STUDIES ON THE OXIDATIVE METABOLISM OF *SACCHAROMYCES CEREVISIAE*

I. Observations on the Fine Structure of the Yeast Cell

EBERHARDS VITOLS, R. J. NORTH, and ANTHONY W. LINNANE, Ph.D.

From the Biochemistry Department and the Electron Microscope Unit, University of Sydney, New South Wales, Australia

ABSTRACT

Vegetative cells of *Saccharomyces cerevisiae* were fixed with potassium permanganate followed by uranyl nitrate, embedded in methacrylate, and studied in electron micrographs of thin sections. Details of the structure of the cell wall, cytoplasmic membrane, nucleus, vacuole, and mitochondria are described. Cell membranes, about 70 to 80 A thick, have been resolved into two dense layers, 20 to 25 A thick, separated by a light layer of the same dimensions, which correspond in thickness and appearance to the components of the "unit membrane" as described by Robertson (15). The cell wall is made up of zones of different electron opacity. Underlying the cell wall is the cytoplasmic membrane, a sinuous structure with numerous invaginations. The nucleoplasm, often of uneven electron opacity, is enclosed in a pair of unit membranes in which nuclear pores are apparent. The vacuole, limited by a single unit membrane, is usually irregular in outline and contains some dense material. Rod-shaped mitochondria, 0.4 to 0.6 μ in length and 0.2 to 0.3 μ in diameter, are smaller in size, but similar in structure to some of those described in plant and animal cells. Attempts to use osmium tetroxide as fixative were unsuccessful, a result similar to that obtained by other workers. It is suggested that yeast cells are impermeable to osmium tetroxide, except when grown under specific conditions.

The fine structure of the yeast cell was first studied in the electron microscope by Agar and Douglas (1). The cells were grown on a special presporulation medium and fixed in osmium tetroxide. These authors found, however, that cells grown on other media failed to fix satisfactorily in osmium tetroxide. We have confirmed this observation, but have found that cells grown on chemically defined media under aerobic conditions and suitable for metabolic studies can be satisfactorily prepared for electron microscopy by the fixation procedure described below. Membrane systems are particularly well preserved by this method.

This communication is a part of a study of the oxidative metabolism in baker's yeast and describes the structure of the yeast cells used for such studies. An accompanying paper (17) describes some of the biochemical properties of isolated yeast particles and relates their morphology to cytoplasmic structures described herein.

MATERIALS AND METHODS

Aerobically grown baker's yeast cells in their early stationary phase were obtained directly from the fermenters of Mauri Bros. & Thomson Ltd., Sydney. They were washed and used directly or transferred onto one of the two following media for further growth. On a small scale the cells were mainly grown

in shake cultures on a glucose–ammonium sulphate–salts medium (16). Cells in larger quantities were grown under forced aeration on a medium adjusted to pH 5.0 and containing molasses, ammonium sulphate, and wheat germ extract.

For cytological studies the cells were washed with several changes of distilled water and then fixed for 10 minutes at 0°C., in a 2 per cent solution of potassium permanganate, buffered at pH 7.2 with 0.02 M veronal acetate. They were then washed in several changes of a 2 per cent aqueous solution of uranyl nitrate and allowed to remain in the final change for 1 hour. After washing in tap water for 15 minutes and dehydration through an alcohol series in the usual manner, the dehydrated material was suspended for 3 hours in a mixture of *n*-butyl and methyl methacrylates in a proportion of 6 to 1, respectively. The suspension was then centrifuged and the cells were resuspended in a partially polymerized methacrylate mixture of the same composition for 30 minutes. The cells were again sedimented by centrifugation and small pieces of this pellet were transferred to the partially polymerized methacrylate mixture in gelatin capsules. Embedding was completed by incubation of the capsules at 60°C. for 48 hours.

Thin sections were cut with a Porter-Blum microtome using a diamond knife, and only those exhibiting silver or golden interference colours were examined. The sections were floated onto copper grids coated with nitrocellulose films stabilized with a carbon layer.

Electron micrographs were taken with a Siemens Elmiskop I electron microscope using a 50 μ objective aperture.

RESULTS AND DISCUSSION

Some of the details of the fine structure of yeast cells fixed with osmium tetroxide have been described by Agar and Douglas (1). Their method, however, was successful only with cells grown on Lindegren's presporulation medium and then stored on gypsum slopes for 20 hours. These results suggest that osmium tetroxide fixes yeast cells only at one particular physiological stage in their development. Using cultures harvested at various growth phases from either of the two media mentioned above, we have so far found osmium tetroxide unsatisfactory as a fixative for the electron microscopic study of vegetative cells. In only an occasional cell was it possible to identify certain cytoplasmic structures, though contrast was poor. A brief pretreatment of the yeast with a detergent (Teepol, Shell Oil Company) resulted in the fixation of a higher proportion of cells. However, such treatment with a detergent may destroy certain cytoplasmic structures and is therefore not suitable for routine use. It appears that the vegetative cells are not easily penetrated by osmium tetroxide, and that the detergent acts by removing some permeability barrier. Yotsuyanagi (18) has also recently indicated that osmium tetroxide is an unsatisfactory fixative for yeast cells. He has described a procedure in which the cells are fixed with formol, digested with ribonuclease, and then postfixed with osmium tetroxide.

We have found that a combination of potassium permanganate and uranyl nitrate is a good and reliable fixative for vegetative cells in all phases of growth, and that fixation is independent of the culture medium on which the cells are grown. When potassium permanganate was used alone as a fixative the cytoplasmic structures were largely preserved, but the membranes were indistinct and contrast within the sections was poor. Uranyl nitrate treatment of the whole cells after permanganate fixation leads to better preservation of the membrane structures and to an improvement in general contrast. This treatment may be regarded as a "postfixation," since staining of permanganate-fixed sections on the grid in the conventional manner with uranyl nitrate fails to reveal the membranes satisfactorily. Kellenberger *et al.* (8, 9) have used uranyl salts in a similar manner after osmium tetroxide fixation of *Escherichia coli*.

A typical section through a budding cell, fixed with potassium permanganate and uranyl nitrate, is shown in Fig. 1, which serves to summarize the cytology of the yeast cell. It confirms the general picture reported by Agar and Douglas (1). A well defined cell wall with prominent bud scars is evident. Immediately below this is a thin, crenated cytoplasmic membrane (better shown in Fig. 2). Mitochondria are present throughout the cytoplasm. The vacuole is irregular in outline, particularly in younger cells, as may be observed in the bud in Fig. 1. The nucleus is usually situated at one pole of the cell, and in Fig. 1 it is shown at an early stage of division by a process already described by Hashimoto *et al.* (6). The ground substance of the cytoplasm has a fine granular appearance.

Cell Wall

Chemical analyses of yeast cell walls (5, 13) have revealed that they are composed of about 70 per cent polysaccharides, 5 to 10 per cent proteins, and

2 to 10 per cent lipids. The electron opacity of the cell wall is considerably less than that of the cytoplasm. This is consistent with the chemical analyses, since permanganate is apparently a protein stain (2). There is no evidence that the cell wall consists of two separate "membranes" as claimed by Bartholomew and Levin (3), but concentrations of electron-opaque material appear to be localized immediately below the outer surface and in the region contiguous with the cytoplasmic membrane (Figs. 2, 3, and 5). This material may be cell wall protein specifically located in those regions, although the electron opacity of the outermost layer could also be due to polysaccharide breakdown products which might react with permanganate. It is interesting to note that the bud scars have a different structure and show a greater concentration of electron-opaque material in the centre.

Cytoplasmic Membrane

As noted by Luft (11) and Mollenhauer (12), permanganate seems to be more specific for the preservation of cell membranes than osmium tetroxide. The cytoplasmic membrane in yeast cells fixed with osmium tetroxide has been described as a single electron-opaque membrane (1). In cells fixed with permanganate–uranyl nitrate the cytoplasmic membrane appears as two dense lines separated by a less dense layer (Figs. 2, 3, and 5). The over-all thickness of this structure is about 80 A, which is of the same order of membrane thickness as that seen with osmium tetroxide–fixed material. Robertson (15) has defined a single unit membrane as one consisting of two dense layers separated by a light one; it has been suggested that these represent two layers of protein separated by a layer of lipid. It seems likely that in permanganate-fixed material the membrane can be resolved into these components. However, it should be pointed out that the nature of the cytoplasmic membrane does appear to be different from that of the other membrane systems —nuclear, vacuolar, mitochondrial—described in this paper. The latter membrane systems all show well delineated electron-opaque layers, separated by a light one, but the cytoplasmic membrane has by comparison two poorly defined outer layers separated by a more pronounced light layer.

Nucleus

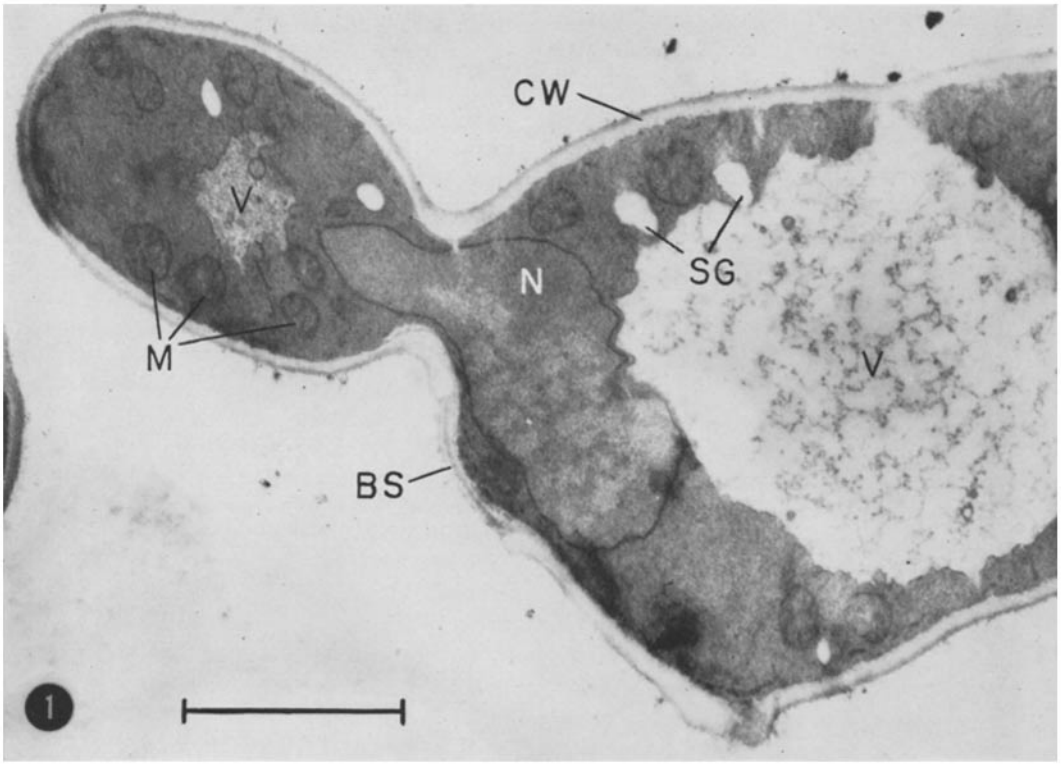
The nature of the yeast nucleus has been the subject of considerable debate over the past decade. Some earlier workers suggested that the central vacuole was a part of the nucleus (10), but recent investigations leave little doubt that they are separate structures (1, 6, 18). This is confirmed by the electron micrograph shown in Fig. 6. The nucleus is enclosed within a pair of unit membranes (the typical nuclear envelope) and is lying close to, but separate from, the vacuole, which has only a single unit membrane. Several breaks in the nuclear envelope are apparent in Figs. 2 and 6. An enlargement of one of these areas (Fig. 7) shows the detailed structure of the nuclear envelope and the discontinuities of the membrane system to form a pore. The total thickness of the nuclear envelope is about 200 A. The unit membranes are some 50 A apart and are made up of two electron-opaque layers, each about 20 to 25 A thick, separated by what is presumably a lipid layer of the same thickness. A through-focus series, however, was not obtained, and these measurements are therefore only approximate.

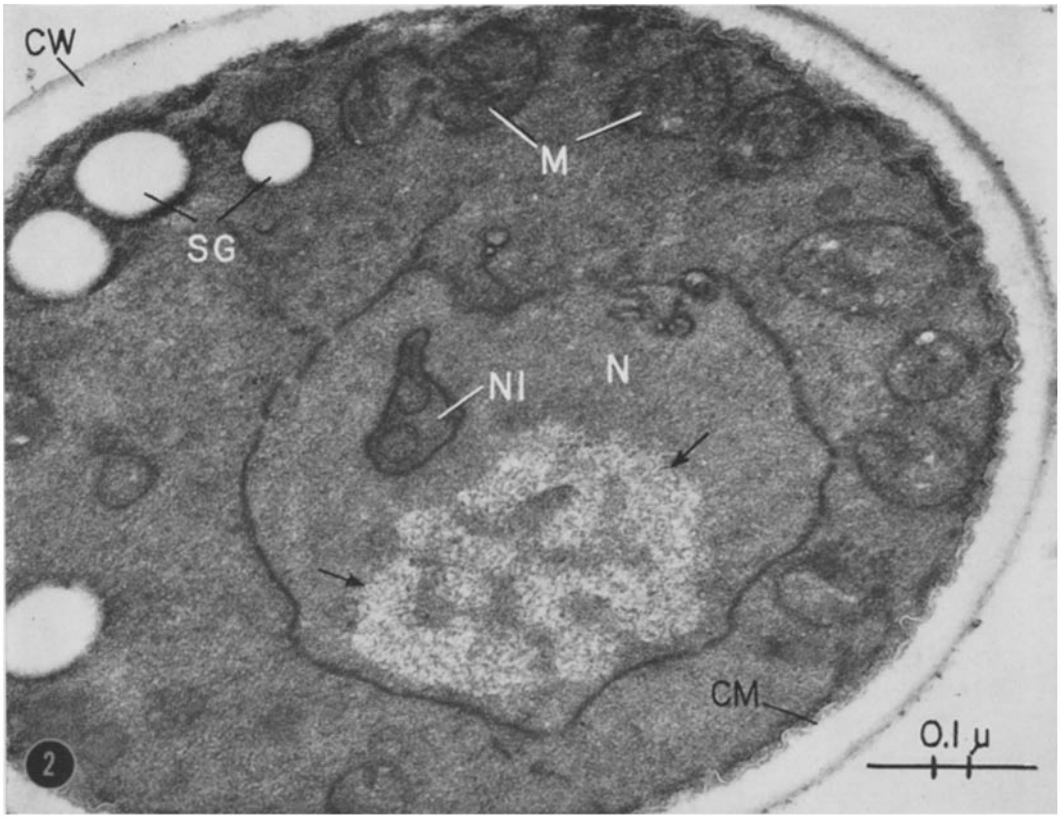
In some sections the nucleoplasm exhibits a non-uniform density (Figs. 1 and 2) which has been previously observed by other workers (7, 18). Yotsuyanagi (19) has described some interesting experiments in which electron microscopic evidence was presented suggesting that the material in areas of low electron opacity is chromosomal in nature.

The membranous structure within the nucleus shown in Fig. 2 is probably a cross-section of an invagination of the nuclear membrane.

Vacuole

The vacuole appears largely as an electron-transparent area limited by a single unit membrane consisting of the three layers described above (Figs. 1, 3, 4, and 8). In some cells the shape of the vacuole may be quite irregular with narrow diverticula extending far into the cytoplasm. A particular section may be cut so as to separate such diverticula from the rest of the vacuole, in which case they would appear as separate membrane systems within the cytoplasm. Buvat (4) from a study of plant cells has presented evidence suggesting that the vacuole is formed by dilations of the endoplasmic reticulum. On this basis the vacuolar membrane might be regarded as a specialization





of the endoplasmic reticulum. However, paired membrane systems separated from the vacuolar membrane, as shown in Fig. 8, are not commonly seen in sections of yeast cells. The rarity of this observation suggests that the extensive endoplasmic reticulum seen in certain cells of higher organisms does not exist in yeast.

Distributed throughout the vacuole are strands and granules of some dense material sometimes linked into a network (Figs. 1, 3, and 8). In the larger cells roughly spherical granules with an average diameter of 0.05 μ and of very high electron opacity are observed (Fig. 6). These granules may represent volutin (polymetaphosphate granules), described by early workers in yeast cytology as occurring in the vacuole (for references see 10).

Mitochondria

Organelles having typical mitochondrial structure are present throughout the cytoplasm (Figs. 1, 2, and 8). In section the mitochondria appear circular or elliptical in outline and thus are probably rod-shaped within the cell, about 0.4 to 0.6 μ in length and 0.2 to 0.3 μ in diameter. The number of cristae in each mitochondrion appears to be of about the same order, per unit area, as that observed in liver mitochondria. The cristae appear to run irregularly in various directions within the mitochondria (Figs. 3 and 6) and not necessarily parallel to their long axes as reported by Agar and Douglas (1). The mitochondria are paired membrane systems as shown in Figs. 3 and 6. Inspection of the mitochondrial section in Fig. 6 (arrow) indicates that the cristae are formed by the infolding of the inner membrane of the mitochondrion as suggested by Palade (14).

Storage Granules

Apart from the cytoplasmic structures so far described, a number of fairly well defined electron-transparent areas are observed in sections of most cells (Figs. 1, 2, and 6). Some of these may be parts of the vacuole, in which case they are enclosed by a single unit membrane (Fig. 6). Others are not enclosed by a membrane (Fig. 2) and probably represent spaces formerly occupied by glycogen or lipid. The "empty" appearance of these granules after permanganate fixation has been noted by other workers (6, 18).

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Explanation of Figures

KEY TO ABBREVIATIONS

BS, bud scar	*NI*, nuclear invagination
CM, cytoplasmic membrane	*NM*, nuclear membrane
CW, cell wall	*NP*, nuclear pore
Cyt., cytoplasm	*SG*, storage granule
G, polymetaphosphate granule	*V*, vacuole
M, mitochondrion	*VM*, vacuolar membrane
N, nucleus	

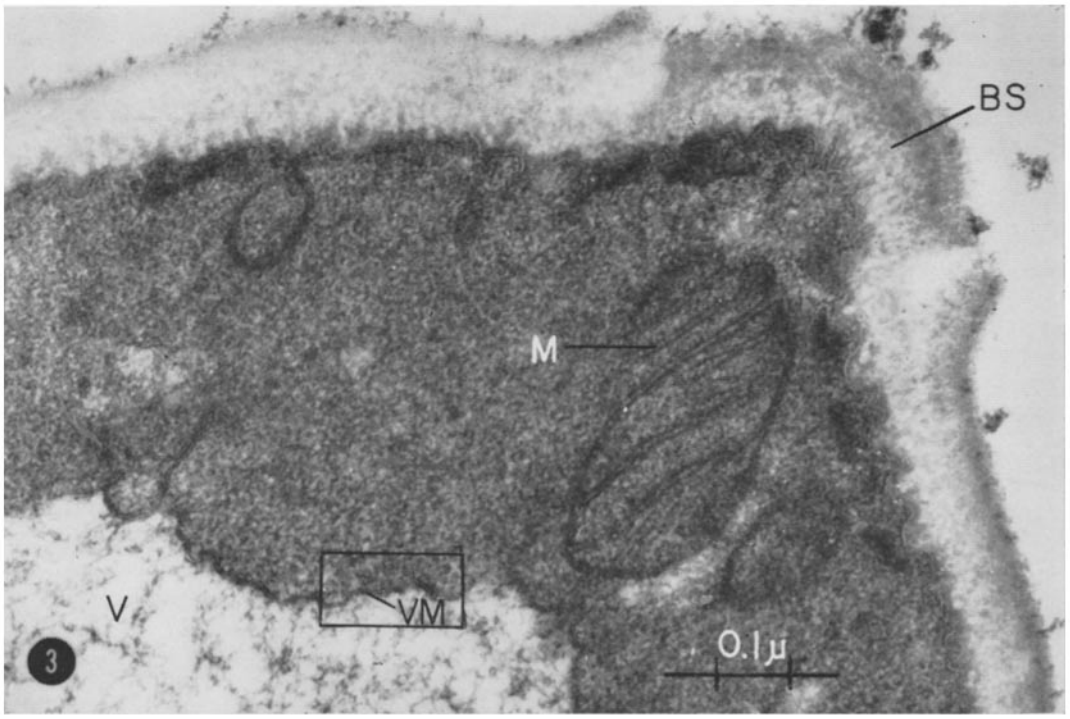
In all figures the length of the black rod represents 1 μ, unless otherwise indicated.

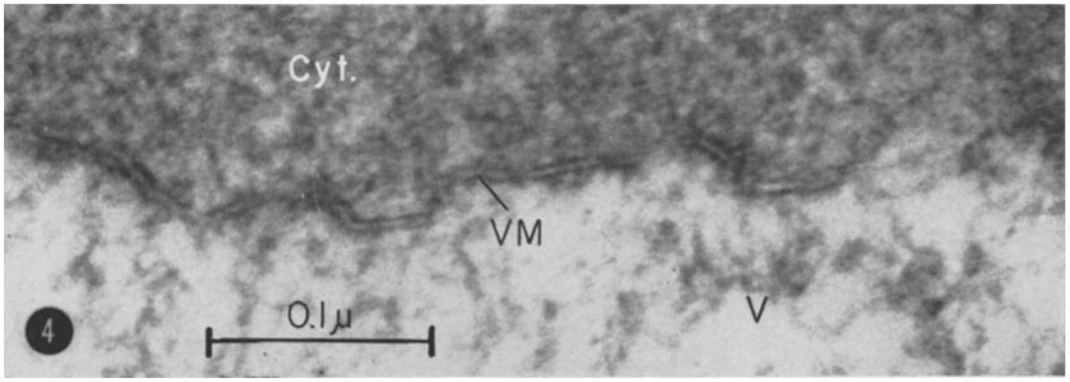
FIGURE 1

Section of a budding yeast cell. The nucleus (*N*) is seen at an early stage of division. The cell wall (*CW*), vacuoles (*V*), mitochondria (*M*), and storage granules (*SG*) are discernible in both the mother cell and the bud. A bud scar (*BS*) in the wall of the mother cell is apparent. × 30,000.

FIGURE 2

Section through a resting yeast cell showing cell wall (*CW*), cytoplasmic membrane (*CM*), storage granules (*SG*), mitochondria (*M*), and nucleus (*N*). Areas of varying density are apparent within the nucleus (arrows). The membranous structure (*NI*) apparently within the nucleus is believed to result from an invagination of the nuclear membrane. × 50,000.





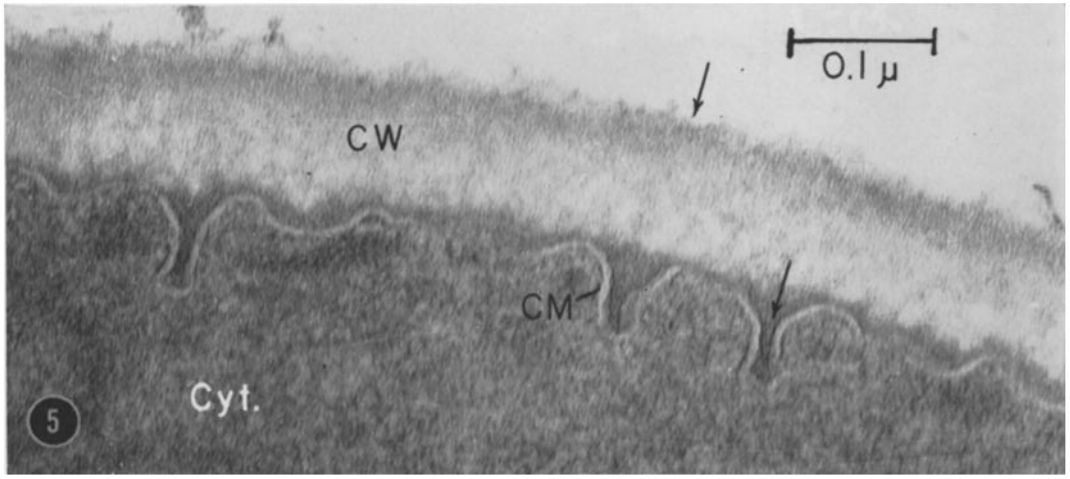


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FIGURE 3

Section through part of a yeast cell to show the fine structure of a mitochondrion (*M*). In addition, a part of the vacuole (*V*) with its limiting single unit membrane (*VM*) and a bud scar (*BS*) are shown. × 100,000.

FIGURE 4

A higher magnification of the area indicated in Fig. 3, showing the unit membrane of the vacuole (*VM*). × 300,000.

FIGURE 5

Cross-section through the cell wall (*CW*) and cytoplasmic membrane (*CM*). The cell wall has two electron-opaque zones, one along the periphery and the other immediately above the cytoplasmic membrane (arrows). The latter structure, a unit membrane, is about 75 A thick and is resolved into two electron-opaque layers separated by a lighter layer. × 200,000.

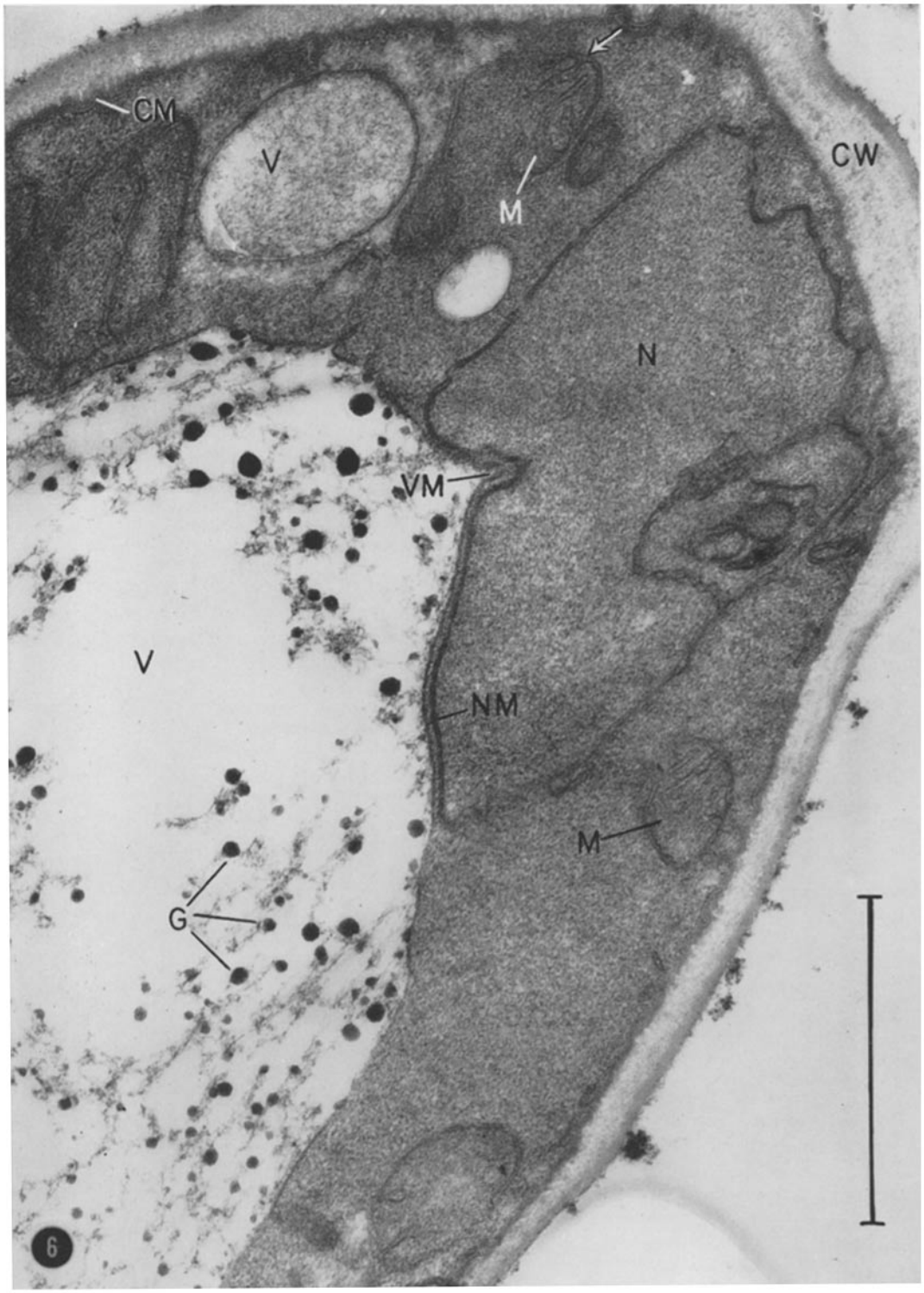
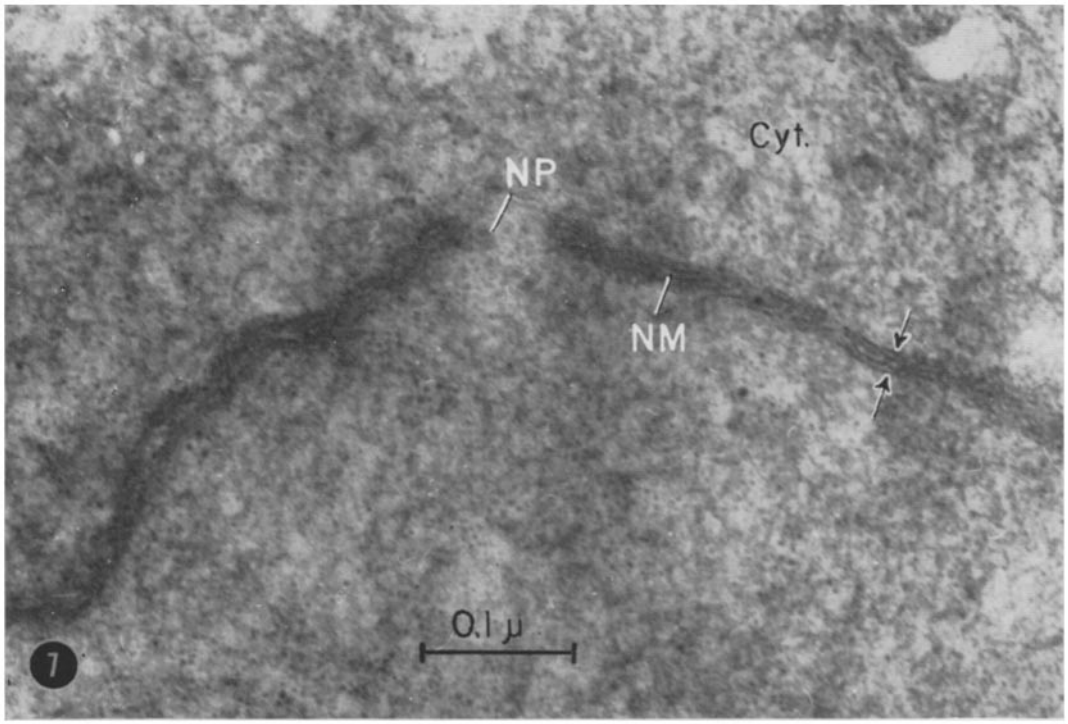


FIGURE 6

Section through part of a yeast cell showing dense granules (*G*) in the vacuole. The vacuolar membrane (*VM*) is clearly separated from the nuclear membrane (*NM*). Infolding of the inner mitochondrial membrane to form cristae can be seen at the top of the figure (arrow). × 60,000.



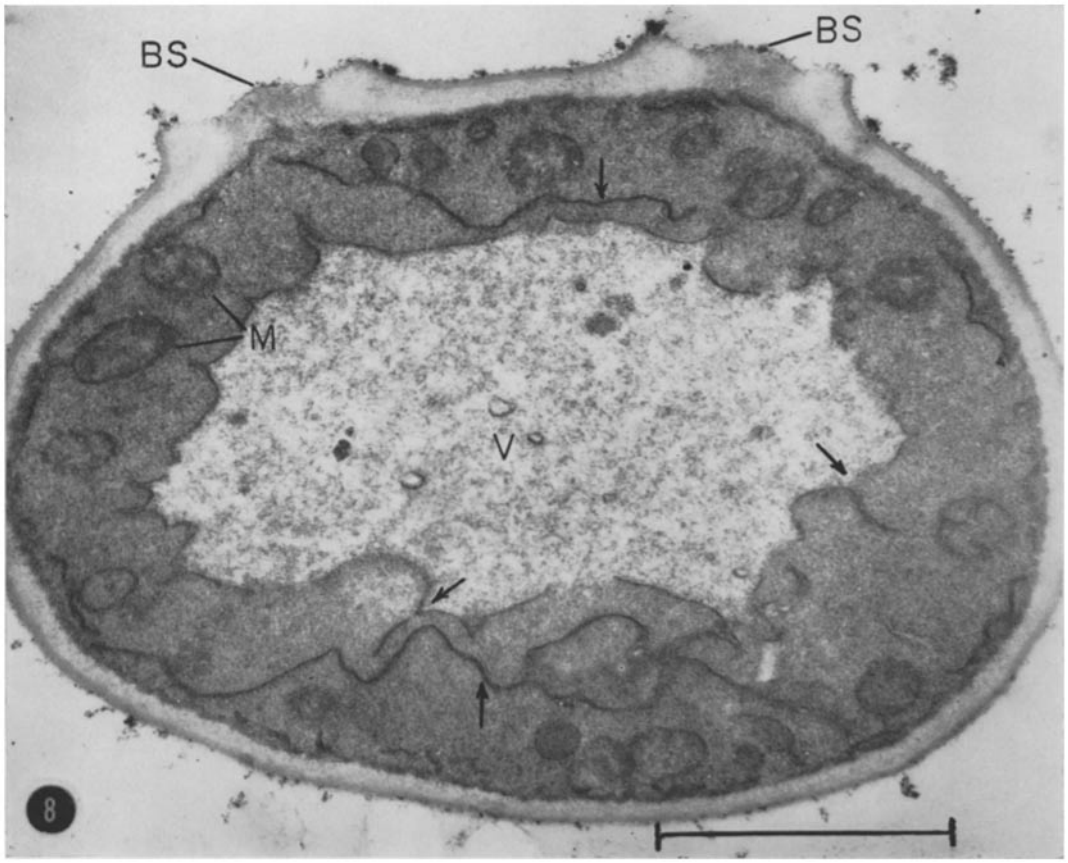


FIGURE 7

Section through the nucleus to show details of the double unit membrane (*NM*) and a nuclear pore (*NP*). × 200,000.

FIGURE 8

Section of a resting yeast cell to illustrate the presence of membranes in the cytoplasm (arrows). The irregular outline of the vacuole (*V*) is apparent and finger-like diverticula can be seen extending into the cytoplasm (arrows). × 40,000.