ELECTRON MICROSCOPICAL STUDIES OF ULTRATHIN SECTIONS IN *PENICILLIUM CHRYSOGENUM*[1]

SEIZO TSUDA

National Institute of Genetics, Misima, Sizuoka-ken, Japan

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The ultrathin sectioning technique, developed since the invention of the electron microscope, made it possible to study the finer structures of microorganisms. Cytological studies using the electron microscope have been made by Hillier *et al.* (1949), Robinow (1953), Chapman and Hillier (1953), Tsujita *et al.* (1954), Maaløe *et al.* (1953*a*, 1953*b*), Higashi (1953), Tsuda *et al.* (1954), and Tsuda (1955*a*, 1955*b*).

Some of the results of the present author's investigations of the fungi have been published (Tsuda, 1953, 1954, 1955*a*, 1955*b*). They showed that the cytoplasm and nuclear materials of these organisms have a more or less reticular structure.

The present paper deals with additional observations of ultrathin sections through the vegetative mycelia of *Penicillium chrysogenum*.

MATERIALS AND METHODS

Penicillium chrysogenum was submerged and grown in a liquid medium for 3 days at 28 C. The culture was centrifuged at a moderately low speed for about 10 minutes and then washed repeatedly with distilled water. To a 1-ml (approximately) suspension of this material, 10 ml of 0.5 per cent osmium tetroxide in phosphate buffered solution was added. The containers were stoppered and put aside for 30 minutes. The fixed mycelia were washed several times with distilled water, then dehydrated by passing through a graded ethyl alcohol series, and then imbedded in a mixture of 95 parts of *n*-butyl metacrylate and 5 parts of methyl metacrylate with 2 per cent benzoyl peroxide as the catalyst. The material in this plastic substance was cut into sections of about 0.1 μ in thickness with an ultramicrotome. Then the sections were carried through amylacetate which removed the plastic substance. The specimens were examined under a J. E. M. type III electron microscope made by Japan Electron Optics Laboratory.

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OBSERVATIONS AND DISCUSSION

Genetic continuity and genetic change of phenomena in microorganisms depend apparently upon the same sequence of events as in the higher organisms. It would be interesting to know the relationship of the metabolic activity of these organisms as compared to that of higher organisms, especially in relation to the utilization of energy and to patterns of synthesis.

Figure 1 is a section of a hypha of *Penicillium chrysogenum*. The cell walls (*C*) are thick, and they seem to be somewhat separated from the cytoplasm. This micrograph illustrates the finer inner structure of the hypha; three nuclei (*N*) are seen in the middle of the cell and many granules (*M*) are scattered in the cytoplasm. The nuclei, as well as the cytoplasm, have a loose filamentous structure. Enveloping the hypha a single coat (*C*) that appears to be homogeneous is observed, and the thickness of the cell wall, though not uniform, is usually considerable.

The resting nuclei as shown in the electron micrograph are round or ellipsoidal, and are filled with smaller granular or filamentous bodies. The nuclear membrane (*NM*) is thin, as can be seen from this figure.

The cross section of the cytoplasmic granules (*M*, figures 1, 2, and 3) is mostly round or oval. A comprehensive electron microscopical study of the cytoplasmic granules in microorganisms has been carried out by Mudd *et al.* (1951*a*, 1951*b*), and Mudd (1954), in whose opinion the cytoplasmic granules of bacteria correspond to mitochondria. The granules which are scattered in the cytoplasm of *Penicillium chrysogenum* as well as in aspergilli and neurospora, already mentioned in the first report, Tsuda (1955*a*), are probably also mitochondria-like bodies.

The varying sizes of the mitochondria-like granules, as shown in figures 1, 2 and 3, suggest differences in growth stages. The inner surface of the inner layer of the limiting membrane exhibits parallel lamellae which seem to protrude

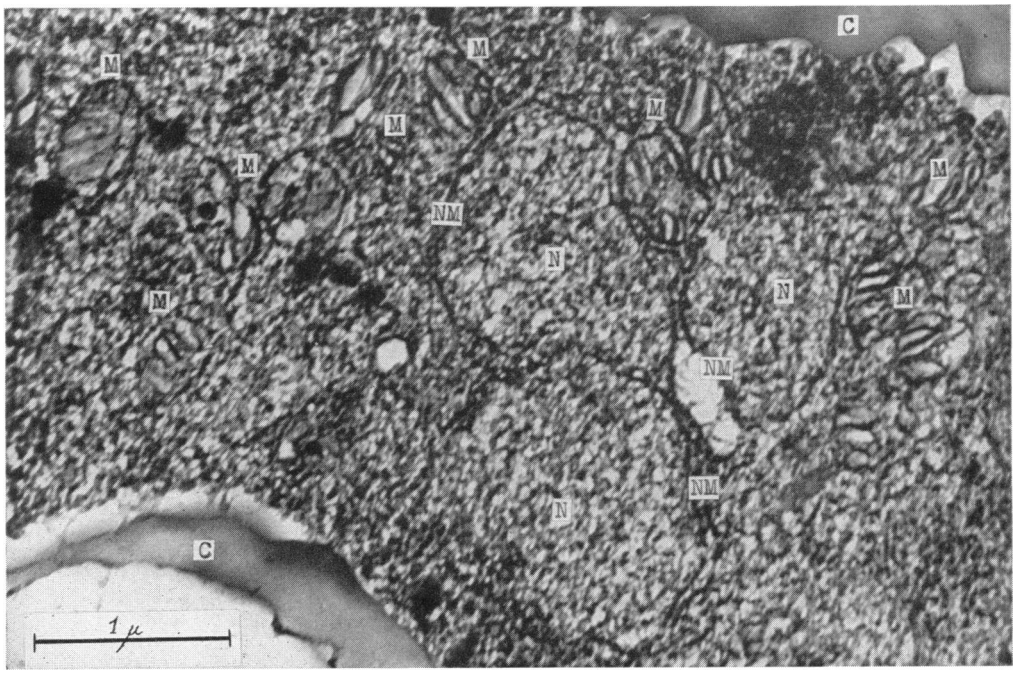

Figure 1. Electron micrograph of ultrathin transverse section of the vegetative mycelium of *Penicillium chrysogenum*. *N*, nucleus; *NM*, nuclear membrane; *M*, cytoplasmic granule; *C*, cell wall.

into the interior of the granules. Also the so-called "cristae mitochondriales" (Palade, 1953) could be observed.

Detailed studies on mitochondria-like granules in bacteria and fungi and their metabolic properties have been carried out by many authors, in mycobacteria, *E. coli*, *B. megaterium*, and *M. cryophilus* by Mudd, *et al.* (1951*a*, 1951*b*); in neurospora by Haskins *et al.* (1953); in *Saccharomyces cerevisiae* by Hartman and Liu (1954), Mundkur (1953), and others. They have found that the mitochondria-like granules are dense bodies possessing limiting membranes and systems of oxidative and reductive enzymes. Subsequently detailed studies carried out on isolated granules, as well as whole cells of microorganisms, showed that they contained oxidase, α-ketoglutaric oxidase, pyruvic oxidase, aconitase, isocitric dehydrooxidase, malic dehydrogenase, and other enzymes.

Figures 2 and 3, the latter representing an enlarged portion of figure 2, illustrate the final stage of division in a hypha. Figure 3 shows a clearly defined transverse wall. Here too, scattered mitochondria-like granules can be seen grouped in the vicinity of the cross wall.

Unfortunately, the observation of the whole process of the nuclear division of fungi has not yet been followed through by electron microscopy. The sections shown represent about one-tenth of the total volume of a cell. The observation of the complete process of nuclear division could therefore not be carried through.

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SUMMARY

By means of ultrathin sections and electron microscopy some new information has been obtained concerning the inner structure of hyphae of *Penicillium chrysogenum*.

The cell wall appears to be thick and homogeneous, although its thickness seems variable.

A number of nuclei were seen in the cross sections of the hyphae, and they were round or ellipsoidal. The nuclear membrane is thin and the nucleus is filled with small granules or filamentous bodies. The nuclear materials and the cytoplasm seem to have a more or less reticular structure.

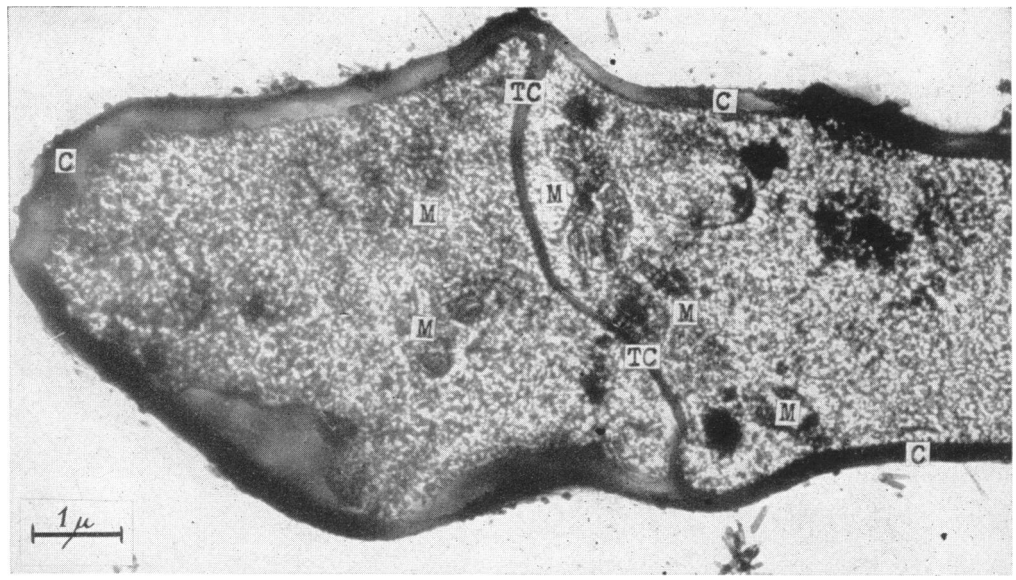

Figure 2. Electron micrograph of ultrathin longitudinal section of vegetative mycelium of *Penicillium chrysogenum*. *M*, cytoplasmic granule; *C*, cell wall; *TC*, transverse cell wall.

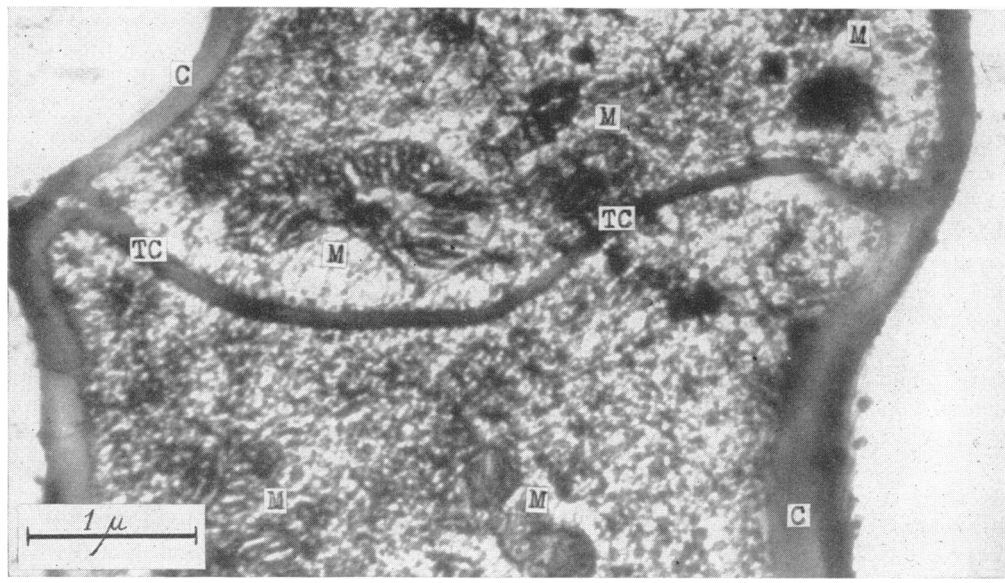

Figure 3. Electronmicrograph of ultrathin section of *Penicillium chrysogenum* enlarged (part of figure 2). *M*, cytoplasmic granule; *C*, cell wall; *TC*, transverse cell wall.

The scattered cytoplasmic granules probably correspond to the mitochondria. They are surrounded by a membrane and the inner surface of the inner layer of the membrane exhibits parallel lamellae and, also, the so-called "cristae mitochondriales" (Palade, 1953).

In the present study, some information has been obtained concerning the finer inner structure of penicillia with regard to the resting nucleus and cytoplasmic mitochondria-like granules.

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