells are characterized extremely by the dense nucleus and cytoplasm, and could readily be identified from the other glial cells. The great density of cytoplasm results from numerous dense particulate materials and dense hyaloplasm. SCHULTZ and others reported that these particulate materials are not attached to the rough surfaced endoplasmic reticulum and randomly scattered without clustering or rosette formation, it is doubtful that these are typical RNA granules.

On the contrary, we observed that these particulate materials are sometimes attached to the endoplasmic reticulum, but these materials are denser and slightly larger than RNA granules of the various cells. On account of the above findings, it is difficult to identify these materials with RNA granules. We anticipate that future studies on cytochemical examination of RNA will help to clarify these particulate materials.

In present study, the macrophages are classified into two types from the difference of density, and these are designated "dark macrophage" and "clear macrophage" respectively.

Although the dark macrophages have phagocytized material and newly produced pseudopods, their fine structural organization are corresponded almost to the resting form of microglial cells.

FARQUHAR and HARTMANN described that the nature of microglial processes were known to vary considerably with phagocytic activity and so nuclear characteristics were found to be of more value in the identification of microglia. SCHULTZ and others had reported that the microglial cell is capable of tremendous enlargement, and becoming bigger it loses its extreme cytoplasmic and nuclear density, so that the cell changes its morphological character quite enor-

mously. BLINZINGER and HAGER also described that in a severely damaged or completely softened tissue, microglial cells showed wards a tendency to a more rounded shape of their cytoplasm body and towards retractions of their processes. FARQUHAR and HARTMANN had reported that the microglial cells are known to be extremely labile, for their forms and contents may vary considerably with the amount of phagocytic activity in which they are engaged.

Therefore, the dark macrophages are considered to be the activated form of microglial cells. By BLINZINGER and HAGER's repors, the resting form of microglial cells are coincided largely, in regard to their cytoplasmic and caryoplasmic fine structure, with the resting mesenchyma cells. The fine structure organization of activated microglial cells corresponds widely to that of histiocytes or macrophages of mesenchymal origin. FARQUHAR and HARTMANN suggested that the microglia was derived from the mesenchyme and was the counterpart of the macrophages in other parts of the body.

In our observation,[10, 11] however, the fine structure of microglial cells differed from the macrophages of the other organs. Namely, the microglial cells are stuffed with numerous dense particulate materials, but the macrophages of the other organs does not contain these particulates, and its cytoplasm is clearer than that of the microglial cell. The Golgi complex of activated microglial cells is considerably developed, but on the other hand, the macrophages have scanty the Golgi complex. Therefore, it is doubtful that the microglial cells are originated from the mesenchyme. We suspected that clear macrophages are derived from the mesenchyme, and detailed observation will be described in the following reports.

SUMMARY

We observed two types of the macrophages which are named "dark macrophage" and "clear macrophage".

The dark macrophage is identified the activated form of microglial cell.

The activated form of microglial cell is distinguished from mesenchymal macrophage.

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EXPLANATION OF FIGURES

Fig. 1. Resting form of microglial cell.
Its extreme overall density is striking. Because of this, it is difficult to delimit the nucleus (n) from the cytoplasm. Neuropil containing glial processes and myelinated axons surrounding the cell. There is a osmiophilic body (↑) in cytoplasm.

Fig. 2. Resting form of microglial cell.
A portion of microglial cytoplasm has numerous dense particulate materials. Because of this, it is impossible to recognize the cell organelles in this photograph. The blood capillary (cap) are present at the lower right corner. The cell is closely surrounded on all sides by the astrocytic processes and neuropils.

Fig. 3. Preactivating form of microglial cell.
The cell is phagocytizing a myelinated axon at the lower field. The cell has relatively large nucleus (n), and small quantity of cytoplasm. There is a laminated osmiophilis body (↑), in cytoplasm.

Fig. 4. Activated form of microglial cell.
The cell has various form of cytoplasmic processes. The nucleus (n) occupies an eccentric position in the cell but it is difficult to delimit the nucleus from cytoplasm. The degenerated myelins, lipid materials (L) and fine granular dense body (↑) are stuffed in cytoplasm.

Fig. 5. Activated form of microglial cell.
The nucleus occupies an eccentric position in the cell. The cell membrane forms many pseudopod processes. The cytoplasm contains small mitochondria (m), numerous dense particulate materials and phagocytized materials.

Fig. 6. Activated form of microglial cell.
There are numerous dense particulate materials which are randomly scattered or attached to rough surfaced endoplasmic reticulum (er). The cell phagocytized the lipid materials (L) and myelin sheaths at different stages of degeneration.

Fig. 7. Activated form of microglial cell.
The cell has elongated nucleus (n), many lipid materials (L) and myelin debris. The dense particulate materials are scattered throughout the cytoplasm.

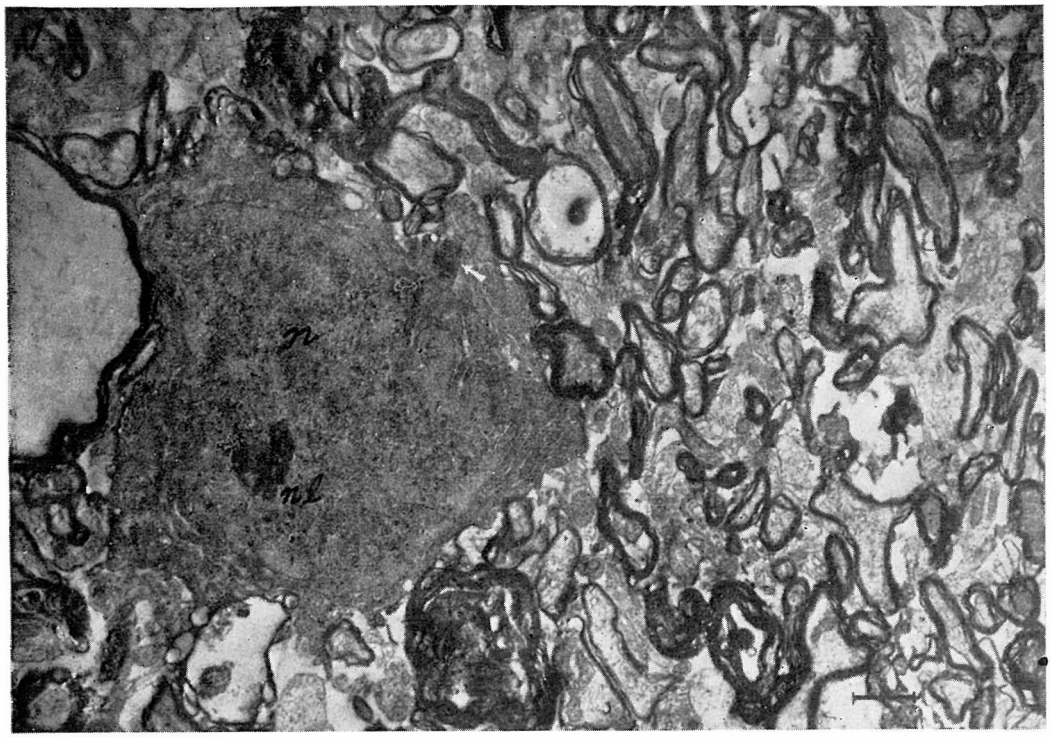
Fig. 8. Activated form microglial cell.
Well-developed Golgi complex (G) can be seen near the nucleus (n). The cytoplasm are stuffed with the lipid materials (L), degenerated myelins and cell debris.

Fig. 9. Activated form of microglial cell.
There is the Golgi complex near the nucleus (n). It has well-developed Golgi lamellae. The phagocytized materials are stored in cytoplasm, but not in the Golgi area. There are no dense particulate materials in the Golgi area.

Fig. 10. Activated form of microglial cell.
There is the Golgi complex surrounded the small mitochondria (m), phagocytized materials and nucleus (n). The Golgi complex (G) consist of well-developed lamellae and vacuoles, and moderately developed vesicles. There are many dense particulate materials in cytoplasm except the Golgi area.

Fig. 11. Activated form of microglial cell.
There is the Golgi complex surrounded with the nucleus (n), the other organellae and phagocytized materials.

Fig. 12. This photograph is high magnification of Fig. 11.
The Golgi complex consists of well-developed lamellae, vacuoles and vesicles. The dense particulate materials and phagocytized materials are not seen in the Golgi area.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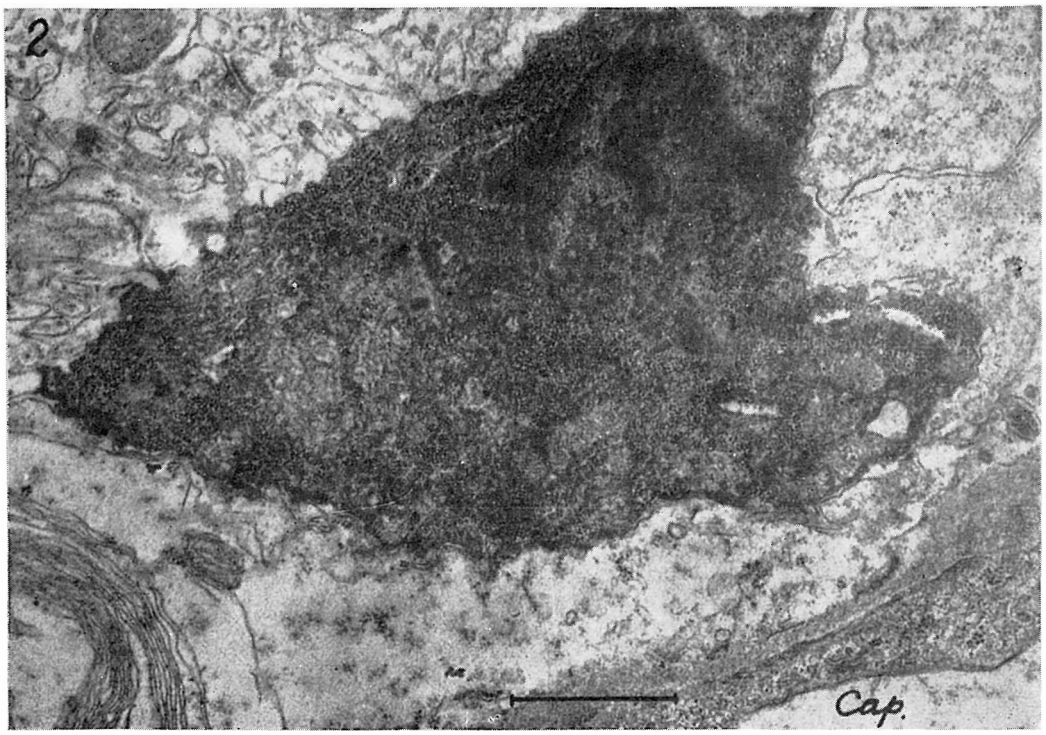
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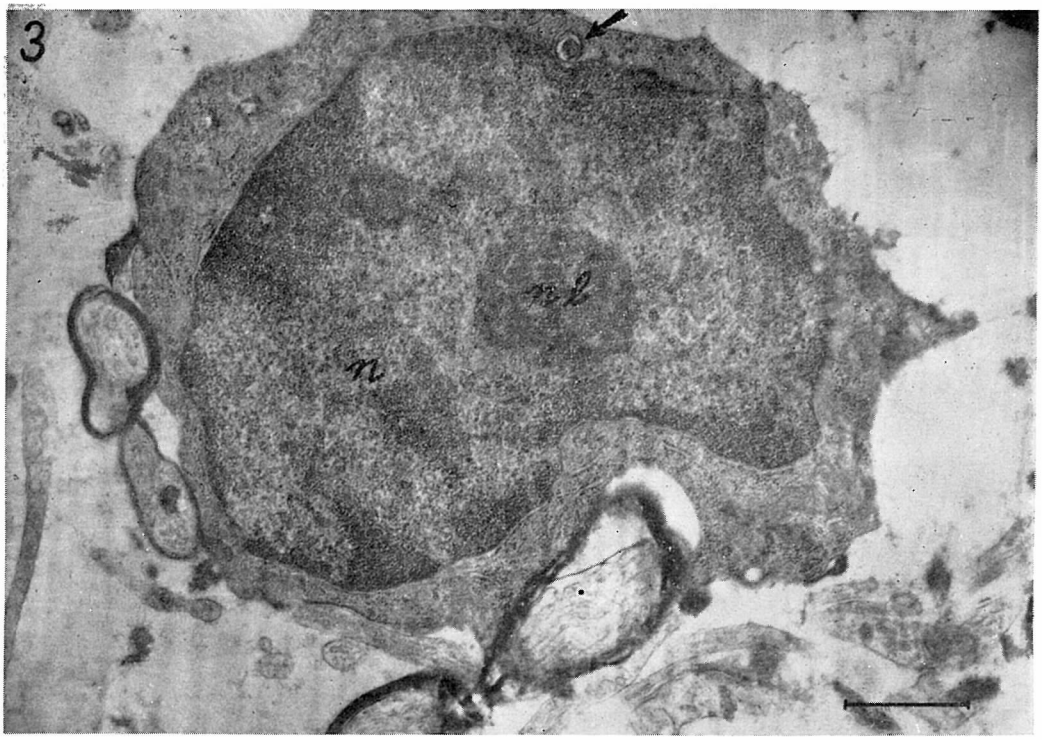
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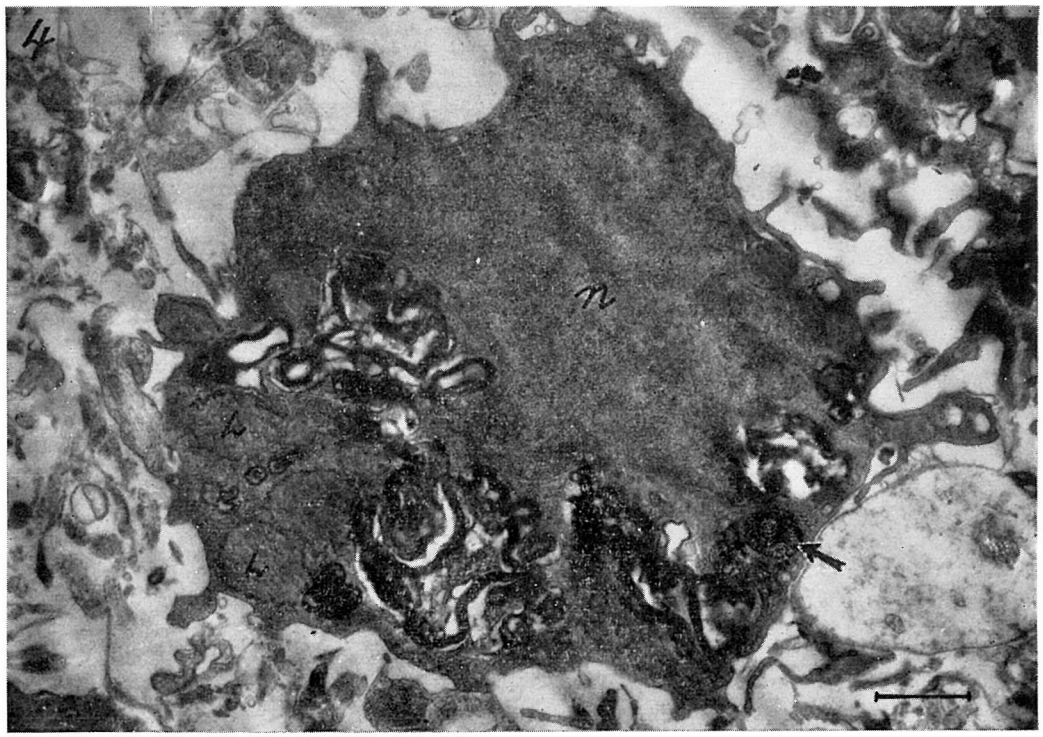
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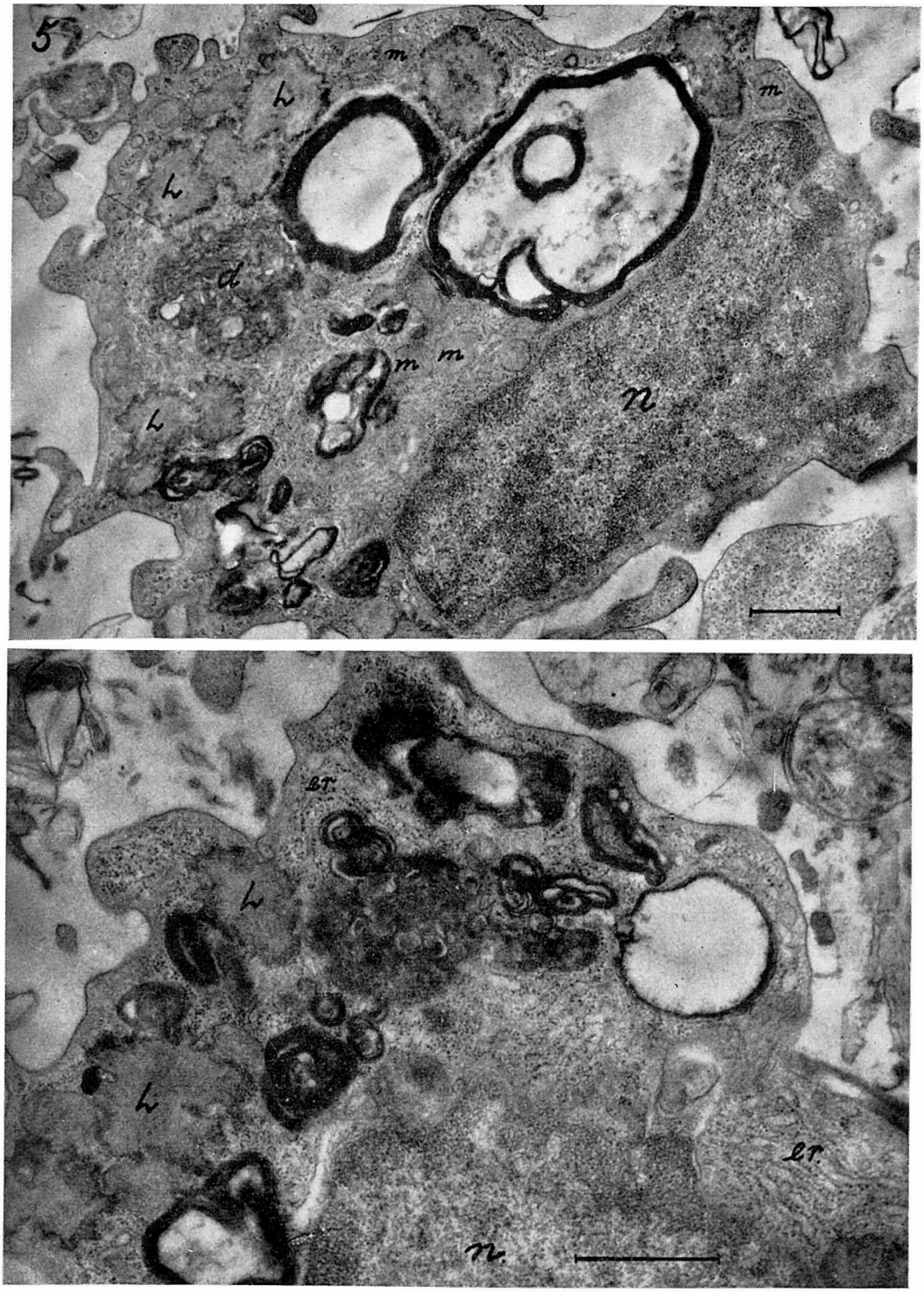
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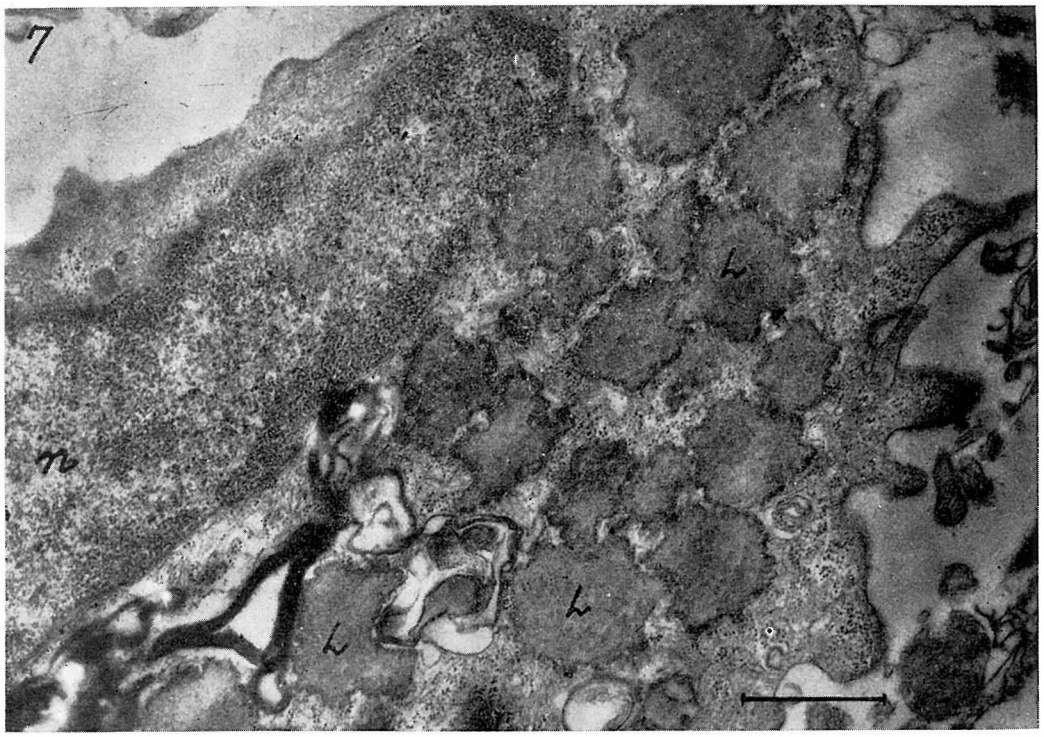
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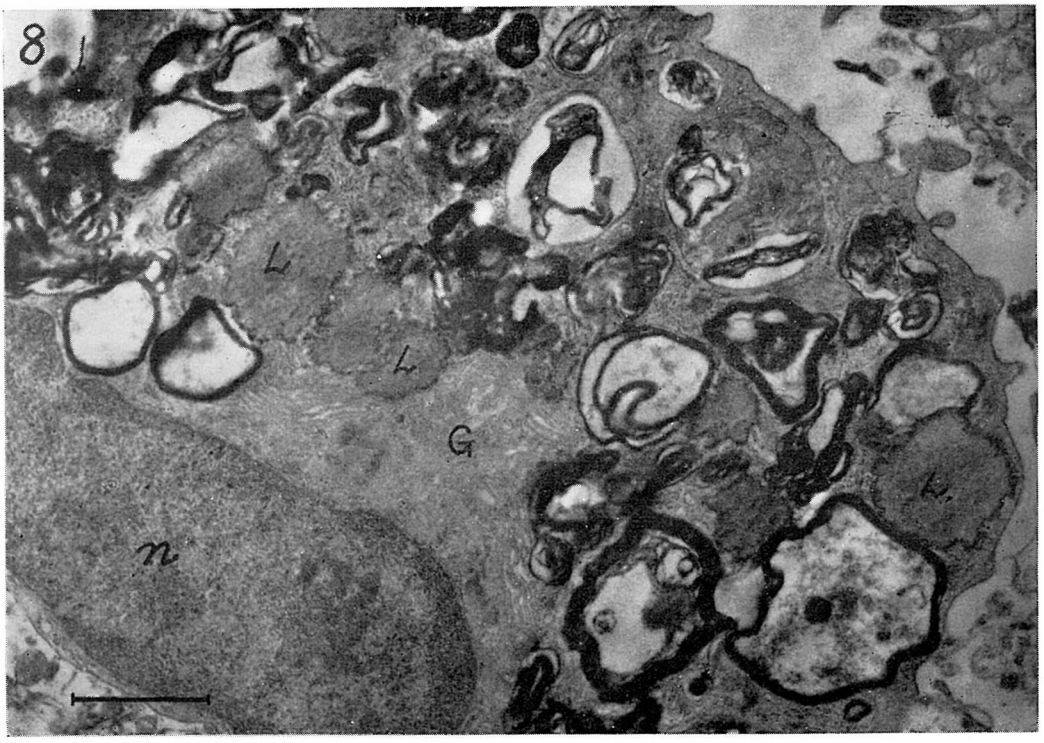
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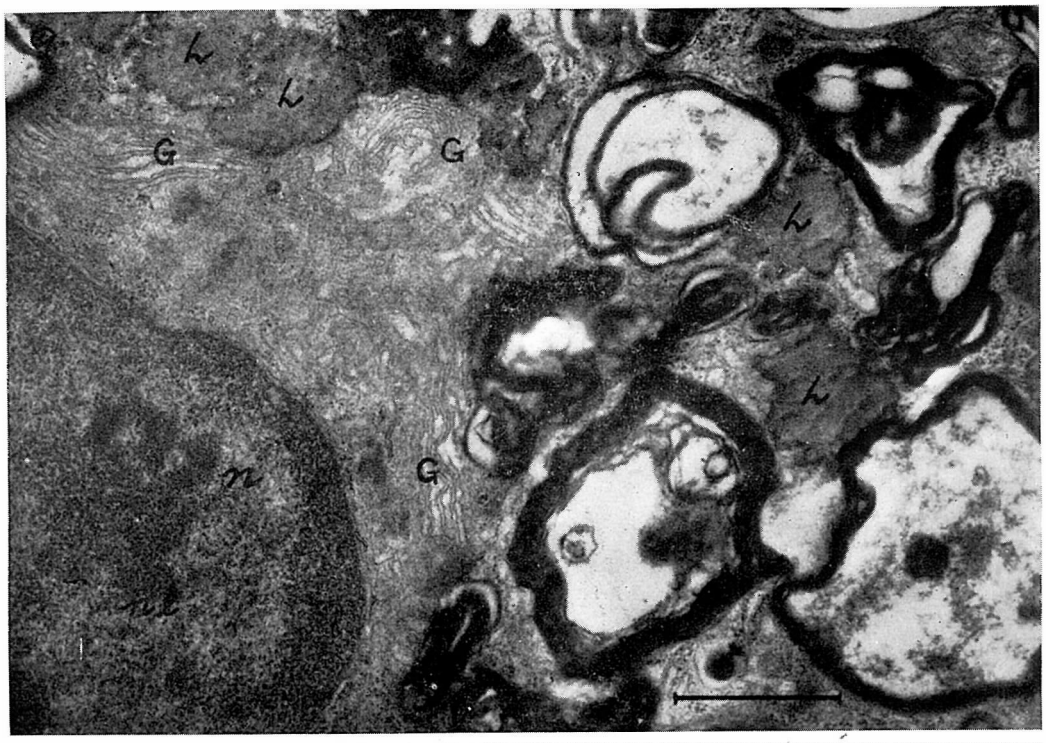
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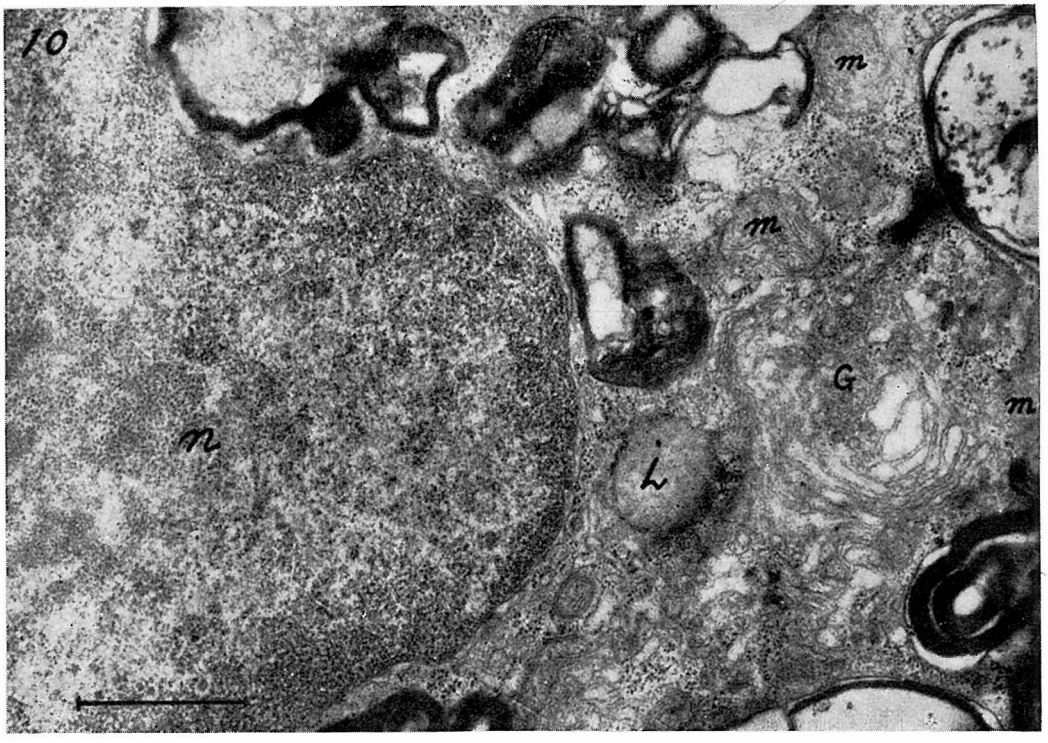
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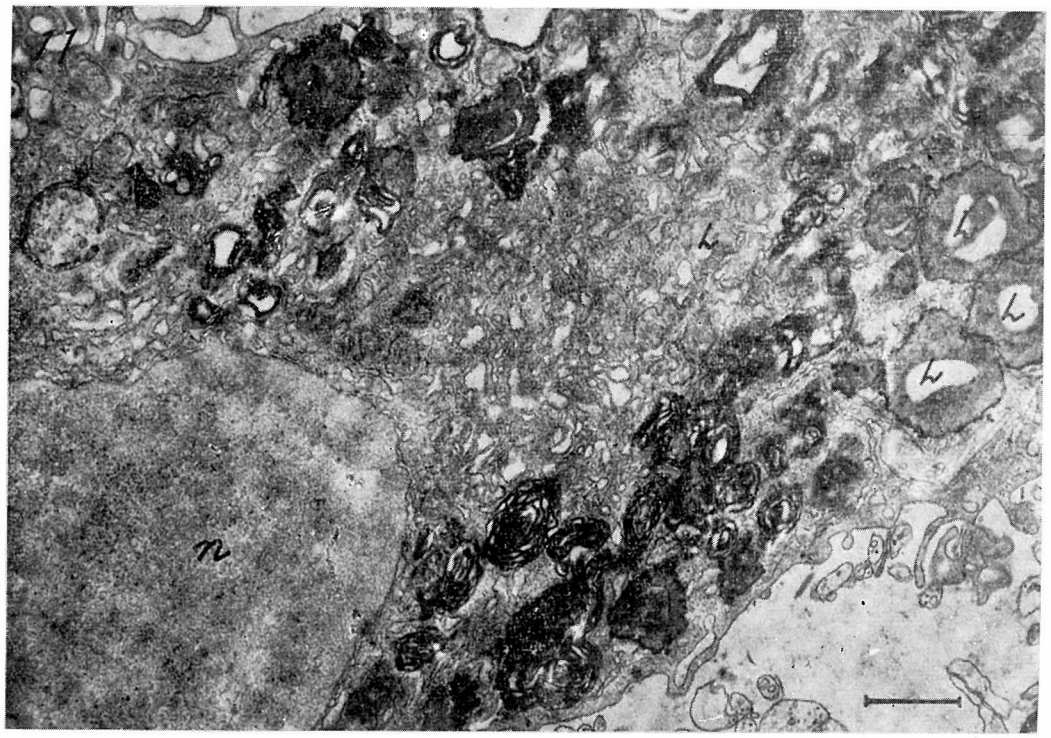
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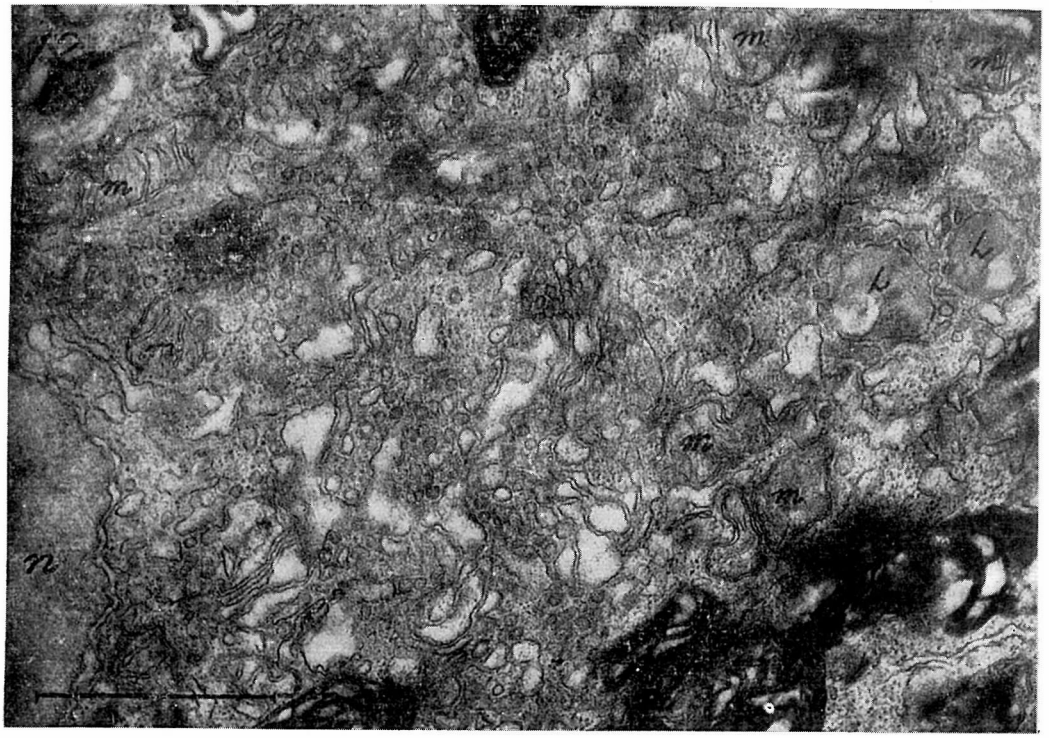
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