A MICROTUBULAR COMPLEX IN THE EPIDERMAL NUCLEUS OF AN INSECT, *CARAUSIUS MOROSUS*

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INTRODUCTION

As is typically the case in insects, the cellular layer underlying the cuticle of the body surface in *Carausius morosus* (Br.) comprises both epidermal cells and enocytes (Wigglesworth, 1953). In this insect a cicadan rhythm of color change occurs, reported to be associated with the movement of pigment granules within the epidermal cells (Schleip, 1911; Giersberg, 1928; and Janda, 1936). In the course of reinvestigation of this problem at the electron microscopic level, a highly organized and well localized complex of microtubules was frequently observed within the nucleus of the epidermal cells. The chief function of these epidermal cells is the secretion of the general body cuticle which is replaced at each molt, whereas the enocytes are believed to be responsible, at least in the hemipteran *Rhodnius*, for production of the lipid or lipoprotein material incorporated into the epicuticle (Wigglesworth, 1953).

MATERIALS AND METHODS

Green adult females of the stick insect *Carausius morosus* were employed in this study. The material (cuticle and underlying epithelium) was fixed in ice cold 2.5 per cent glutaraldehyde in phosphate-buffered 0.15 M sucrose at pH 7.2, postfixed in 1 per cent osmium tetroxide at the same pH, dehydrated in ethanol, and embedded in Araldite. Sections were cut on a Huxley microtome, stained with a lead-tartrate complex (Millonig, 1961), and examined in a Philips EM 200.

OBSERVATIONS

In electron micrographs the epidermal cells of *Carausius* are very clearly distinguished from the enocytes accompanying them. Whereas the latter have abundant cytoplasm rich in tubular elements of the agranular reticulum and very large spherical nuclei, the epidermal cells comprise a central region containing a deeply lobate nucleus, and a greatly dissected cytoplasmic portion laden with pigment granules.

Sections of epidermal cell nuclei (Fig. 1) may include one or more dense nucleoli and, in many instances, profiles of microtubules in close association with nucleolar material. These microtubules form clusters of discretely localized circular or ovoid groups attaining an over-all length of *ca.* 0.5 μ. Within each cluster the constituent tubular elements may be sectioned in transverse, longitudinal, or oblique planes, or in combinations of these. In no instance has a limiting membrane been detected surrounding these structures.

Transverse sections of the complex (Fig. 3) in-

dicate that the individual tubules are *ca.* 180 to 200 A in diameter with a center-to-center spacing of *ca.* 270 to 290 A. Each tubule exhibits an electron-transparent core, *ca.* 70 to 90 A in diameter. The complex illustrated in Fig. 3 includes about 220 units. In Fig. 2 the lattice of strongly curved tubules is sectioned in a generally longitudinal manner, and again, in this plane of section, the core of the tubules is resolved. Both longitudinal and transverse tubule profiles are seen together in the micrograph shown in Fig. 4, while transverse and oblique profiles predominate in Figs. 5 and 6: in each of these instances, despite the variable plane of section, it is evident that the hexagonal organization of the complex is retained.

High-magnification micrographs of transversely sectioned tubules provide some evidence that they consist of small subunits, while longitudinal profiles suggest that the electron-opaque cortex of the tubules exhibits a repeating substructure with a periodicity of *ca.* 130 to 140 A (Fig. 7) which appears to be distinct from the apparent periodicity seen in oblique sections (Figs. 5 and 6) due to superimposition of successive rows of tubule profiles.

A striking feature of the organization of this intranuclear complex is its relatively uniform maximum size. The transverse section shown in Fig. 3 includes 16 to 17 tubules situated along each diametric traverse: an approximately similar number of tubule rows are present in the profiles shown in Figs. 2 and 5. Thus it is possible that this structure contains a characteristic number of constituent tubules. The profiles shown in Figs. 4 and 7 may represent either grazing sections of complexes similar in size to those described above, or alternatively, median transects of smaller complexes.

Despite this degree of uncertainty concerning the size of the complex, it appears that the various configurations illustrated here are consistent with a model of the composite body as a spheroidal or slightly tapering association of tubular units, preserving a basic hexagonal symmetry, whilst exhibiting marked distortion or curvature along the tubule axis.

DISCUSSION

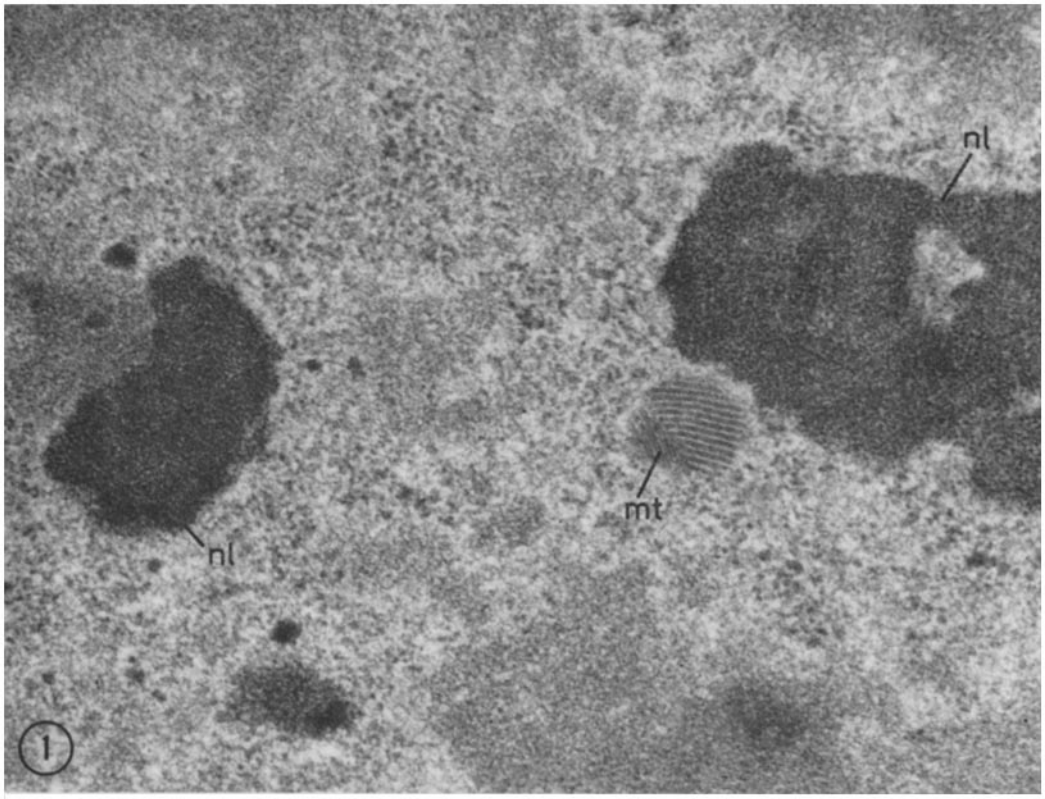
Recently, several authors (Slautterback, 1963; Ledbetter and Porter, 1963; de-Thé, 1964) have produced evidence that microtubules, morphologically resembling the oriented elements of cilia and flagella, are a well defined cytoplasmic component of a wide variety of animal and plant cells. The ubiquity of these structures is such that, at present, it is not possible to assign a common role to them.

In addition, the literature now contains numerous descriptions of intranuclear tubular systems, which are of more direct relevance to the present discussion. Of these, the spindle fibrils of dividing nuclei (Roth and Daniels, 1962) are the best known. Roth and Shigenaka (1964) describe morphologically similar tubules (150 A in diameter) in the dividing macro- and micronuclei of the ciliates *Diplodinium ecaudatum* and *Ophryosocolex caudatus*, and these authors were unable to find these structures in interphase nuclei. Vivier and André (1961) described a system of filaments (250 A in diameter), which were not clearly resolved as tubular, in the macronucleus of *Paramecium caudatum*. These structures were found to be of undetermined length (at least 2 μ), and collected into bundles 1 to 2 μ in diameter showing some evidence of organization into an hexagonal array. Vivier and André consider that these structures may represent an intranuclear protein material.

Although the nuclear tubules described here are similar in diameter to those mentioned above, it should be pointed out that the epidermal cells of adult *Carausius* are not engaged in division, and there is no suggestion that the tubular complexes are associated with a mitotic apparatus. Furthermore, the constancy of size and shape of these complexes, together with the preservation of hex-

FIGURE 1 A low-power field within the nucleus of an epidermal cell of the stick insect *Carausius morosus*. Two nucleoli (*nl*) are included in this micrograph, and a microtubular complex (*mt*) is seen, in close association with nucleolar material. × 37,000.

FIGURE 2 A microtubular complex within a *Carausius* epidermal cell nucleus (*mt*). In this instance, the individual microtubules are seen predominantly in longitudinal section, and (as in the regions indicated by arrows) the electron-transparent "core" of the tubules is resolved. × 150,000.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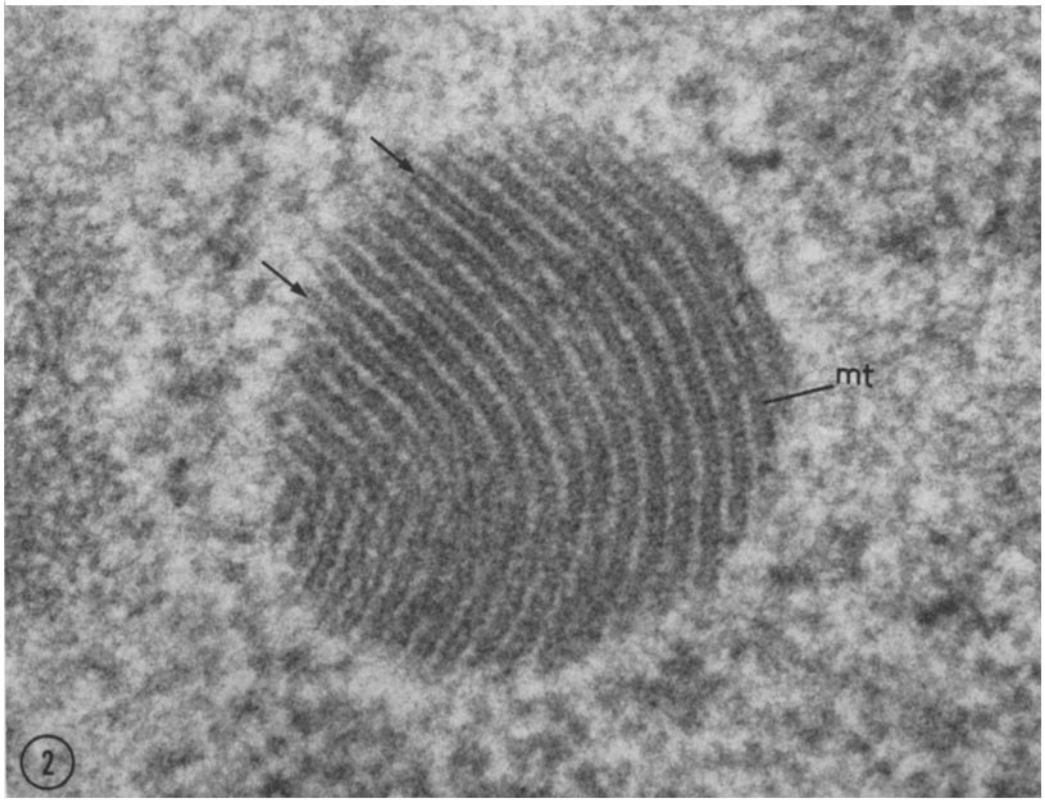
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agonal symmetry in an angled array, clearly distinguishes these structures from configurations of microtubules which have been described in either nuclear or cytoplasmic locations. In addition, the diameter of the tubular units, together with the loose packing of the array, appears to demarcate the complex from such crystalloid bodies as those described in rabbit blastocyst cells by Hadek and Swift (1960).

In the absence of information on the physiological role of this epidermal cell inclusion, we are at present in a position to define only its unusual structural organization.

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REFERENCES

DE-THÉ, G., 1964, *J. Cell Biol.*, **23,** 265.

GIERSBERG, H., 1928, *Z. Vergleich. Physiol.*, **7,** 657.

HADEK, R., and SWIFT, H., 1960, *J. Biophysic. and Biochem. Cytol.*, **8,** 836.

JANDA, V., 1936, *Bull. Acad. Sc. Bohème*, 1–4.

LEDBETTER, M. C., and PORTER, K. R., 1963, *J. Cell Biol.*, **19,** 239.

MILLONIG, G., 1961, *J. Biophysic. and Biochem. Cytol.*, **11,** 736.

ROTH, L. E., and DANIELS, E. W., 1962, *J. Cell Biol.*, **12,** 57.

ROTH, L. E., and SHIGENAKA, Y., 1964, *J. Cell Biol.*, **20,** 249.

SCHLEIP, W., 1911, *Zool. Jahrb.*, **30,** 45.

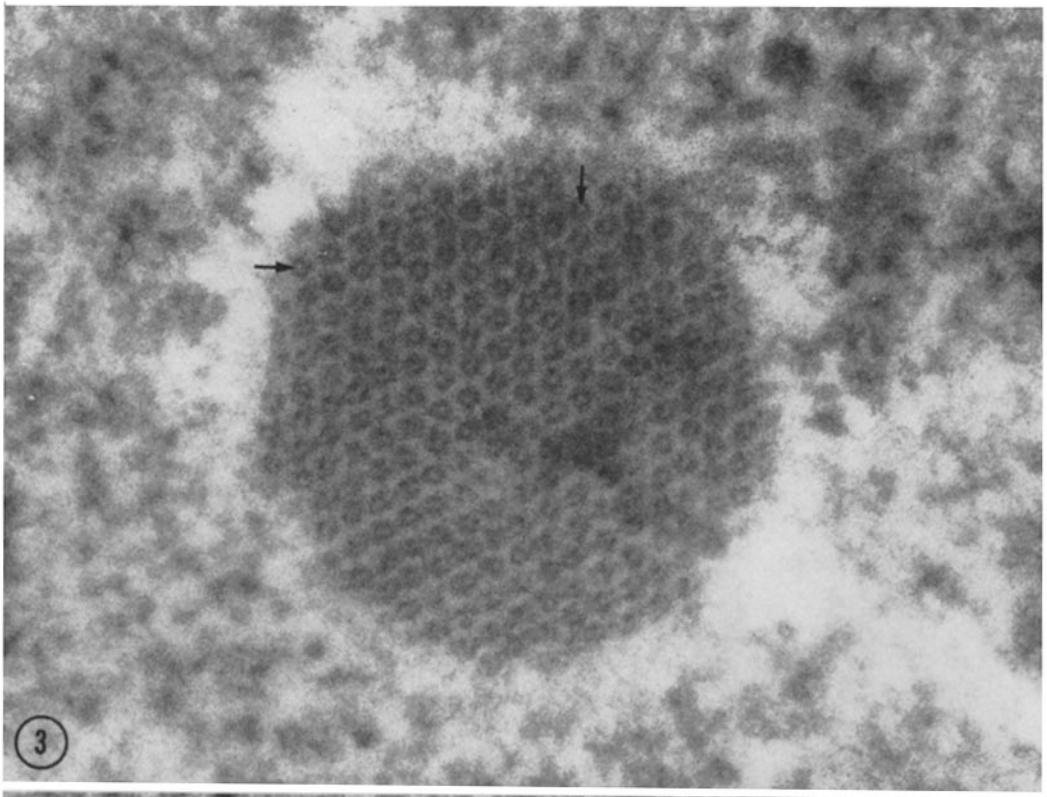
SLAUTTERBACK, D. B., 1963, *J. Cell Biol.*, **18,** 367.

VIVIER, E., and ANDRÉ, J., 1961, *Compt. rend. Acad. Sc.*, **252,** 1848.

WIGGLESWORTH, V. B., 1953, Principles of Insect Physiology, London, Methuen and Co., Ltd., 5th edition, 296.

FIGURE 3 A microtubular complex illustrating the precise orientation of the tubular elements. In this instance, the tubules are seen in transverse profile, and form a hexagonal array. Each microtubule is *ca.* 170 to 200 A in diameter, the center-to-center distance in the lattice is *ca.* 270 to 290 A, and the electron-transparent central region of the tubules is clearly illustrated (arrows). × 150,000.

FIGURE 4 A microtubular complex in a *Carausius* epidermal nucleus closely associated with nucleolar material (*nl*) (*cf.* Fig. 1). In this field, the complex comprises both transversely (*1*) and longitudinally (*2*) sectioned tubular elements. A similarly oriented complex is illustrated in Fig. 2. × 150,000.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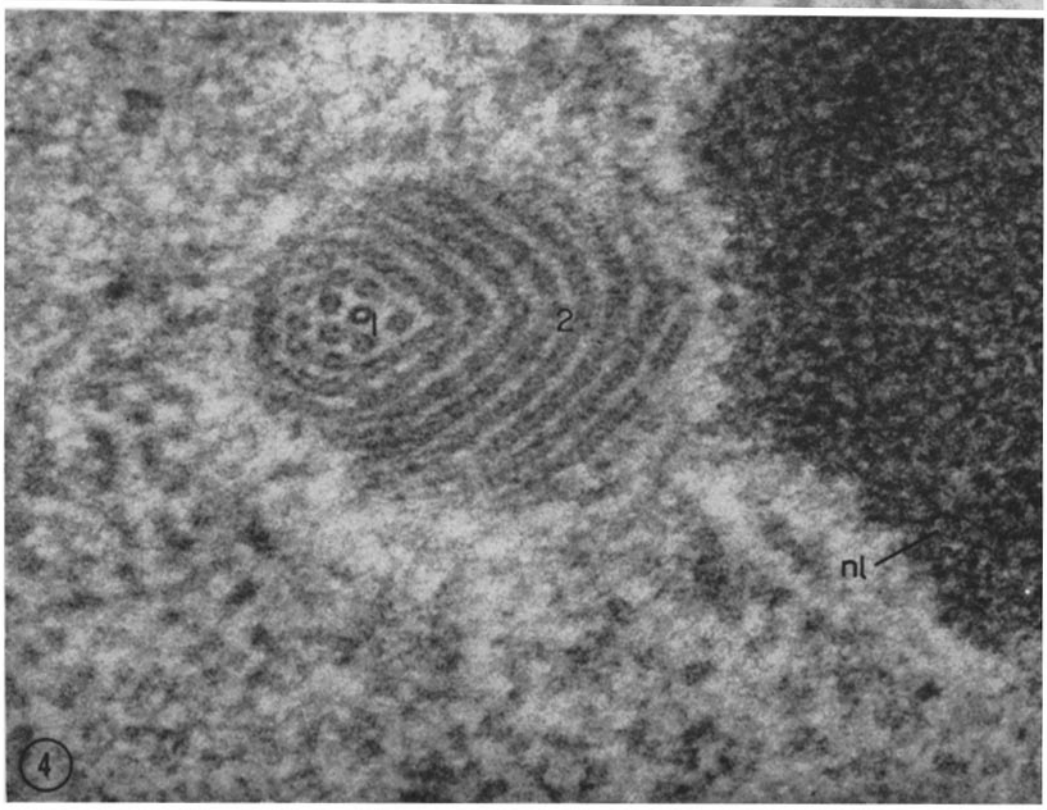
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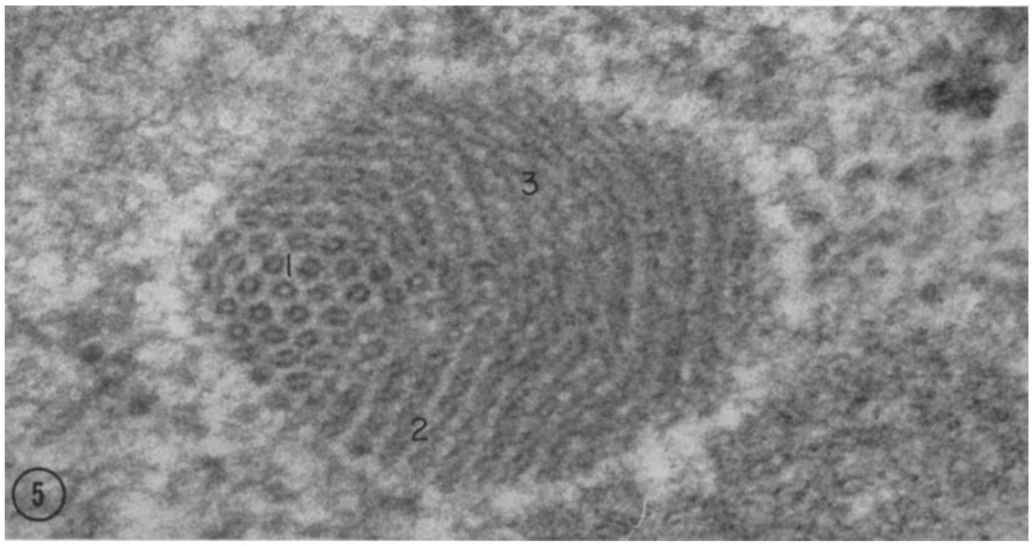
FIGURE 5 The microtubular complex illustrated in this micrograph includes profiles in transverse (*1*), largely oblique (*2*), and oblique (*3*) section. Note the precise orientation of these components. × 150,000.

FIGURE 6 An enlargement of the field illustrated in Fig. 5. Note the suggestion of substructure in the transverse profiles of the tubules (arrow). × 280,000.

FIGURE 7 A group of longitudinally sectioned microtubules within the nucleus of an epidermal cell of *Carausius*. In this instance, the tubule profiles show some evidence of periodic substructure (as in the region between arrows). × 150,000.



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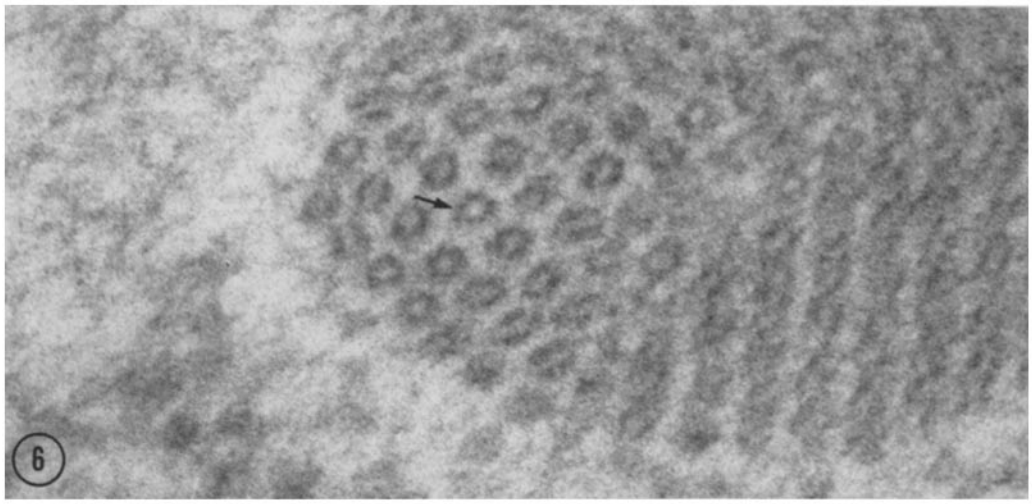

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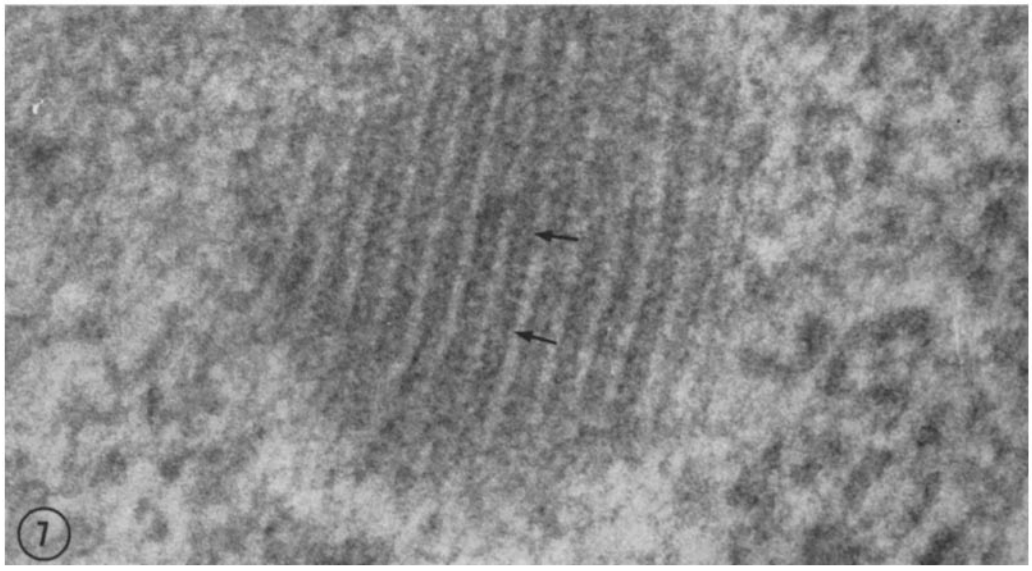

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