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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Botanica. — *Septal pore apparatus of the ascomycete* Morchella rotunda *(Persoon ex Fries) Boudier; ultrastructural and chemical observations.* Nota (*) di GIUSEPPE PATRIGNANI e STEFANIA PELLEGRINI (**), presentata dal Corrisp. M. GEROLA.

RIASSUNTO. — Sono stati analizzati, al microscopio elettronico, i pori del fungo ascomicete *Morchella rotunda* (Persoon ex Fries) Boudier. Essi sono classici pori semplici con, associati, corpuscoli sferici simili ai corpi del Woronin, cristalli esagonali e « pore plug » di natura granulare. In molti casi, l'occlusione del poro viene effettuata da uno o più cristalli esagonali mentre il « pore plug » è ancora presente in tracce. Quando si verifica questo caso, una delle due cellule presenta una ricca vacuolizzazione, alcune vescicole e minore densità di ribosomi. La digestione effettuata con un enzima proteolitico, la pronasi, dimostra che, in *Morchella rotunda*, sia il « pore plug » che i cristalli esagonali hanno contenuto di natura proteica mentre i corpi sferoidali non vengono rimossi. Questi perciò non possono essere ritenuti corpi del Woronin ma « microbodies » più simili ai corpi lipidici.

INTRODUCTION

Many studies have been made regarding Basidiomycetes dolipore ultrastructure that presents several variations in the pore cap and lends itself to remarks of systematic and phylogenetic nature. The Ascomycetes simple pore, on the contrary, appears to present less interesting characteristics. In fact, it appears as a simple break in the transversal septum of cells constituting the dykaryotic hyphae and presents always associated electrondense spheroidal organelles, too generically named Woronin bodies.

Besides, just on the pore opening, we can see electron dense materials, sometimes limited by a well differentiated membrane; these were named " pore plugs " or " septal plugs " (Reichle and Alexander, 1965; Trinci, 1974).

Crystals, which took the form of elongated hexagons, were commonly associated with mature septa in some species of Ascomycetes. Hexagonal crystals have been observed in *Neurospora crassa* by Shatkin and Tatum (1959) and they were isolated, analyzed and shown to be composed of ergosterol by Tsuda and Tatum (1961). H. C. Hock and Maxwell (1974) studied proteinaceous

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hexagonal inclusions in hyphae of *Whetzelinia sclerotiorum* and *Neurospora crassa*. Armentrout and Maxwell (1974) examined hexagonal inclusions in an ergosterol-free mutant of *Neurospora crassa*.

Regarding the microbodies named Woronin bodies, there are numerous publications which strive to identify their origins, functions and chemical nature. Dickson (1963) mentioned Woronin bodies in his study of *Pithomyces chartarum*, Reichle and Alexander (1965) described round or oval Woronin bodies associated with multiperforate septal pores of different species of gen. *Fusarium*; Lowry and Sussman (1966) in the intrahyphal hyphae in " clock " mutans of *Neurospora*; Shatkin and Tatum (1959) in *Neurospora crassa*; Scannerini (1968) associated with the septa of *Tuber magnatum*; McKeen (1971) in *Erysiphe graminis* and so on.

However, other fine structural studies have designated the inclusions that lie near the septal pore of Ascomycetes as granules (Dickson, 1963), lipoid granules (Zacharuk, 1970), lysosomes (Wilson *et al.*, 1970), lipid bodies (Mitchell and McKeen, 1970), spherosomes (Wilson *et al.*, 1970), Woronin-like bodies (Cutler and Erke, 1971) and crystal-containing microbodies (Maxwell *et al.*, 1972).

Consequently, Woronin bodies may be erroneously designated by ambiguous terms, especially as, at present, very little is known about their origin and development.

The ascomycete *Morchella rotunda* also presents, associated with septa, microbodies, Woronin-like bodies, hexagonal crystals and pore plugs. Wishing to explain the alternative function of these three different structures in pore plugging processes, we also wanted to study their chemical nature by digestion with a proteinic enzyme, the pronase.

Materials and Methods

Fruiting bodies of *Morchella rotunda* (Persoon ex Fries) Boudier were collected by members of the Mycological Society of Milano and of Borgo Val di Taro (Italy). Small pieces (1 mm^3) of these fruiting bodies were fixed in 1,5% glutaraldehyde in 0,1 M phosphate buffer, pH 7,0 and then post-fixed in 2% osmium tetroxide for 2 hours. After dehydration in ethanol series, the specimens were embedded in Epon Araldite (1 : 1). An ultramicrotome was used for obtaining thin sections which were picked with golden grids. Thin sections, double stained with 2% aqueous uranyl acetate and lead citrate, were examined using a Siemens Elmiskop I A electron microscope.

Protease digestion of pore plugs and other organelles in thin sections of fixed fungal hyphae was performed at 36° C. Golden grids, supporting thin sections, were treated previously with 3% H_2O_2 for 40 minutes and placed in two different pronase solutions the first at 1% for 16 hours, the second at 2% for 16 hours (McKeen, 1971). After washing with distilled H_2O, thin sections were ready for observations.

OBSERVATION

In *Morchella rotunda*, the simple pore presents, in normal conditions, a granular pore plug (*p*) (Plate I, fig. 1). Near the pore, some hexagonal crystals are always present. In transversal section, they present a characteristic small stick-like appearance (*h*) and are limited by a well-defined membrane. In addition to pore plug and hexagonal microbodies, spheroidal structures are present. They are delimited by a membrane and resemble Woronin bodies (Plate II, fig. 3: inset). Their diameter measures approximately 0,5 μ and their contents are granular and not homogeneous.

When all kinds of material or organelles pass through a pore, from one cell to another, the pore plug disappears (Plate I, fig. 2).

In many cells of the mycelium, a single crystal or several hexagonal crystals get into a pore opening to form a rapid occlusion (Plate II, fig. 3). In such cases, the cytoplasm of one in two cells shows vacuoles (*va*) and vesicles (*v*).

Electron dense material was often associated with such a crystal (Plate II, fig. 4).

A thin section of *Morchella rotunda*-control, before pronase digestion, shows a large granular pore plug just on the pore opening and, near the septal wall, a Woronin-like corpuscle and a characteristic hexagonal crystal (Plate III, fig. 5).

Digestion, effected by 1% pronase solution for 16 hours at 36° C, does not suffice to completely remove the proteinic material that constitutes hexagonal crystals and pore plugs (Plate III, fig. 6). In fact, crystal contents appear still partially electrondense. The Woronin-like bodies are quite recognizable because they are not modified by the proteolythic enzyme.

Proteinic contents digestion of hexagonal crystals is complete when sections are treated with a 2% pronase solution for 16 hours at 36° C (Plate III, fig. 7). The electrondense material trace, which together with crystals, is sometimes present near the pore opening, also disappears when the pore plug is replaced by crystal rapid occlusion.

The hexagonal crystal digestions do not correspond to alterations for Woronin-like microbodies that show up the pale background micrographies (Plate III, fig. 7: inset).

DISCUSSION

Morchella rotunda transversal septa present a simple pore normally plugged by granular electrondense material which pronase digestion has proved to be proteinaceous. Near the pore opening there are hexagonal crystals containing proteinic materials, well delimited by a membrane. Plunged in cytoplasm, then, there are always distinguishable spheroidal organelles which pronase digestion does not change, and which therefore contain no protein so, according to McKeen (1971) and Reichle and Alexander (1965), it is impossible to call them Woronin bodies.

The author's histochemical studies, in fact, have proved that pepsin and other proteolythic enzymes destroy the Woronin bodies.

Woronin bodies being absent in *Morchella rotunda*, it should be impossible, in this case, to assert, according to Wergin (1973), that a pore plug may be a derivation from a Woronin body that changes and loses its identity.

Besides, as observed by Trinci and Collinge (1974) in *Neurospora crassa*, electrondense material remains near the pore opening, when the occlusion is constituted by hexagonal crystals, while one of two cells presents always an abundantly vacuolized cytoplasm and some vesicles that the authors affirm to contain the enzymes and precursors which are essential for the formation of the primary hyphal wall. A reduced ribosome density is also observable here.

Trinci and Collinge (1974), observed septal plugs constituted by hexagonal crystals in *Neurospora crassa* when the hyphae were purposely damaged with a razor edge.

According to the above mentioned authors, occlusions of septal pores of damaged hyphae by hexagonal crystals or some other solid body will drastically reduce loss of cytoplasm, and hyphal damage caused by a sudden osmotic shock due to flooding will be probably a common event in nature. " The two different methods of septal pore plugging described for *Neurospora crassa*, may also occur in other Ascomycetes ", assert Trinci and Collinge (1974): one of these Ascomycetes is *Morchella rotunda*, even if we have not artificially damaged the hyphae, before the fixation, but used material collected from a fruiting body naturally grown and picked in a woodland area in North Italy.

Since pronase digestion proves that hexagonal crystals are proteinaceous, we too can affirm, in agreement with other authors (Reichle and Alexander, 1965; Brenner and Carroll, 1968; Wergin, 1973; Hoch and Maxwell, 1974; Trinci and Collinge, 1974), that when found in *Morchella rotunda*, they appear to be functionally analogous with the spherical Woronin bodies found in other different Ascomycetes. " Indeed they should perhaps be regarded as hexagonal Woronin bodies ". In fact, both are homogeneous and electron opaque; both are sometimes seen in microbodies; both are bounded by a unit membrane; both are removed with proteases after glutaraldehyde fixation; both are associated with septa of Ascomycetes and may function in the plugging of the septal pore.

This hypothesis is strengthened since, in *Morchella rotunda*, there are not present Woronin bodies but non-proteinaceous spherical microbodies, as was demonstrated by the digestion performed. Moreover, these microbodies were never observed directly on the pore opening but only near to it or to the transversal septal wall or in other parts of the hyphal cells.

From our observations, we may not obtain other data concerning Woronin-like bodies functions or regarding the granular and inhomogeneous nature of their contents that could recall the microbodies which are commonly found in plant cells (Frederik *et al.*, 1968), whether for their non-proteinaceous nature which might recall the lipid bodies (Mitchell and McKeen, 1970), or for their size which is more similar to that of Woronin bodies described for different

ascomycetes (Schrantz, 1964; Reichle and Alexander, 1965; Carroll, 1966; Bracker, 1967; McKeen, 1971), than to those described for lipid bodies (McKeen, 1971).

Our Study points out, once more, a possible equivocation between a true Woronin body and another spherical microbody associated with an ascomycete simple pore. This exchange could lead to erroneous conclusions because different organelles present opposite significance and varying functions in relation to the pore occlusion mechanism of these fungi. Consequently it should be necessary to name " Woronin body " only that organelle whose chemical nature is certainly proteinaceous and to reserve the name " Woronin-like body " for the remaining ones.

On the other hand, since Woronin, in his time, did not analyze the chemical contents of spherical homonymous microbodies, we might go on naming " Woronin body ", every spherical electrondense organelle, delimited by membrane, associated with the ascomycete simple pores, but mentioning explicitly their chemical nature which can characterize their own functionality and different properties.

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EXPLANATION OF PLATE I-III

PLATE I

Fig. 1. - Simple pore of *Morchella rotunda*. Pore opening presents a granular pore plug (p). Near it, several hexagonal crystals appear like small sticks (h) when dissected longitudinally. × 50000.

Poro semplice di *Morchella rotunda*. L'apertura del poro è chiusa da un pore plug di natura granulare (p). Accanto ad esso, alcuni inclusi esagonali che appaiono a forma di bastoncello (h) quando sono sezionati longitudinalmente. × 50000.

Fig. 2. – Simple pore of *Morchella rotunda* when a cellular organelle seems to pass from a cell to another. × 22400.

Poro semplice di *Morchella rotunda* mentre un organulo cellulare sembra stia passando da una cellula all'altra. × 22400.

PLATE II

Fig. 3. – Pore's occlusion by an hexagonal crystal. In this case, one of two cells appears very rich in vacuoles (va). × 44000.

Occlusione del poro mediante un cristallo esagonale. In questo caso, una delle due cellule appare molto vacuolizzata (va). × 44000.

Fig. 3 inset. - Spheroidal Woronin-like body near hyphal transversal wall. × 44000.

Organello sferoidale simile a un corpo del Woronin, accanto al setto trasversale di un'ifa. × 44000.

Fig. 4. - An hexagonal crystal forms the pore occlusion. Ap ore plug trace is visible while a cell presents many vacuoles (va), vesicles (v) and less density in ribosomes. × 44000.

Un cristallo esagonale forma l'occlusione del poro. Permane una traccia del pore plug mentre una cellula è molto vacuolizzata (va), presenta vescicole (v) e minore densità di ribosomi. × 44000.

PLATE III

Fig. 5. – Transversal septum of *Morchella rotunda*-control with granular pore plug (p) on its opening. There are associated hexagonal crystal (h) and spheroidal organelle. × 30000.

Setto trasversale di *Morchella rotunda*-controllo con la apertura del poro chiusa da un « pore plug » granulare (p). Vi sono associati un cristallo esagonale (h) e un organulo sferoidale. × 30000.

Fig. 6. – Septal pore apparatus of *Morchella rotunda* after digestion by 1% pronase solution. The pore plug (p) appears digested, the hexagonal crystal is partially digested while Woronin-like body appears complete. × 30000.

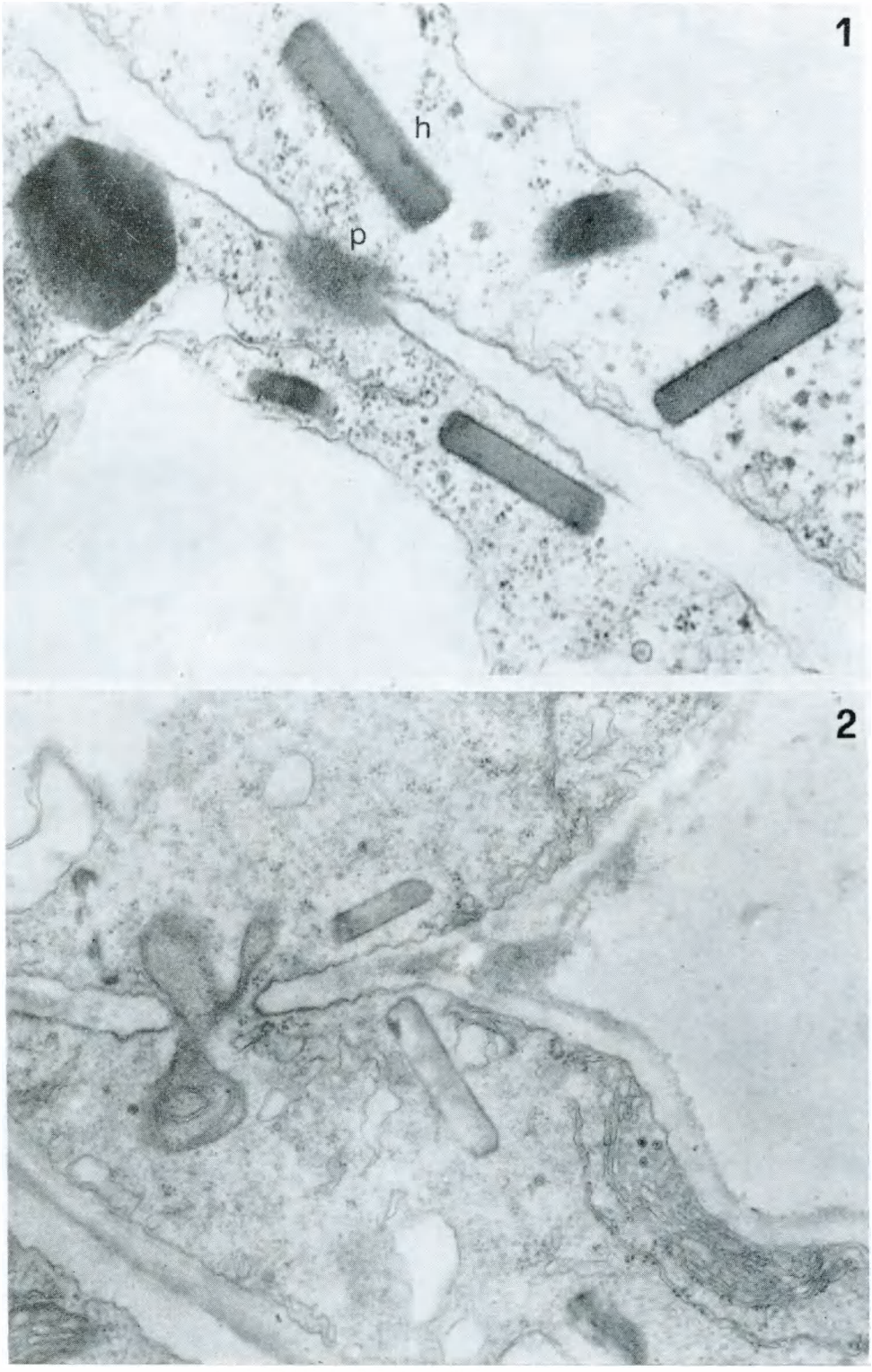
Apparato settale di *Morchella rotunda* dopo digestione con una soluzione all'1% di pronasi. Il pore plug (p) è digerito, il cristallo esagonale è parzialmente digerito mentre appare integro il corpuscolo simile a un Woronin. × 30000.

Fig. 7. - Hexagonal crystals as pore occlusion after digestion by 2% pronase solution. They appear electron transparent. × 48000.

Cristalli esagonali che formano l'occlusione del poro dopo digestione con una soluzione al 2% di pronasi. Essi appaiono trasparenti agli elettroni. × 48000.

Fig. 7 inset. – Undigested spherical microbody after 2% pronase treatment. × 44000.

Microbody sferico indigerito, dopo trattamento con una soluzione al 2% di pronasi. × 44000.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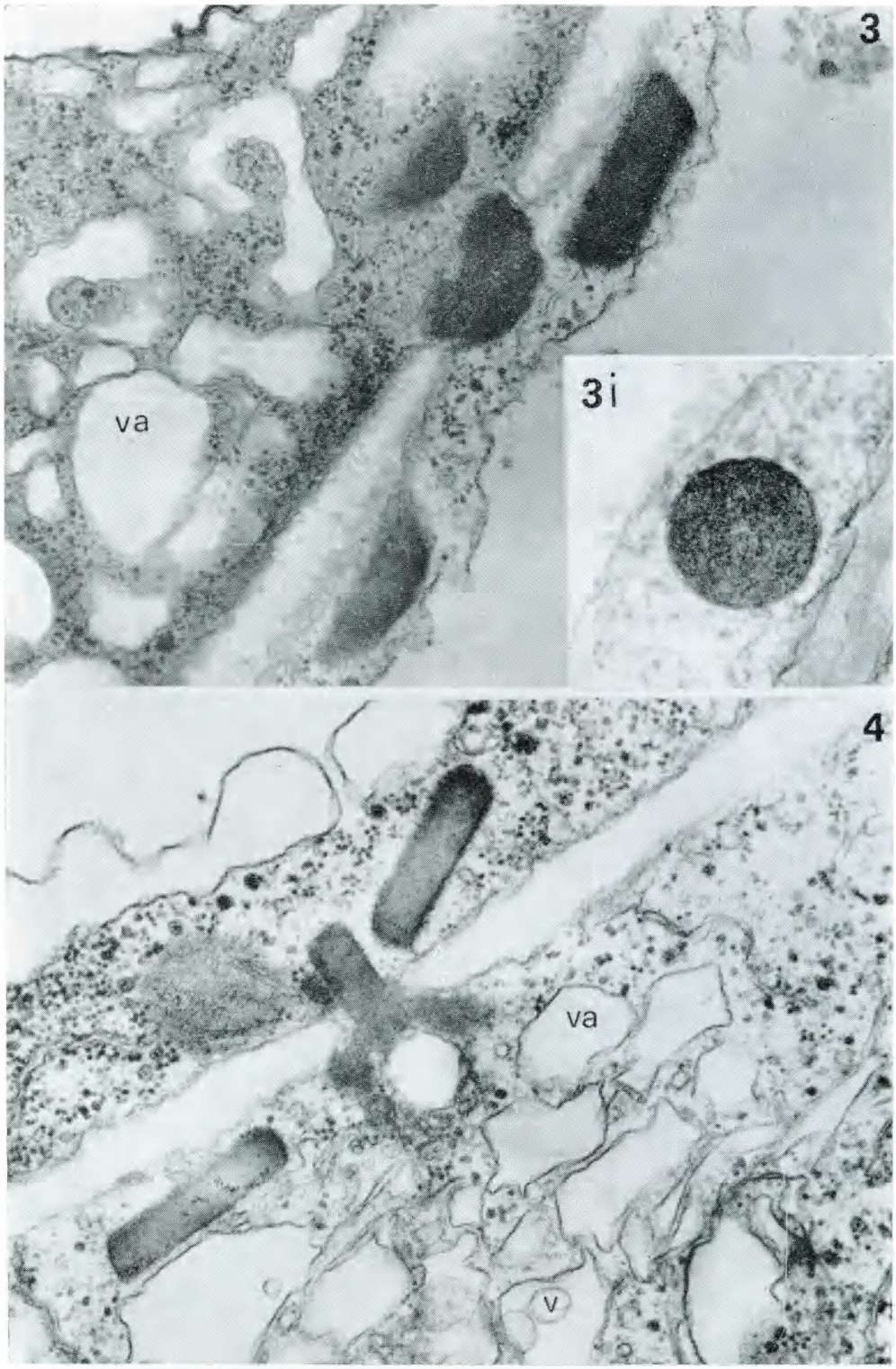
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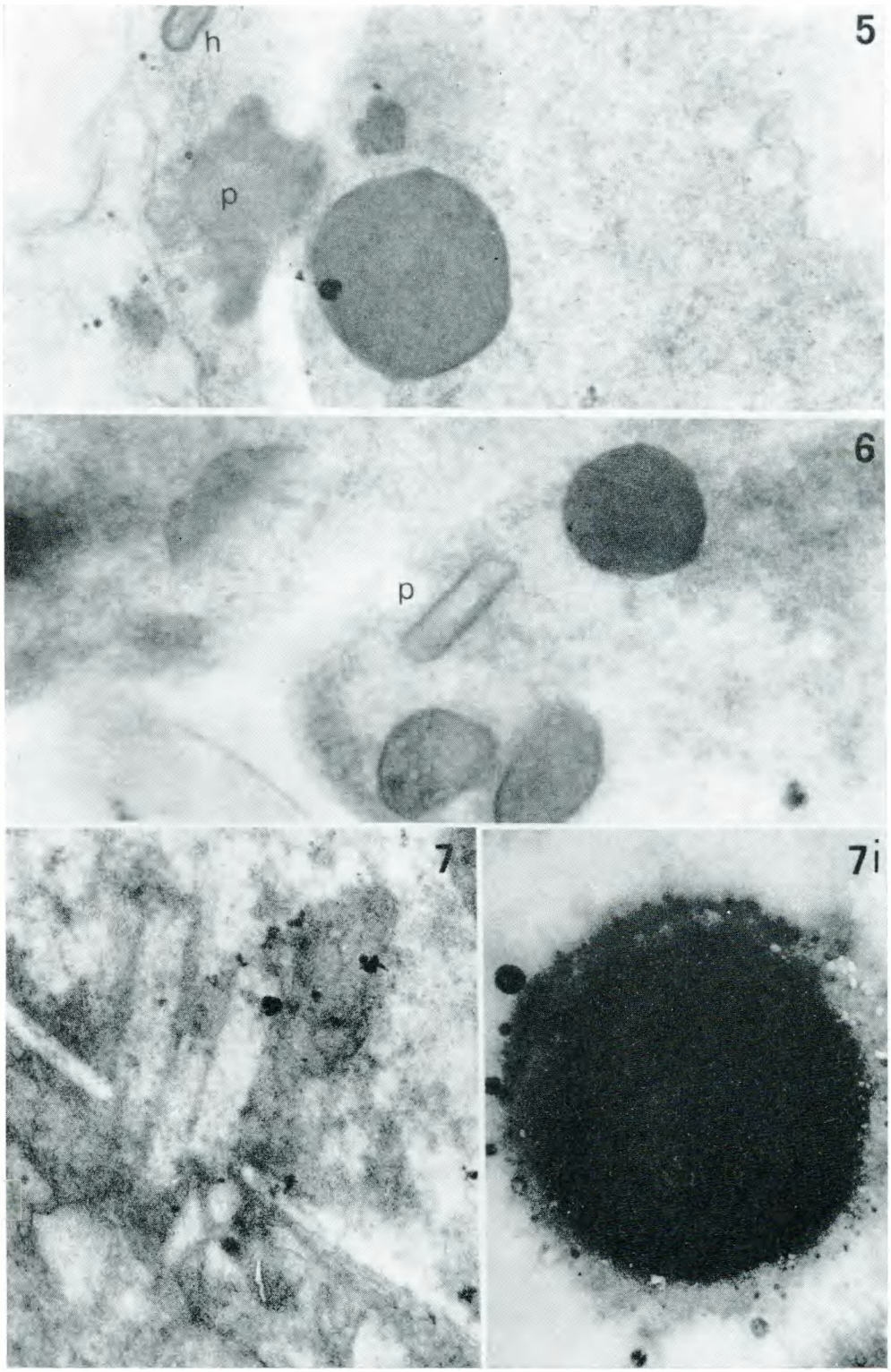
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