MYELINIC FIGURES IN LEPRA CELLS EXAMINED BY PHASE-CONTRAST AND ELECTRON MICROSCOPY[1]

M. Nishiura, M.D., J. Ogawa, M.D., F. Kanetsuna, M.D., and E. Toda, M.D., with the technical assistance of N. Isowa

Leprosy Research Laboratory
Kyoto University School of Medicine
Kyoto, Japan

Myelinic figures (onion-like bodies) are frequently observed in necrobiotic cells of the tuberculoid nerve lesions of leprosy ([11]). In 1961, Imaeda *et al.* ([6]) reported the presence of convoluting structures resembling onion-like bodies in a case of lepromatous leprosy accompanied by xanthoma tuberosum. Usually, however, these onion-like bodies are absent in ordinary lepromatous lesions of the skin and peripheral nerves.

In a case of lepromatous leprosy that we present here, we found myelinic figures in lepra cells of typical lepromas that have shown no clinical picture of xanthoma. Although myelinic figure formation is a very rare occurrence in lepromatous lesions, it seemed interesting that this process can take place even in lepra cells when some degenerative factors affect them.

MATERIALS AND METHODS

Case Report.—The case reported is that of a female patient, 52 years old, with nodular lepromatous lesions on her face, upper- and lower-extremities, breasts and shoulders. Lepromatous macules were observed also on her back. The lepromin reaction was negative. All smears from several lepromas showed numbers of globi.

Phase-contrast microscopy: A small chip of leproma was suspended in a drop of physiologic saline on a microslide, and covered immediately with a coverslip. As the lepromas were very soft, slight pressure on the coverslip was enough to reduce the chip to a thin monolayer of lepra cells, in which the cytoplasmic details of lepra cells could be observed clearly with a phase-contrast microscope. As all lepra cells were alive during the microscopic observation, they appeared free from artifact except for blister formation of some lepra cells due to the saline used.

Electron microscopy: In order to demonstrate membranous structures clearly, the specimens were fixed in $KMnO_4$ for three hours. As methacrylate embedding is not suitable for observation of myelinic figures because of "explosion artifacts" ([3]), the material was embedded in Epon according to Uchida's method ([15]), (a modification of Luft's method ([8])). The embedding procedure was as follows:

1. Fix the specimens in 1% $KMnO_4$ without buffer for 3 hours;
2. Dehydrate them in graded series of ethanol (50%, 75% and 95%, 10 minutes in each grade);
3. Pass them through two changes of absolute ethanol (15 minutes in each);
4. Pass them through two changes of propylene oxide (10 minutes in each);
5. Immerse them in a 1:2 mixture of propylene oxide and polymerizing resin mixture for 3 hours;

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6. Place them in capsules in a polymerizing mixture composed of Epon 815 (5 parts), Epon 812 (5 parts), DDSA (dodecenyl succinic anyhydride, 16 parts) and DMP-30 (2,4,6-tri (dimethylaminomethyl) phenol, 0.45 parts), and heat for 20 hours at 37°C and then 10 hours at 60°C.[2]

FINDINGS OF PHASE-CONTRAST AND ELECTRON MICROSCOPY

Phase-contrast microscopy revealed myelinic figures in this case, but the number was small as compared with those seen in tuberculoid nerve lesions ([11]). Figure 1 shows a myelinic figure resembling a myelin sheath as observed in the cytoplasm of a lepra cell by phase-contrast microscopy.

Electron microscopy of the specimen revealed varieties of myelinic figures. Some of these are shown in the accompanying illustrations.

Embedding in Epon is quite suitable for preservation of the lamellar structure of myelinic figures. The "explosion artifact" of myelinic structure, as commonly observed in methacrylate-embedded material, was never seen in Epon embedding. Because of their striking resemblance to phase-contrast images, the myelinic figures as observed in Epon-embedded specimens seemed more natural than the onion-like figures in methacrylate-embedded specimens.

There appear to be numerous ways in which myelinic figures are formed in the cytoplasm of degenerating cells. In some of the myelinic figures lamellar structure seemed to have been derived from endoplasmic reticulum, and in others from a Golgi complex or by paracrystallization of phospholipids in microbodies.

DISCUSSION

Myelinic figure formation is a common occurrence in degenerative process of cell cytoplasm caused by various agents. It is observed as an early feature of cytoplasmic degeneration in a number of processes,

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[2]"Epon" is the commercial name of an epoxy resin sold by the Shell Chemical Corporation. When it is baked at 60°C with DDSA a hardener, and DMP-30, an accelerator, a hard, cured resin is formed. As Epon 815 is harder than Epon 812 when they are cured, the hardness of the final plastic blocks can be controlled by changing the proportions of Epon 815 and Epon 812. Because of the low shrinkage of Epon upon cure, it is particularly suitable for embedding the lamellar ultrastructure of myelinic figures. The only disadvantage of epoxy resin in the electron microscopic study of leprosy is its poor penetration into leprosy bacilli located in the intracytoplasmic foamy structure of lepra cells. For this reason Epon embedding is more suitable for studies of tuberculoid lesions and nerve lesions of leprosy than for study of the leprosy bacillus.

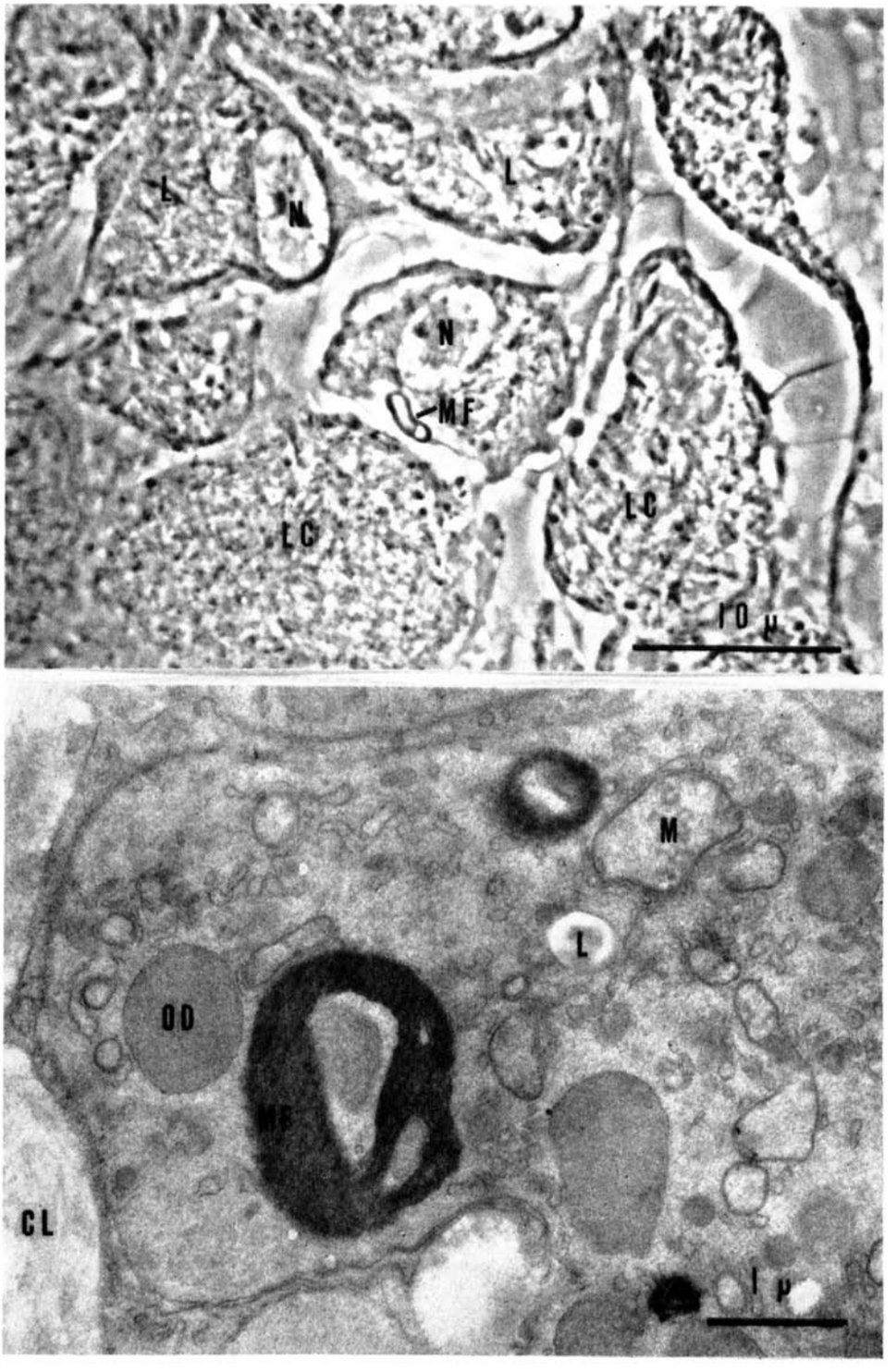
DESCRIPTION OF PLATE

FIG. 1. Fresh lepra cells suspended in saline and observed with the phase-contrast microscope. A myelinic figure is observed in a lepra cell. Magnification: 3,000×.

Symbols: **L,** leprosy bacilli; **LC,** lepra cell; **MF,** myelinic figure; **N,** nucleus.

FIG. 2. Two myelinic figures can be seen in the cytoplasm of a lepra cell. They can be differentiated easily from myelin sheath by the absence of mesaxon. Because of the fixation in $KMnO_4$, a leprosy bacillus shows very weak electron density. Magnification; 20,000×.

Symbols: **CL,** collagen fibrils; **L,** leprosy bacillus; **OD,** opaque droplet; **M,** mitochondria; **MF,** myelinic figure.


L
N
L
N
MF
LC
LC
10 μ
M
L
OD
MF
CL
1 μ

including bacterial infections (tuberculosis ([2]), leprosy ([11]), viral infections (adenovïrus type 3 ([1]), ECHO virus ([12]), lipophanerosis caused by colloidal silicone ([13]), pancreatic degeneration due to dl-ethionine ([5]), the effect of dimycin on otocysts ([4]), and vital staining with neutral red ([14]).

Because of their morphologic similarity to myelin sheath material, the predominant chemical component of these myelinic figures seems to be phospholipid. Mercer ([10]) obtained myelinic figures by allowing small lumps of phospholipid to imbibe water. According to his report myelinic figures appear to be paracrystals of the hydrated phospholipid, possibly representing the least active form, or perhaps a storage form of the lipid.

The role of serum in the formation of lamellar bodies is interesting, but tissue culture experiments by several investigators have given divergent results in this respect. Menefee and Evans ([9]) have reported that in tissue culture of human epithelial cells, lamellar bodies were noted in cells growing in media containing protein, but never in a protein-free medium. In contrast, Kojima and Kozuka ([7]) observed that lamellar bodies were abundant in HeLa cells grown in serum-free medium, but were not found in normal HeLa cells grown in standard medium.

Myelinic figures are derived frequently from mitochondria ([7,9]), but our findings show that they can be derived from many other cytoplasmic organelles.

Although the possibility of myelinic figure formation in lepra cells was confirmed in this case, this process should not be considered as a common ultrastructural feature of lepromatous lesions.

Why such myelinic figures occurred in lepra cells in this case is obscure. The patient had been taking large doses of bromvaleryl urea for several years because of insomnia, but we are not sure if this practice had any bearing on the myelin figure formation observed in lepra cells.

SUMMARY

Myelinic figures found in lepra cells of a lepromatous case were examined by phase-contrast and electron microscopy. They appeared to have been derived from various cytoplasmic organelles, such as mitochondria, Golgi complexes, endoplasmic reticulum and microbodies.

Although it was confirmed that myelinic figures can be found even in lepra cells, it is not a common occurrence in lepromatous leprosy.

RESUMEN

Fueron examinadas con el microscopio de contraste de fase y electrónico las figuras mielínicas encontradas en células leprosas. Ellas parecen haber derivado de varias organelas citoplasmáticas, tales como mitocondrias, complejo de Golgi, retículo endoplásmico y microcuerpos (microbodies).

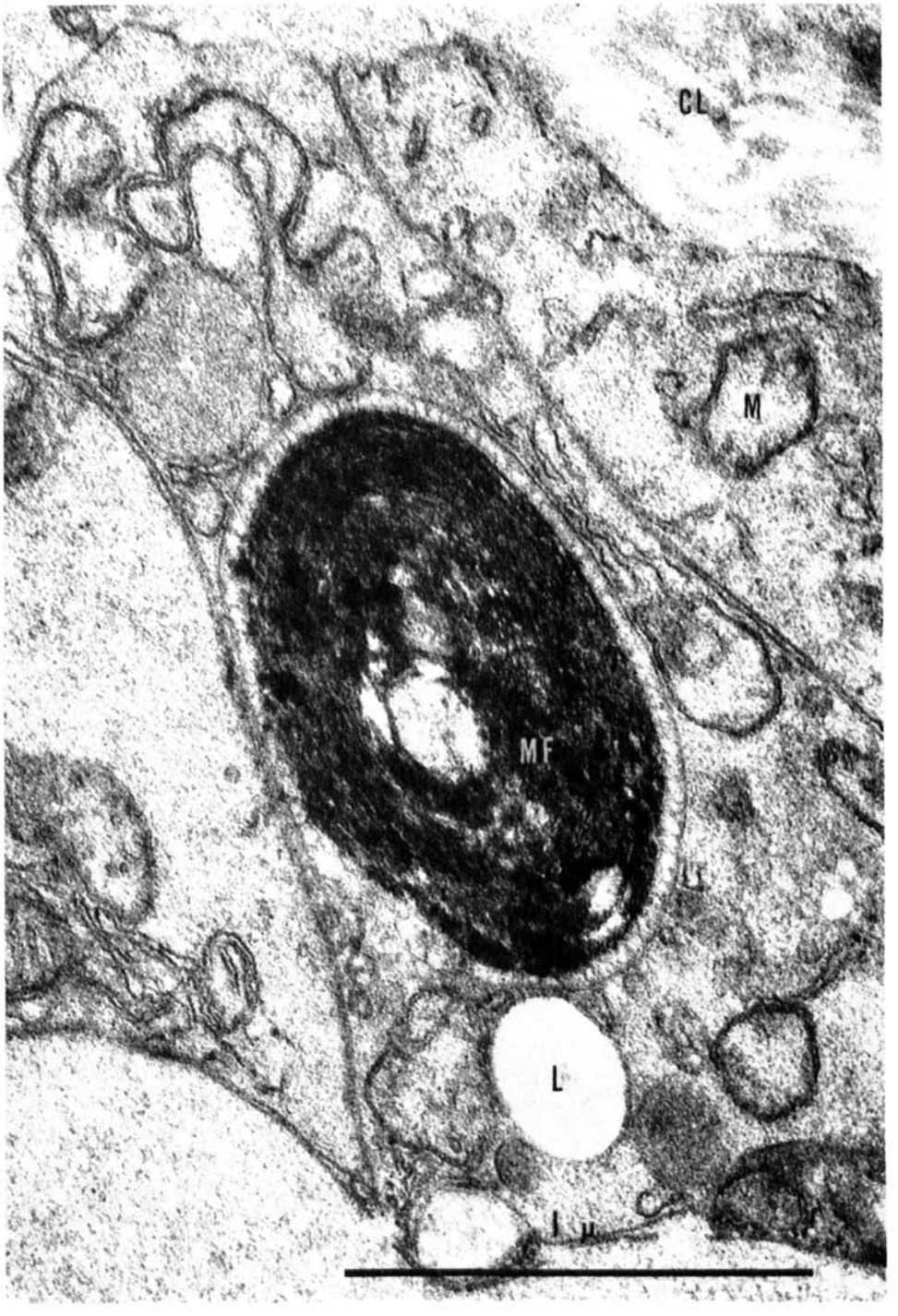


FIG. 3. A myelinic figure in a lepra cell. A peculiar space can be seen between the myelinic figure and the cytoplasm of the lepra cell. Many connecting bridges are observed in this space. The myelinic figure is composed of concentric lamellae. Magnification: 67,000×.
Symbols: **CL**, collagen fibrils; **L**, leprosy bacillus; **M**, mitochondria; **MF**, myelinic figure.

Aunque se ha confirmado que las figuras mielínicas pueden ser encontradas aún en células leprosas, no es frecuente en la lepra lepromatosa.

RESUMÉ

Des images myéliniques observées dans les cellules lépreuses d'un cas lépromateux ont été examinées par la microscopie à contraste de phase et au microscope électronique. Elles apparaisent comme provenant de diverses parties du cytoplasme, telles que mitochondries, appareils de Golgi, réseau endoplasmique et autres particules de petites dimensions.

Quoiqu'il soit ainsi confirmé que des images myéliniques peuvent survenir même dans des cellules lépreuses, il ne s'agit pas là d'un phénomène fréquent dans la lèpre lépromateuse.

REFERENCES

1. Andres, K. H. and Nielsen, G. Beobachtungen an Adenovirus (Typ 3) -infizierten HeLa-Zellkulturen nach Fixierung mit Kaliumpermanganat. Fourth Internat. Conf. Electron Microscopy, Berlin. Berlin: Springer-Verlag, 1960, 602-605.
2. Cedergren, B. The lung tissue in mice infected by tubercle bacilli. Electron-microcopy. Proc. Stockholm Conf., September 1956. Stockholm: Almqvist & Wilksell, 248-249.
3. Condie, R. M., Howell, A. E., and Good, R. A. Studies on the problem of preservation of myelin sheath ultrastructure; evaluation of fixation, dehydration, and embedding techniques. J. Biophisic. & Biochem. Cytol. **9** (1961) 429-443.
4. Friedmann, I. Pleomorphic cytoplasmic inclusion bodies in. tissue cultures of the otocyst exposed to dimycin. Fourth Internat. Conf. Electron Microscopy, Berlin. Berlin: Springer-Verlag, 1960, 266-269.
5. Herman, L., Fitzgerald, P. J., Weiss, M., and Polevoy, I. S. Electron microscope observations of degenerating and regenerating pancreas following ethionine administration. Fourth Internat. Conf. Electron Microscopy, Berlin. Berlin: Springer-Verlag, 1960, 372-378.
6. Imaeda, T., Convit, J., Mendoza, S., and Arvelo, J. J. Electron microscope study of xanthoma cells in a lepromatous leprosy lesion. Internat. J. Leprosy **29** (1961) 343-354.
7. Kojima, K. and Kosuka, S. Structural changes in HeLa cells cultivated in serum-free medium. J. Cell Biol. **14** (1962) 141-144.
8. Luft, J. H. Improvements in epoxy resin embedding methods. J. Biophysic. & Biochem. Cytol. **9** (1961) 409-414.
9. Menefee, M. G., and Evans, V. J. Structural differences produced in mammalian cells by changes in their environment. J. Nat. Cancer Inst. **25** (1960) 1303-1324.
10. Mercer, E. H. Comparison of natural biological membranes with artificial models. Electron microscopy in anatomy. London: Edward Arnold Ltd., 1961, 100-113.

DESCRIPTION OF PLATE

Fig. 4. Varieties of myelinic figures found in lepromas of the case reported. All illustrations have the same magnification, i.e., 30,000×.

Symbols: **M,** mitochondria; **MF,** myelinic figure; **SMF,** a small myelinic figure formed by paracrystallization inside a microbody.

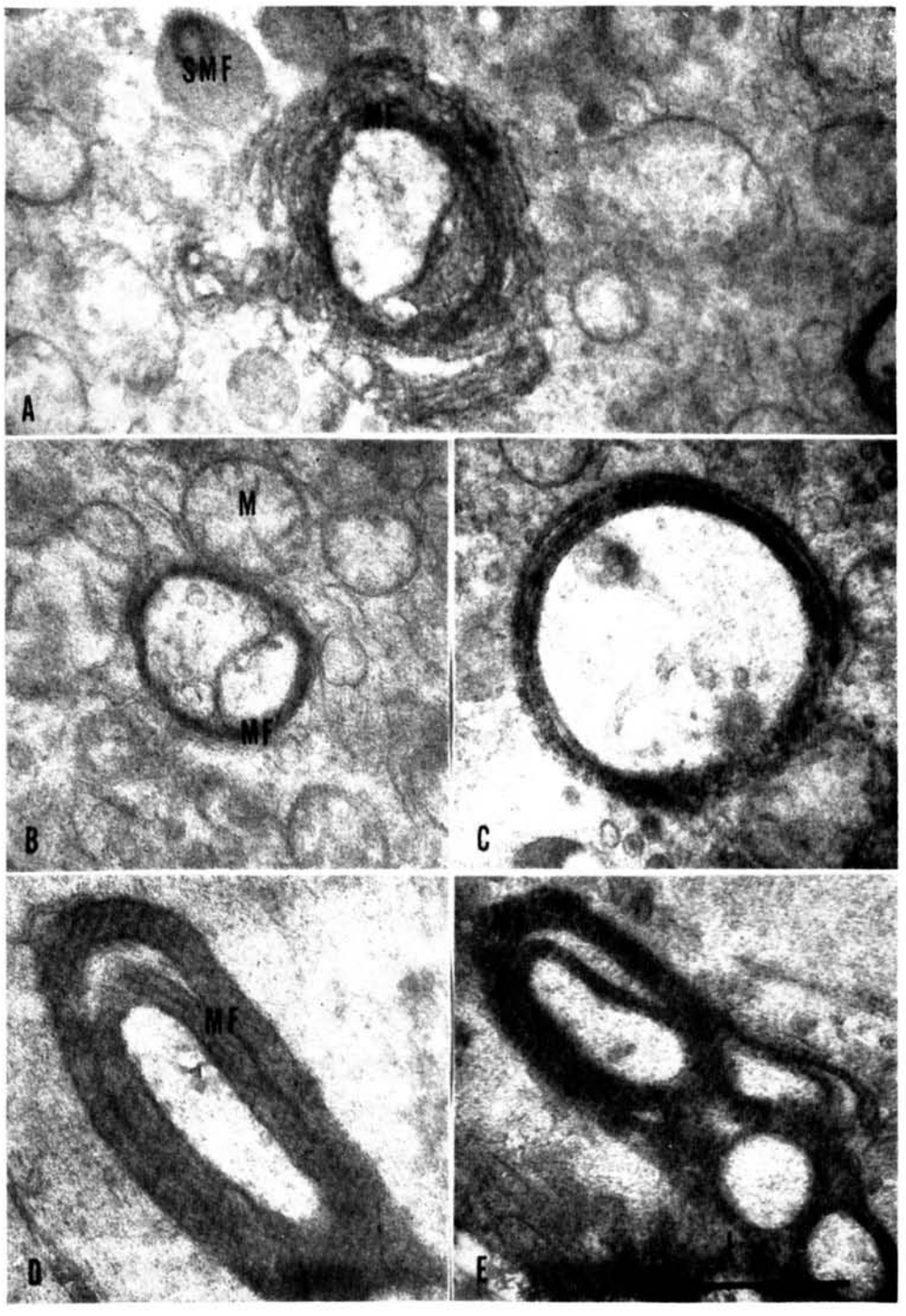
(A) An irregular-shaped myelinic figure, suggesting probable derivation from a Golgi complex. A small myelinic figure is observed also within a microbody formed apparently by paracrystallization of phospholipids.

(B) A very small myelinic figure found in the cytoplasm.

(C) A myelinic figure composed of concentrically arranged paired lamellae.

(D) A myelinic figure in which the lamellae do not show tight arrangement.

(E) A myelinic figure suggesting probable derivation from endoplasmic reticulum.


SMF
MF
A
M
MF
B
C
MF
D
E

11. Nishiura, M. The electron microscopic basis of the pathology of leprosy. Internat. J. Leprosy **28** (1960) 357-400.
12. Nunez-Montiel, O., Weibel, J., and Vitelli-Flores, J. Electron microscopic study of the cytopathology of ECHO virus infection in cultivated cells. J. Biophysic. & Biochem. Cytol. **11** (1961) 457-467.
13. Policard, A., Collet, A., and Pregermain, S. Étude au microscope électronique de la lipophanerose cytoplasmique. Fourth Internat. Conf. Electron Microscopy, Berlin. Berlin: Springer-Verlag, 1960, 258-261.
14. Tanaka, H. Segresome and the cellular uptake of varied substances as revealed in the electron microscope. Ann. Rept. Inst. Virus Res., Kyoto University, Japan. 1961, 118-152.
15. Uchida, Y. Electron microscopic study on Wallerian degeneration of the spinal cord after section. Arch. f. Japan. Chir. **31** (1962) 479-500.