

ELECTRON MICROSCOPY OF A FISSION YEAST, *SCHIZOSACCHAROMYCES POMBE*

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ABSTRACT

MACLEAN, NORMAN (University of Edinburgh, Edinburgh, Scotland). Electron microscopy of a fission yeast, *Schizosaccharomyces pombe*. *J. Bacteriol.* **88**:1459-1466. 1964.—The structure of the fission yeast, *Schizosaccharomyces pombe*, was studied electron microscopically, with potassium permanganate and osmium tetroxide as fixatives. The cell was found to be bounded by a cell wall, 1,000 to 2,000 Å thick, and a cell membrane. A layer of material was found between the cell membrane and the wall. The central nucleus, 2 to 3 μ in diameter, was bounded by a nuclear membrane, seen in some pictures to be double. Osmium tetroxide fixation revealed a granular body within the nucleus, identified as a nucleolus. Cytoplasmic structures included numerous vacuoles (probably normally containing lipid), a number of membranes and vesicles (which may represent a poorly organized mitochondrial system), and numerous granules (probably representing ribosomes).

Studies of budding yeast by light microscopy are numerous (Lindgren, 1949; Leupold, 1956; Ganesan, 1959), and electron micrographs of *Saccharomyces cerevisiae* have been published (Hashimoto, Conti, and Naylor, 1959; Yotsuyanagi, 1960, 1962; Hirano and Lindgren, 1963; Vitols, North, and Linnane, 1961; Hirano, 1962; Koehler, 1962; Tanaka, 1962; Mundkur, 1961). An electron microscopic study of frozen etched cells of this yeast was published by Moor and Muhlethaler (1963); electron micrographs of *Schizosaccharomyces octosporus* (Conti and Naylor, 1960), *Torulopsis utilis* (Linnane, Vitols, and Nowland, 1962), and *Pichia* (van Iterson, 1960) have appeared.

Work on the structure of *Schizosaccharomyces pombe* is sparse. The position of its nucleus was demonstrated by Ganesan and Swaminathan (1958), using staining and light microscopy, and by Rustad (1958), using fluorescent microscopy.

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Nuclear division in *S. pombe* was studied by Sando and Tanaka (1963) and Schopfer, Wustefeld, and Turian (1963). One paper on the electron microscopy of this cell has been published (Tanaka, 1963).

MATERIALS AND METHODS

Organism. *S. pombe* (National Collection of Yeast Cultures 132) is a fission yeast, cylindrical in shape, with the ends of the cell hemispherical. Normal cells vary between 8 and 20 μ in length, maintaining a fairly constant diameter of 3.5 μ. Although sexual reproduction by conjugation and resulting spore formation is known in this species, all the cells examined were growing vegetatively.

Culture. Cells were grown in 2% (w/v) Oxoid wort broth (malt-extract broth) in distilled water. All the cells described were in the exponential phase of growth from cultures containing between 3×10^8 and 5×10^6 cells per ml. Cultures were grown at 33 C; the generation time at this temperature is slightly over 2 hr. The method of culture was to inoculate sterile 1-liter flasks containing 400 ml of medium, and the flasks were never aerated or agitated during growth.

Electron microscopy. Cultures were harvested by centrifugation at $2,700 \times g$, and were fixed in the following ways: (i) cells were washed twice in 0.25 M sucrose, buffered with Veronal acetate at pH 7.2, and fixed for 1 hr at 0 C in 2% osmium tetroxide in the Veronal acetate buffer; (ii) cells were washed twice in 1.5 M saline, followed by fixation for 1 hr at 0 C in 1.5 M potassium permanganate in the 1.5 M saline.

After fixation, cells were dehydrated in a graded series of alcohols (from 20% to absolute alcohol), the dehydration being completed by three washes in absolute alcohol. Methacrylate was employed as an embedding medium (a mixture of 92% butyl methacrylate, 7% methyl methacrylate, and 1% benzoyl peroxide). Material fixed in permanganate was much more prone

to "explosion" during polymerization than that fixed in osmium tetroxide, and Borysko's technique with prepolymerized methacrylate proved essential to the embedding of such material. Polymerization of the embedded material was carried out at 58 C.

Sections were cut with glass knives on a Huxley ultramicrotome and were floated onto a distilled water reservoir, from which they were picked up on carbon-coated grids after expansion by contact with xylene vapor.

After sections had been transferred to grids and dried, some of the grids were immersed in a staining solution, to improve contrast in the microscope. Both uranyl acetate and potassium permanganate were used in this way on different sections of both types of fixed material. Staining was carried out in petri dishes at 20 C for 30 min to 1 hr.

A Siemens Elmiskop I electron microscope was used in examining sections, at 40 or 60 kv and with a 30 μ objective aperture. Photographs were taken on Ilford N 40 plates at instrumental magnifications of 15,000 to 30,000 \times .

RESULTS AND DISCUSSION

Cell wall. Growth of a cell of *S. pombe* is lengthwise and usually at only one end of the cell. The growing end appears to be the end opposite to the most recent division scar, at the periphery of the cell plate. It is therefore not surprising to find that cell-wall thickness varies from one part of the cell to another. The general dimensions of the cell wall along the length of the cell are between 1,000 and 2,000 A (Fig. 2 and 3). Old division scars may result in areas of greater thickness. Some electron micrographs reveal two layers to the wall (Fig. 3), a thin outer coat of about 400 A which appears coarse and granular when fixed, and a thick inner layer of around 1,000 A and with very low electron density in unstained sections. The work of Vitols et al. (1961) revealed a cell wall of similar appearance in *Saccharomyces cerevisiae*.

Cell membrane. The cell membrane as revealed by permanganate fixation consists of a very pronounced central light layer bounded by two more indistinct dark lines. This structure measures about 80 A, which is the general dimension of the single membrane occurring in most cells fixed with permanganate. Figures 2 and 3 demonstrate this membrane fairly well. Material fixed with osmium tetroxide shows only poorly defined

cell membranes; both Agar and Douglas (1957) and Vitols et al. (1961) commented on the difficulty of fixing yeast cells with osmium tetroxide.

Between the cell wall and the cell membrane is to be found a layer of material staining darkly with uranyl acetate and sending protrusions of varying size into the cell (Fig. 3). Although remaining outside the protoplast, this layer does not seem to be an integral part of the cell wall.

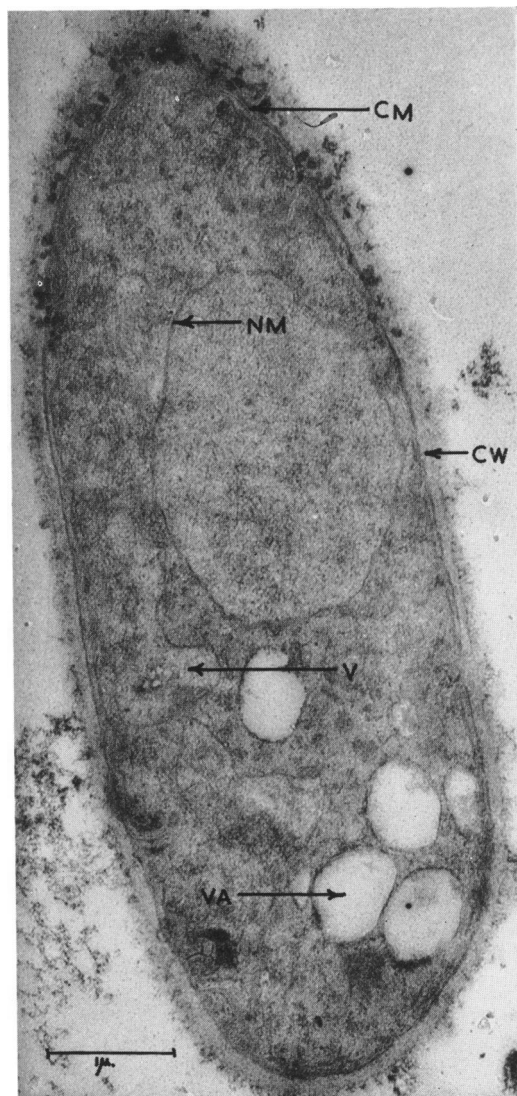


FIG. 1. Whole cell in longitudinal section; potassium permanganate fixation with no postfixation staining. CM = cell membrane; CW = cell wall; NM = nuclear membrane; V = vesicle; VA = vacuole.

A similar layer of material was noticed by Lindegren (1963) in *S. cerevisiae*, and he identified it as nucleoprotein on the basis of its dark staining with uranyl acetate.

Cytoplasmic membranes and vesicles. Throughout the cytoplasm of *S. pombe* there is a mem-

branous system, much of which is organized into closed vesicles but which also includes apparently loose membranes dispersed throughout the cytoplasm (Fig. 2 and 4). The possibility that some of these open membranes represent vesicles disrupted during fixation or embedding cannot of

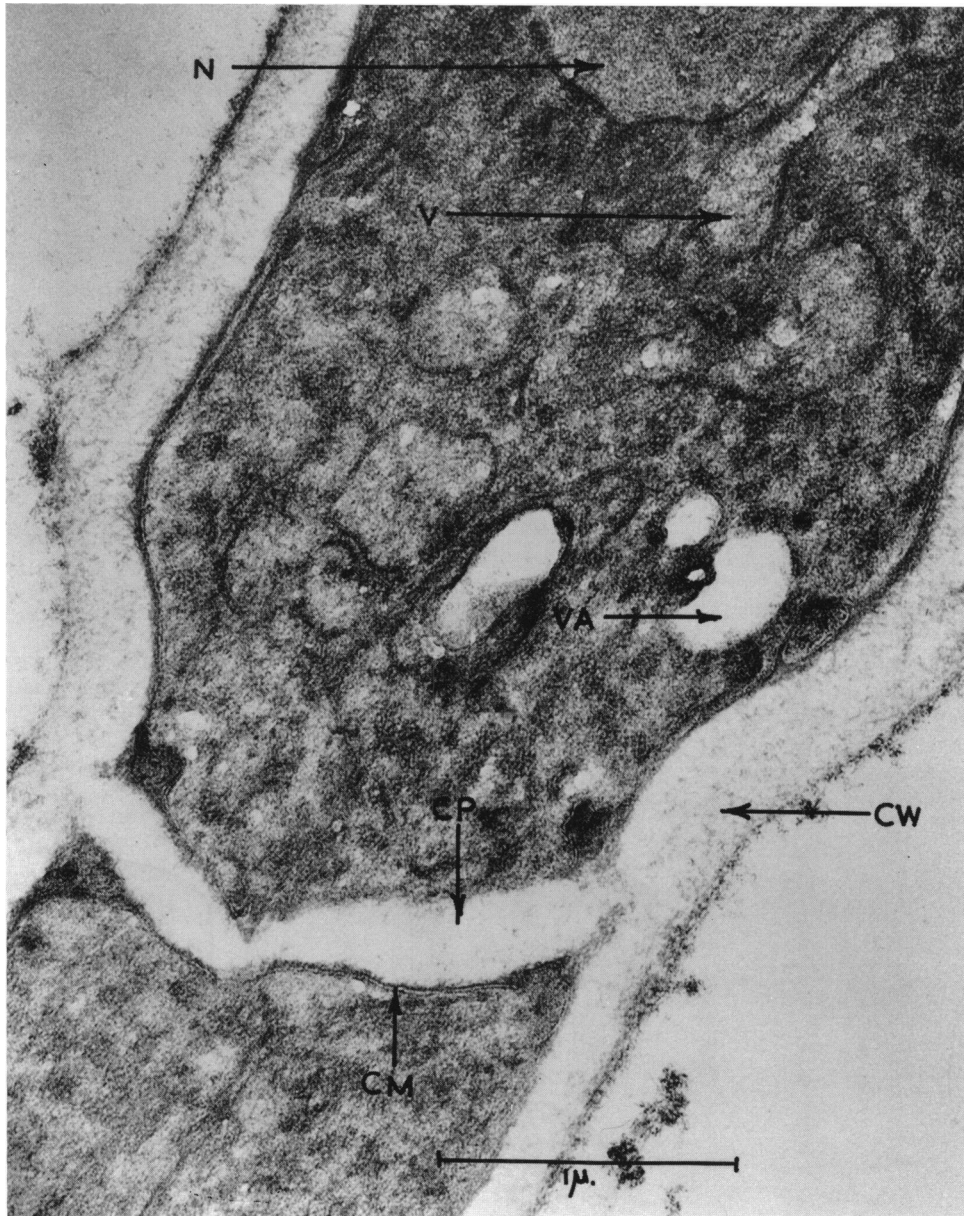


FIG. 2. Section of cell just prior to division, with cell plate formed; potassium permanganate fixation followed by potassium permanganate staining. CM = cell membrane; CP = cell plate; CW = cell wall; N = nucleus; V = vesicle; VA = vacuole.

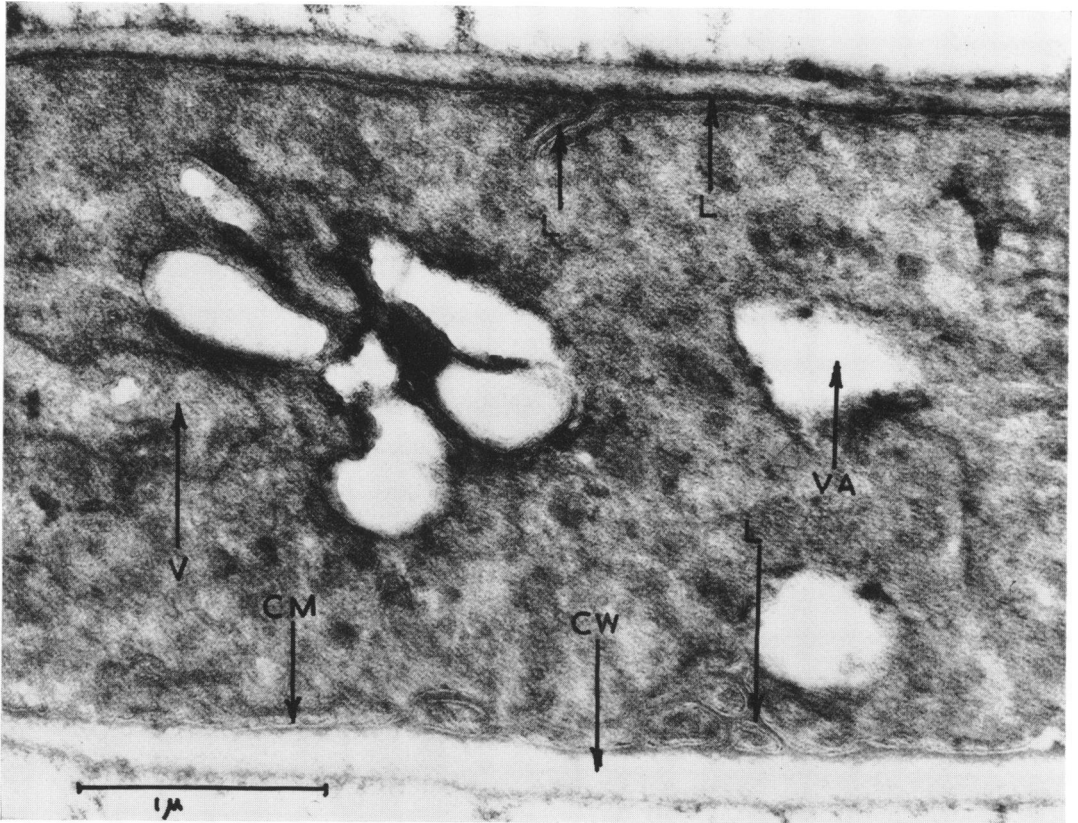


FIG. 3. Central portion of a cell; potassium permanganate fixation followed by potassium permanganate staining. CM = cell membrane; CW = cell wall; L = layer of material between cell membrane and wall; V = vesicle; VA = vacuole.

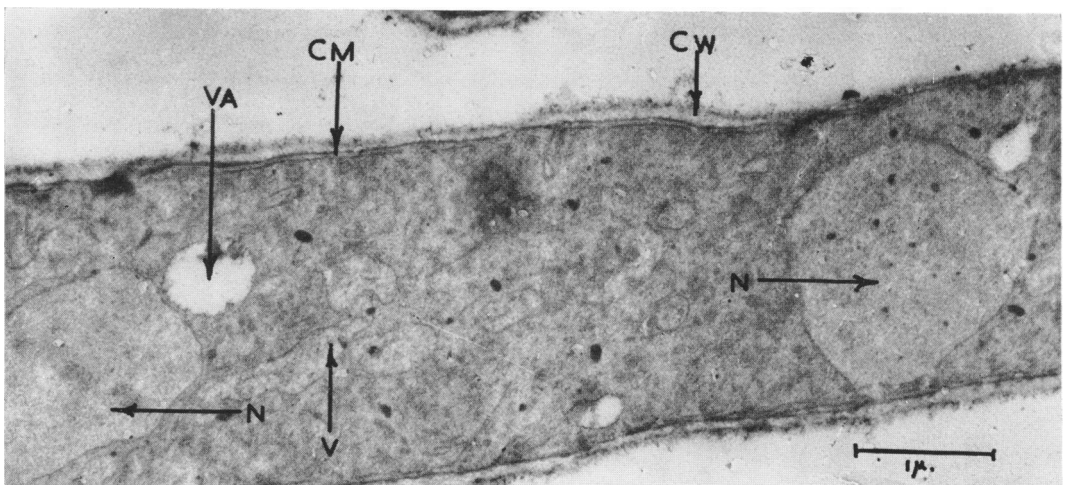


FIG. 4. Cell after nuclear division but before cell plate formation; potassium permanganate fixation with no postfixation staining. CM = cell membrane; CW = cell wall; N = nucleus; V = vesicle; VA = vacuole.

course be ruled out. Studies of bacterial cytoplasm have revealed the frequent presence of whorled formations of unit membranes in the cytoplasm (Glauert and Hopwood, 1960). The work of Linnane et al. (1962) on *Torulopsis* grown under anaerobic conditions revealed a system of straggling cytoplasmic membranes; these workers suggested that yeast cells grown under anaerobic conditions do not develop

mitochondria, but instead possess this straggling membranous and vesicular system. It has now been amply demonstrated that budding yeasts do possess mitochondrial vesicles with numerous cristae; but, the membranous vesicles in *S. pombe* do not appear to possess cristae, and their function remains to be discovered. It is also apparent from the cells fixed in osmium tetroxide (Fig. 8) that ribosomes do not occur inside these

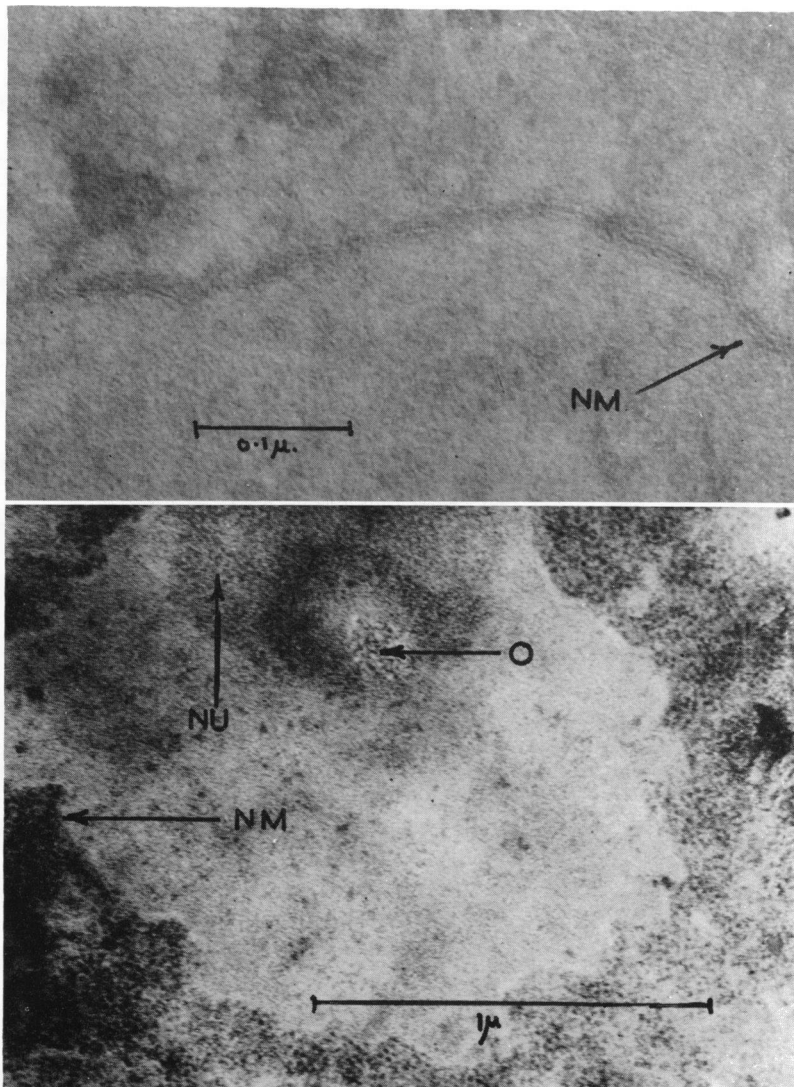


FIG. 5. (top) High magnification of nuclear membrane to show double-membrane structure at arrow; potassium permanganate fixation with no postfixation staining.

FIG. 6. (bottom) Nuclear region of cell; fixation with osmium tetroxide and stained after fixation with uranyl acetate. NM = nuclear membrane; NU = nucleolus; O = fibrillar object associated with the nucleolus. Note ribosomes in the cytoplasm and the poorly stained nuclear membrane.

vesicles. In the work of Yotsuyanagi (1962) on budding yeast at different stages of growth, it seems that true mitochondria with obvious cristae appeared only in cells entering or in stationary phase, and that log-phase cells normally had no mitochondria whatever and only some scattered membranes. Since the cells of *S. pombe* under study were from log-phase cultures, it would appear that the lack of true mitochondria is not an isolated example.

As found by Koehler (1962) in *S. cerevisiae*, there is often present in *S. pombe* a membranous layer extending around the periphery of the cell in close proximity to the cell membrane itself. This is particularly obvious in Fig. 4.

Storage granules. Living cells of *S. pombe*, when viewed by phase-contrast microscopy, display highly refractile particles which are especially frequent near to the ends of the cell. These particles give staining reactions suggestive of lipid material (J. M. Mitchison, *personal communication*), and the dimensions and location of these particles in the light microscope are in keeping with the holes present in sections after

fixation for electron microscopy (Fig. 1 and 2). The correlation between similar holes and lipid storage material has been suggested in work on bacteria by Wyss, Neumann, and Socolofsky (1961), and holes are present also in electron micrographs of budding yeast (Koehler 1962). The large vacuole present in budding yeast, situated near to, and in the past often confused with, its nucleus (Lindegren, 1949), is absent from *S. pombe*.

Nucleus. The nucleus is fairly central, and between 2 and 3 μ in diameter (Fig. 1, 6, and 7). In material fixed with osmium tetroxide or potassium permanganate, its contents are less electron-dense than is the cytoplasm.

Nuclear membranes. It is less easy to achieve clarity of definition of the nuclear membranes than it is of the cell membrane, but in some sections a double-membrane structure is discernible (Fig. 5). By a double membrane is meant a structure measuring about 150 A and consisting of three lines of electron-dense material alternating with two lines of rather electron-transparent material, the central dark line normally

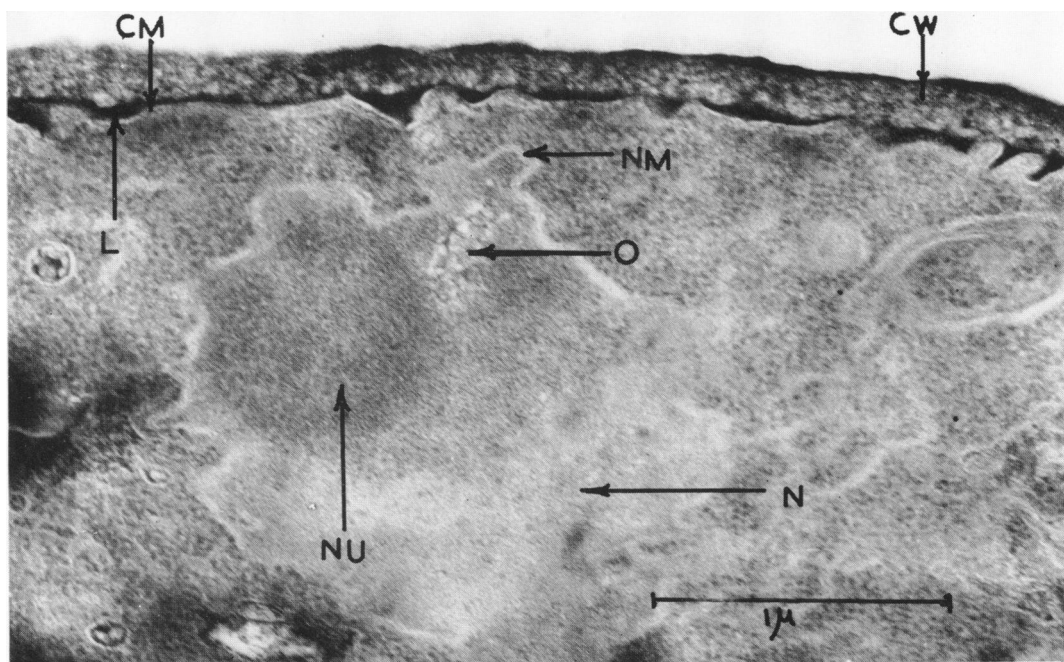


FIG. 7. Part of cell showing nucleus; fixation with osmium tetroxide and stained after fixation with uranyl acetate. CM = cell membrane; CW = cell wall; L = layer between cell membrane and wall; N = nucleus; NM = nuclear membrane; NU = nucleolus; O = fibrillar object associated with the nucleolus. Note ribosomes in the cytoplasm and poorly stained membranes.

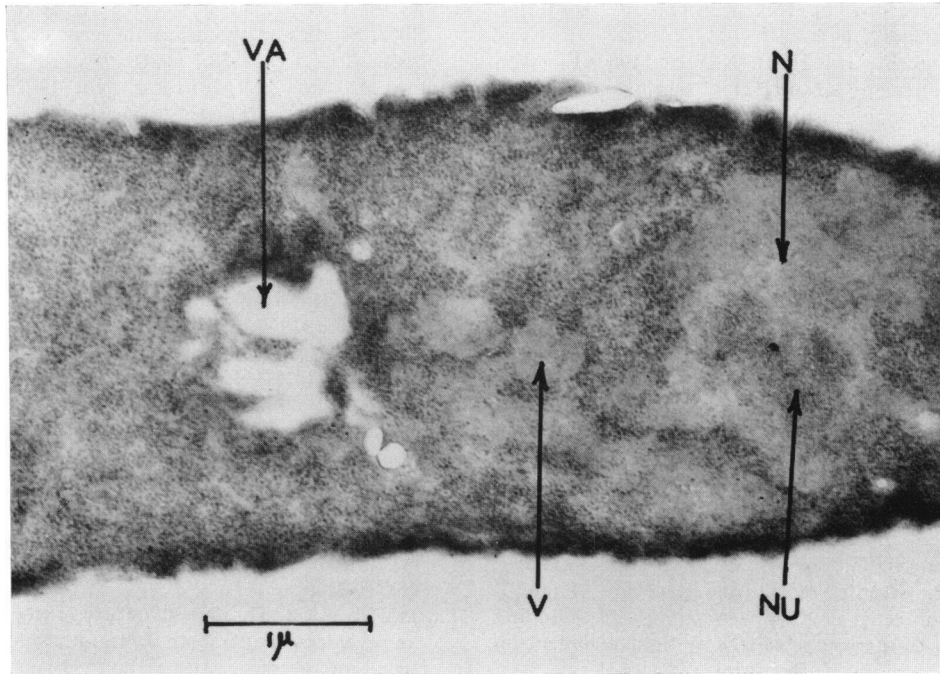


FIG. 8. Part of cell fixed in osmium tetroxide but with no postfixation staining. *N* = nucleus; *NU* = nucleolus; *V* = vesicle; *VA* = vacuole. The cell wall is not sufficiently stained to appear in the print. Note ribosomes in the cytoplasm.

being the thickest (Robertson, 1959). The results of the osmium tetroxide fixation on the nuclear membranes and on the cytoplasmic membranes are very curious in *S. pombe*. In Fig. 6, 7, and 8, these membranes are poorly defined and less dense to electrons than either the nuclear or cytoplasmic contents. The reason for this apparent osmiophobic reaction is obscure.

Nucleolus. When fixed with potassium permanganate, the nuclear contents of *S. pombe* are uniformly unorganized and rather less dense than the cytoplasm; with osmium tetroxide fixation, a large and very dense nucleolus becomes visible (Fig. 6 and 7), measuring 1.2 to 1.4 μ in diameter. No enclosing membrane can be detected at its surface. In all osmium tetroxide-fixed cells, the nucleolus is seen to be finely particulate in structure; in some sections, a fibrillar organelle is associated with it (Fig. 6 and 7). Since all the recent electron micrographs of *S. cerevisiae* have involved fixation with potassium permanganate, the presence of a nucleolus in this cell has not been demonstrated. Edwards, Hazen, and Edwards (1959) displayed

it very clearly in the yeastlike cells of *Histoplasma* fixed with osmium tetroxide. The work of Mundkur (1961) on *S. cerevisiae* (freeze-drying fixation) displays a dark and highly granular object in the nucleus that is probably the nucleolus.

Ribosomes. After fixation with potassium permanganate, the cytoplasm shows little or no trace of granularity. After osmium tetroxide fixation (Fig. 6, 7, and 8), the cytoplasm is particulate; the particles presumably represent ribosomes, being about 150 A in diameter. These ribosomes appear to be evenly distributed in the cytoplasm, and are not significantly related to any membranes.

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