ELECTRON MICROSCOPY OF CENTRIFUGED HYPHAE OF *NEUROSPORA*

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ABSTRACT

Normal and centrifuged hyphae of *Neurospora* were studied with the electron microscope. The following cell structures could be identified: nuclei with nucleoli, mitochondria, endoplasmic reticulum, ribosomes, glycogen, fat bodies, vacuoles, and vesicles with an inner canalicular system, of unknown nature. In centrifuged hyphae, the glycogen layer appeared as a light area, with a slight indication of granular structure. The ribosome layer consisted of densely packed ribosomes without any membranes. The mitochondrial layer contained spaces filled with ribosomes. The nuclei were loosely packed, with endoplasmic reticulum between them. The "enchylema" layer was composed of vesicles belonging to the endoplasmic reticulum. The vacuolar layer was poorly preserved and consisted of double-walled vesicles. Fat appeared as stellate osmiophilic droplets. These observations were compared with previous observations under the optical microscope and their meaning for cell physiology was discussed.

INTRODUCTION

In recent publications (1, 2) I have described the cytochemistry and function of cell organelles in *Neurospora*, studied by the use of a technique of centrifugation of living hyphae. The present report complements these studies with the observation of centrifuged cells under the electron microscope. This allows better identification of sedimented cell organelles and determination of the degree of purity of each centrifuged layer. Centrifugation has been used by other workers as an aid in the study of cellular particles under the electron microscope (3–6). *Neurospora* is an advantageous material for such studies because of its relatively simple cell structure and because the cell constituents can be separated completely by centrifugation.

METHODS

Wild type *Neurospora crassa*, strain 2597*a*, was used in all preparations. Normal and centrifuged hyphae were of the type found at the growing periphery of the mycelia. Methods of centrifugation were described earlier (1). Centrifuged mycelia were fixed in 1 per cent osmium tetroxide in veronal buffer (pH 6.0) for 16 hours at room temperature, dehydrated in acetone, and embedded in Vestopal (7). For sectioning, hyphae were oriented parallel to the knife edge, so that longitudinal sections encompassing several sedimented layers were possible. Sections were observed under an RCA electron microscope model EMU 2E at ×6450 direct magnification, and were enlarged further photographically

RESULTS

Normal Hyphae

Neurospora is being studied with the electron microscope in several laboratories and one of these studies referring to young, thin hyphae has been published (8). Since the hyphae used for centrifugation come from older mycelia and are thicker, it was necessary to study their normal fine struc-

ture first. Further study of the fine structure of such hyphae is planned, and only the general appearance under the electron microscope will be given here (Fig. 1). The usual cell organelles could be easily identified. The nuclei were surrounded by a well defined double membrane and showed a granular structure, denser and darker in nucleoli. The mitochondria were of typical appearance with double membranes and cristae. The cytoplasm contained vesicles of various sizes, tentatively identified as endoplasmic reticulum (9). Ribosomes were scattered throughout the cytoplasm, no clear connections with membranes being evident. Glycogen appeared as irregular aggregates, lighter than the background. The aggregation of glycogen was probably a fixation artifact, as it has been noticed in cytochemical studies (10). Fat droplets could be seen as dark, osmiophilic bodies of uniform size, around 0.3 μ in diameter. They presented finely granular structure, probably reflecting non-homogeneous precipitation of reduced osmium. Vacuoles could be found as large vesicles without prominent inner structures. Besides vacuoles, vesicles with a peculiar inner ramified canal system were observed. Their exact nature is not known.

Centrifuged Hyphae

Glycogen Layer (*Fig. 2*): It appeared as a light area, dotted with many dark spots of different sizes, which could be best interpreted as irregular deposits of reduced osmium. Glycogen itself did not reduce osmium tetroxide and was lighter than the background of the embedding medium. There was a slight indication that glycogen was present in the form of granules, but their exact size could not be resolved.

Ribosome Layer (*Fig. 3*): The layer which was described in previous work (1) as ergastoplasm turned out to be composed of densely packed ribosomes, appearing as dark granules approximately 120 to 150 A in diameter. At the boundary between glycogen and ribosomes, both components were incompletely separated. A few glycogen granules were interspersed deep inside the ribosome layer. No membranous formations could be detected.

Mitochondrial Layer (*Fig. 4*): Mitochondria could be readily recognized by their typical structure. They were generally oriented parallel to the length of the hyphae. They were loosely packed, as if centrifugal forces could not disfigure them in order to pack them in a contiguous mass. Spaces left between individual mitochondria were filled with ribosomes. At the boundary between the ribosome and mitochondrial layers these spaces were wider. There was no indication of any differences between mitochondria at the centripetal and centrifugal end of the layer.

Nuclear Layer (*Fig. 5*): Nuclei were only loosely packed. The nucleolus was found at the centrifugal end; it appeared dark with a lighter area (vacuole) in the center. The spaces between the nuclei were filled with cytoplasmic vesicles, like those found in the next layer. The boundary between the nuclei and the mitochondria was distinct and no ribosomes could be seen remaining in the nuclear layer.

Endoplasmic Reticulum (*Fig. 6*): This layer was previously (1) called "enchylema," since under the optical microscope it was of hyaline appearance. The electron microscope revealed a population of vesicles of different sizes, measuring between 500 and 1000 A, each surrounded by a simple membrane. Rarely, a few lamellar structures, irregularly folded across the entire width of the hypha, could be observed. Between vesicles were small granules, similar to ribosomes, but more irregular in size, more diffuse in outline, and not as dark.

Vacuoles (*Fig. 7*): Fewer preparations were available of the vacuolar layer and it was not possible to detect any well preserved vacuoles. Osmium tetroxide fixation and other treatments may have

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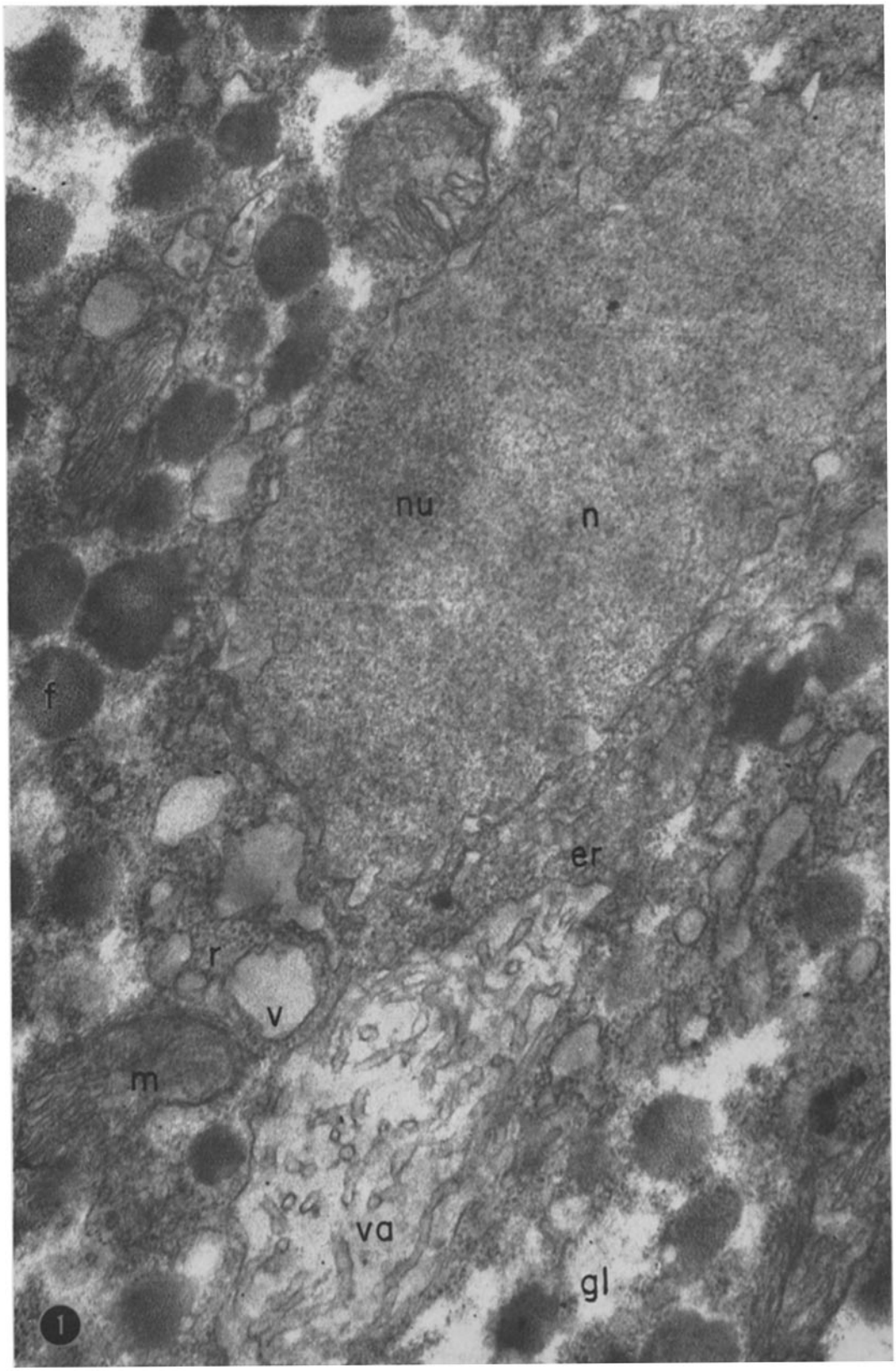
Explanation of Figures

The following abbreviations are used in all figures: *n*, nucleus; *nu*, nucleolus; *m*, mitochondria; *er*, endoplasmic reticulum; *r*, ribosomes; *v*, vacuole; *va*, vesicle with internal canalicular system; *gl*, glycogen; *f*, fat droplets.

FIGURE 1

Section of normal hypha of *Neurospora*, near the growing tip. × 47,700.


nu
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contributed to their collapse, since only irregular membranes and vesicles could be seen at the site of vacuoles. Some of these vesicles were double, one within another, possibly a cross-section of a collapsed vacuole. The exact meaning of these structures will not be clear until preparations fixed by other methods are available.

Fat Layer (*Fig. 7*): The centripetal layer contains, according to cytochemical observations, fats and lipids. As seen in the electron microscope, the layer is packed with large osmiophilic bodies of irregular outline. These bodies could be recognized as corresponding to the rounded fat droplets of uncentrifuged preparations; the irregular outline must have been due to less successful fixation or other factors which need further analysis. Such irregular fat bodies were found in some of the centrifuged preparations and were also observed in other fungi (11).

Cell walls showed no remarkable structures. They appeared clearer than the embedding medium. The cross walls showed a darker intermediate lamella. In a few preparations, sections containing the central opening were obtained; some cytoplasmic lamellae were protruding through the opening into the glycogen layer of the next cell.

CONCLUSION

Centrifugation helped in the recognition of cellular structures under the electron microscope by separating and assorting them inside the cell. Thus, it has not been easy to decide what represents glycogen under the electron microscope (12), and whether it appears light or darkened by osmium tetroxide fixation. Glycogen, which can be easily identified in a centrifuged cell, does not reduce osmium tetroxide substantially and is less electron-dense than the embedding medium. Similar low density is characteristic for cell walls.

Several authors (13, 14) have expressed doubt about the reality of ribosomes as a distinct cytoplasmic structure. The fact that ribosomes can be centrifuged into a distinct layer in a living cell and that they still appear as separate granules after fixation implies their independent existence. The centrifugal sedimentation of a pure ribosome layer is at variance with the observation of Monné (15) on sea urchin eggs, where ribosomes were centrifuged together with the cytoplasmic lamellae. In *Neurospora*, ribosomes are not attached to the endoplasmic reticulum but are suspended freely in the cytoplasm, as has been observed in *Allomyces* (11). On the basis of present observations and since the term ribosome has become widely accepted, I feel compelled to abandon the proposal in a previous paper (2) to name these granules "chromidia."

The endoplasmic reticulum consists of vesicles and tubules similar to the ones described in *Allomyces* (11). It remains a question whether the observed vesicles exist independently or are a part of an interconnected canalicular system, although it can be asserted that the *Neurospora* hypha does not have any extensive lamellar system.

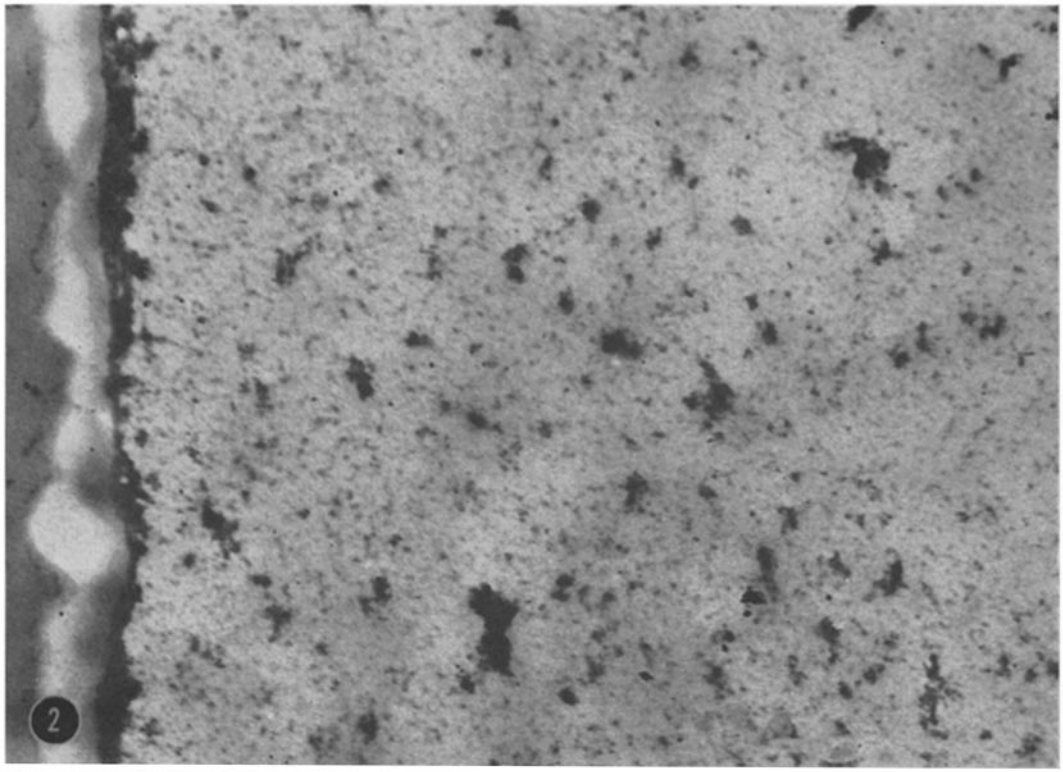
The present observations also shed more light on the results of cytochemical and autoradiographic studies (1, 2). It becomes clear that ribosomes are the seat of the cellular ribonucleic acid and the main site of protein synthesis. The presence of ribonucleic acid and of protein synthesis in the mitochondrial layer can now be explained by the persistence of ribosomes between the spaces of loosely assorted mitochondria. The lack of exact quantitative measurements precludes a final conclusion as to whether ribonucleic acid is present in the mitochondria and whether protein synthesis occurs in them. The fact that the ribosomes become separated from the endoplasmic reticulum by centrifugation may explain the lack of protein synthesis in a centrifuged cell. Interaction of the two components is probably necessary for the synthesis in a living cell. On the other hand, the contact of ribosomes with mitochondria is not sufficient to support protein synthesis.

FIGURE 2

Glycogen layer of centrifuged hypha. Black areas are probably deposits of osmium tetroxide. × 36,000.

FIGURE 3

Ribosome layer of centrifuged hypha. White spots are incompletely centrifuged glycogen granules. × 61,000.



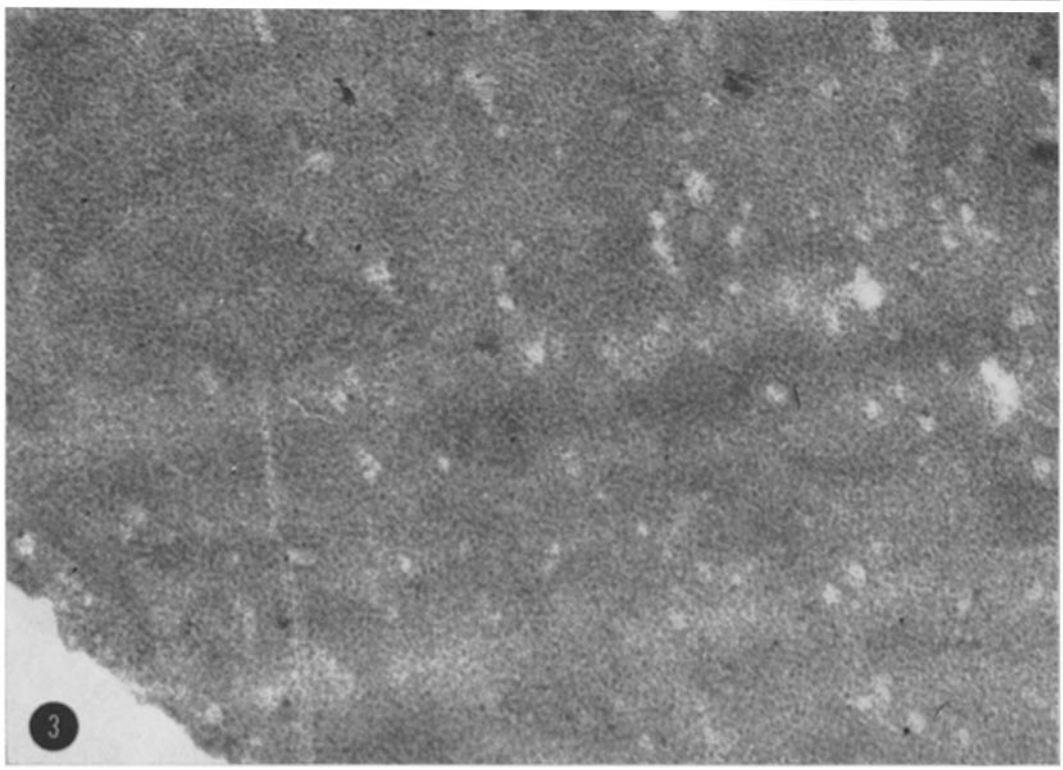


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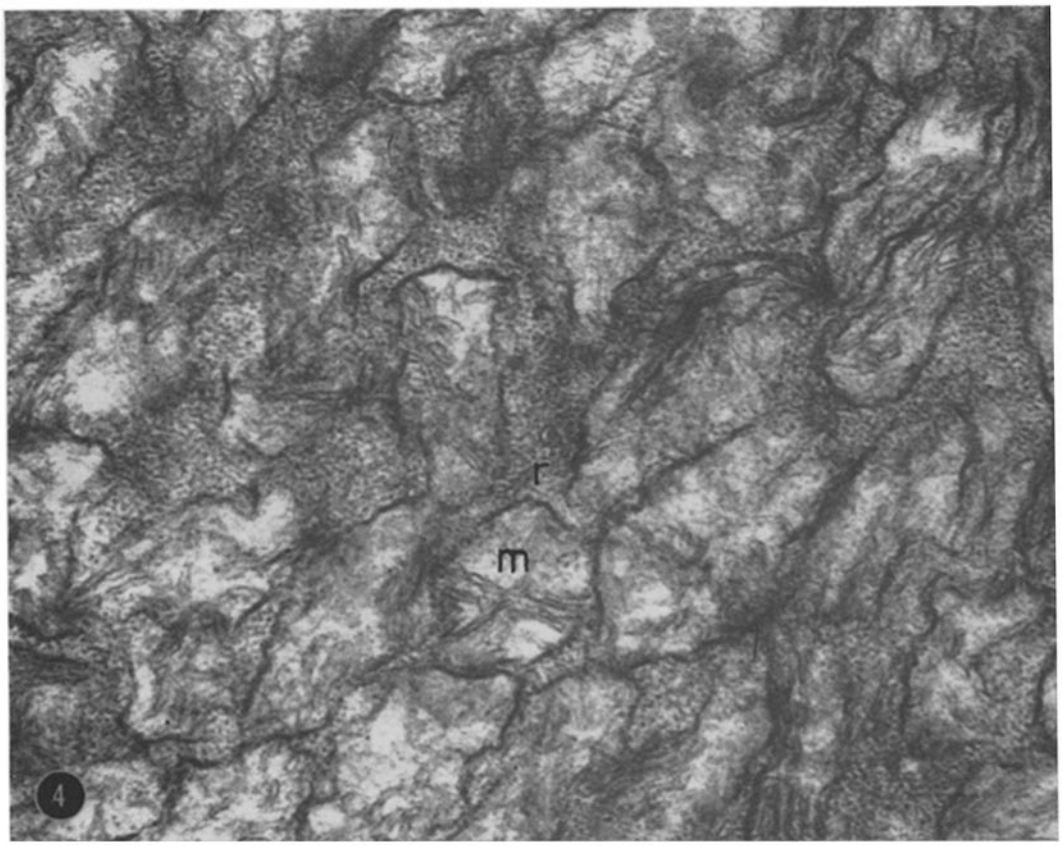
FIGURE 4

Mitochondrial layer of centrifuged hypha. Ribosomes fill spaces between mitochondria. × 61,000.

FIGURE 5

Nuclear layer of centrifuged hypha. Spaces between nuclei are occupied by endoplasmic reticulum. Black spots are contaminants. × 36,000.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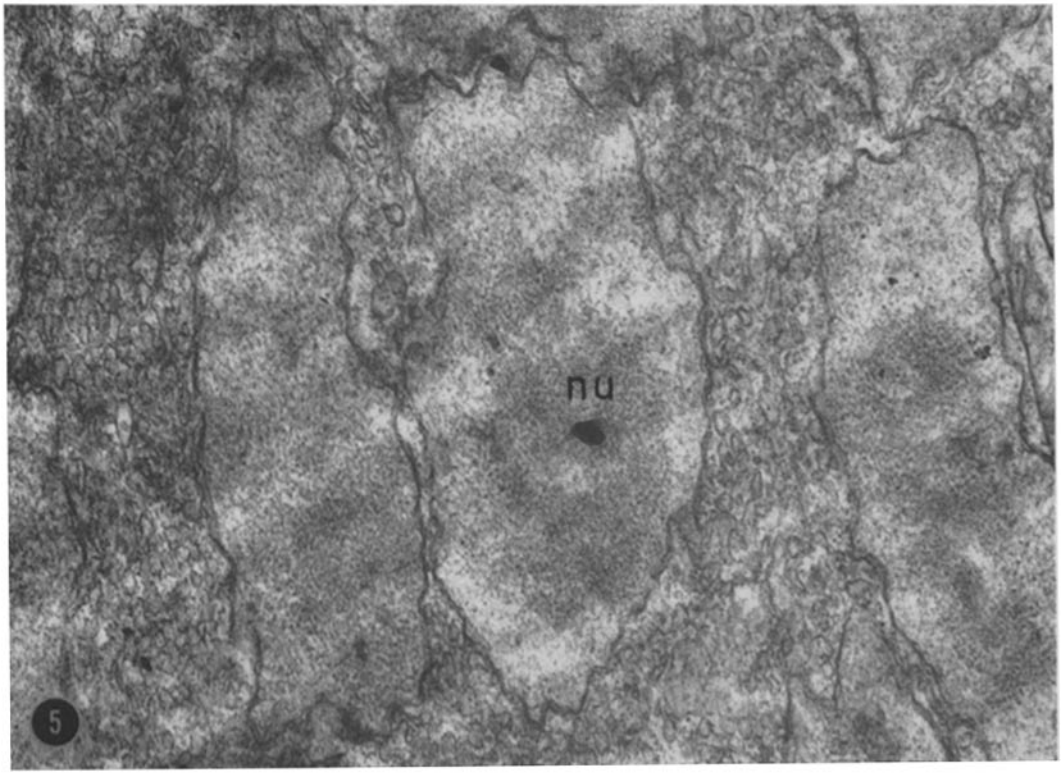
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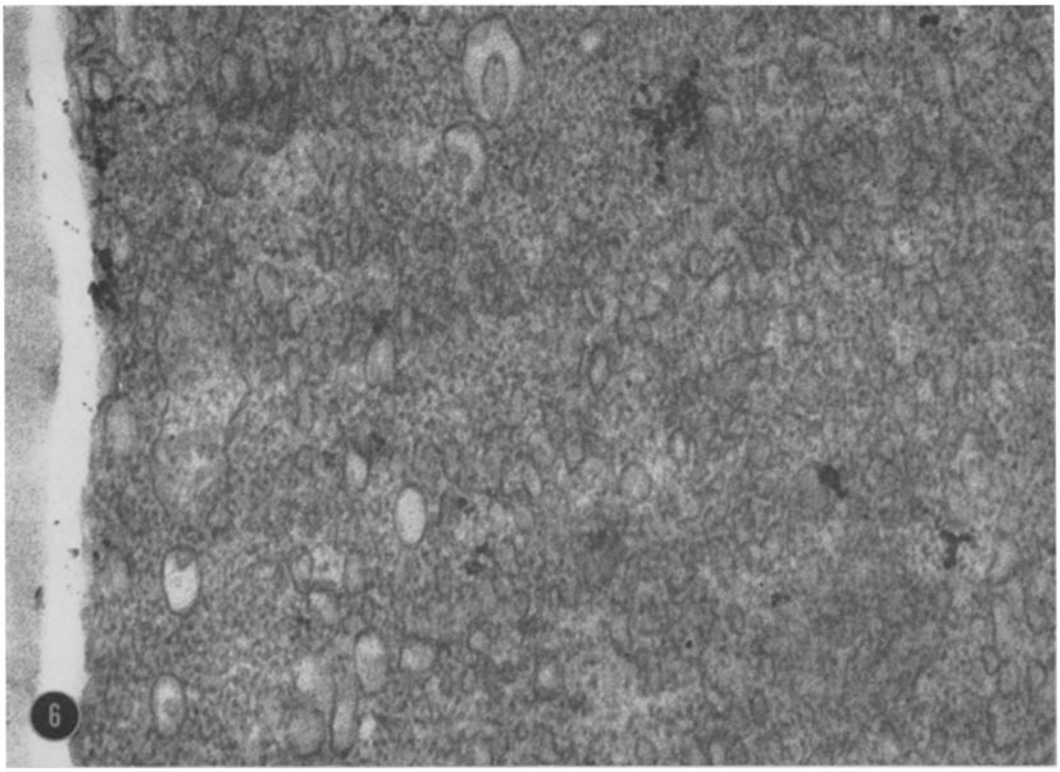
FIGURE 6

Endoplasmic reticulum of centrifuged hypha. Note cell wall to the left. × 61,000.

FIGURE 7

Vacuolar and fatty layer of centrifuged hypha. Collapsed (?) vacuoles produce double vesicles. Fat granules with irregular outline. × 61,000.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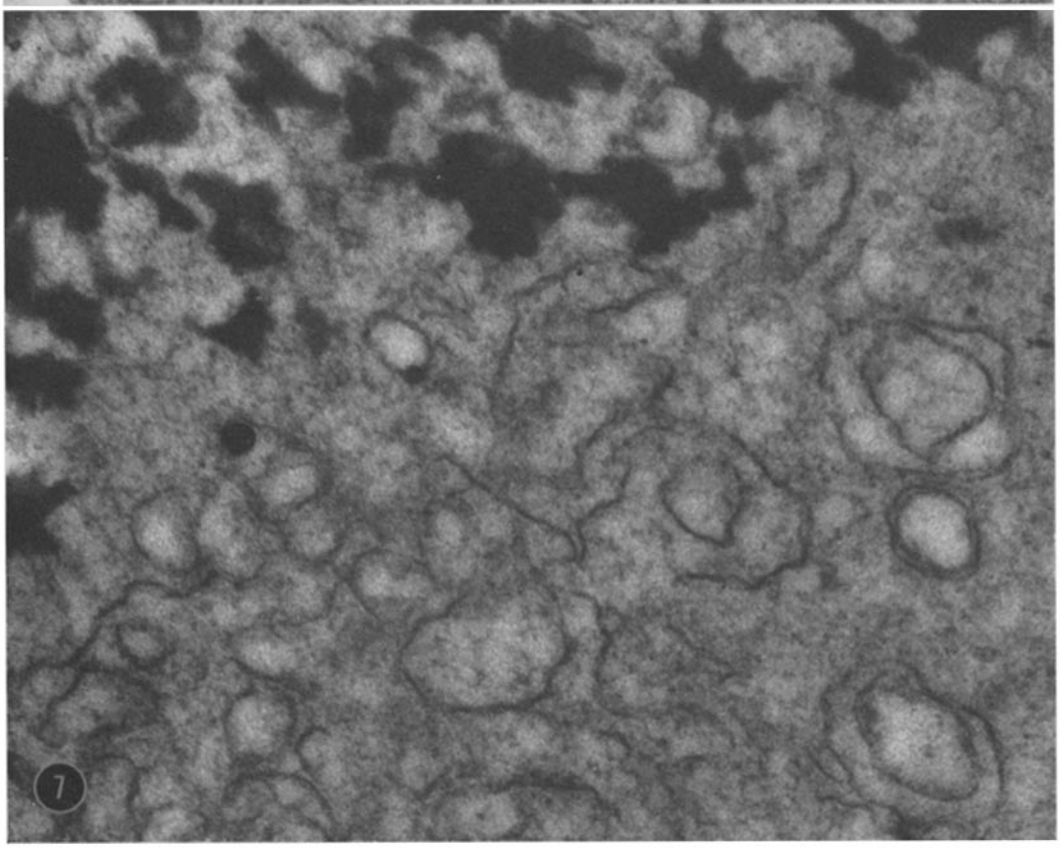
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