

STUDIES OF BUDDING AND CELL WALL STRUCTURE OF YEAST

ELECTRON MICROSCOPY OF THIN SECTIONS¹

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Observations of intact yeast cells with the electron microscope have yielded little information on cellular structure because of the impenetrability of the cells to the electron beam. The development of techniques for the preparation of ultra-thin sections promises to obviate this difficulty, and several papers have already appeared in which this approach has been applied in studies of the structure of microorganisms (Chapman and Hillier, 1953; Birch-Anderson *et al.*, 1953; Wolken and Palade, 1953; Gustafson *et al.*, 1954).

In this paper we describe details of the budding process and the structure of the cell wall of *Saccharomyces cerevisiae* as observed in thin sections of intact cells and purified cell walls.

METHODS

A tetraploid *S. cerevisiae* strain IF-5 was used for the major part of this study, although some work was also done with a haploid strain. The tetraploid strain was chosen because of its larger size and because it had been studied cytologically and genetically by other workers in this department (Hawthorne, 1955). Vigorously budding cultures in the logarithmic phase were used. These were obtained by transferring cells to tubes of broth (1 per cent yeast extract, 1 per cent peptone, 2 per cent glucose) from an overnight culture and incubating on a shaker at room temperature for 4 to 6 hours. The cultures were centrifuged and the supernatant discarded and replaced by an osmium tetroxide fixative solution. The 1.0 per cent buffered fixative of Palade (1952) and the fixative of Chapman and Hillier (1953) gave equally good results if the fixation time was prolonged to 16 to 20 hours. Osmium concentrations below 1 per cent and shorter

fixation times resulted in swelling, vacuolization, and disruption of the cells.

Following fixation, the cells were dehydrated by passage through a graded series of ethanol concentrations, impregnated with methacrylate monomer, and polymerized in gelatin capsules as described by Newman *et al.* (1949). A mixture of 4 parts N-butyl methacrylate and 1 part methyl methacrylate monomer gave blocks of the most satisfactory hardness for thin sectioning.

Sections were cut with a modified Spencer Rotary Microtome (advanced by thermal expansion) and more recently with a Porter Blum microtome.² Glass knives prepared as described by Latta and Hartmann (1950) were used with both microtomes. The sections, as cut, were floated off on the surface of a 50 per cent acetone-water solution behind the knife edge and picked up on formvar coated specimen grids. The sections were drained on filter paper, air dried, and selected for suitable thinness by their interference colors when viewed under a direct illumination microscope at 80 ×. The sections were studied with an RCA model EMU-2b electron microscope equipped with a self-biased gun and a limiting aperture in the objective pole piece to improve contrast. Electron micrographs were taken at initial magnifications of 3,800 × to 9,000 × with further photographic enlargement as indicated.

Cell walls were prepared by disrupting yeast suspensions in a Mickel tissue disintegrator, followed by differential centrifugation and washing. The cell walls were then fixed, dehydrated, and embedded in methacrylate as described above.

RESULTS AND DISCUSSION

A longitudinal section through a mother cell with two buds (A and B) at different stages of

² Manufactured by Ivan Sorvall Inc., P.O. Box 230, Pearl Street, Norwalk, Conn.

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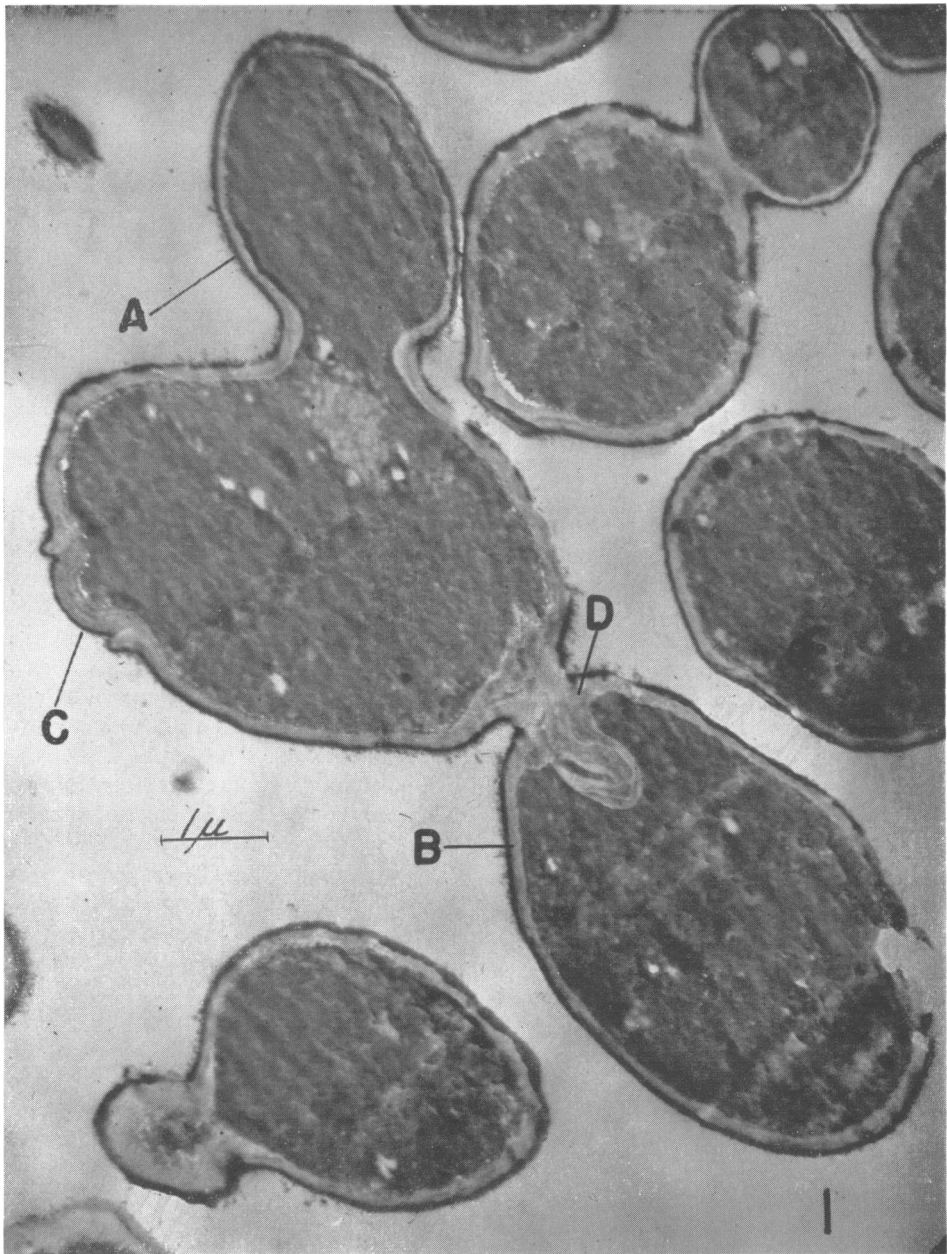
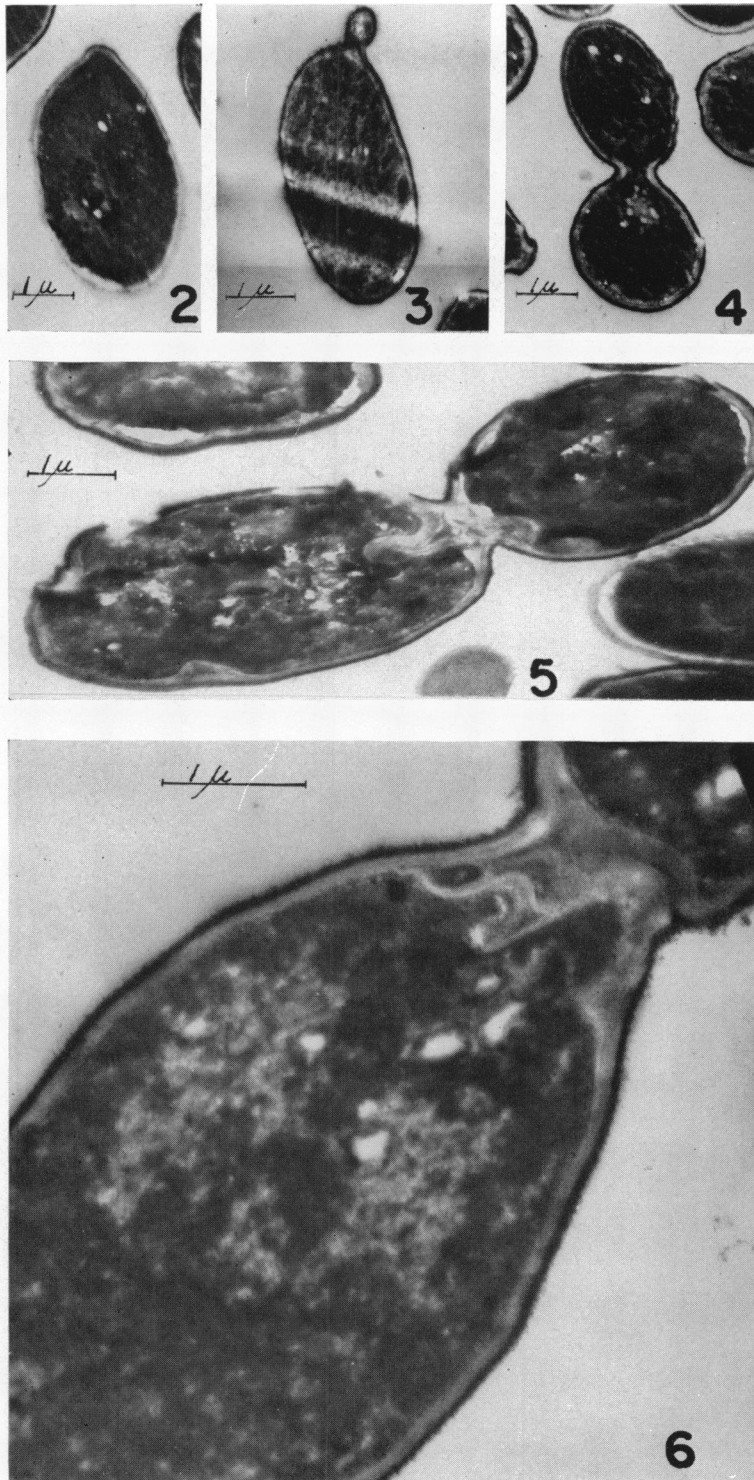


Figure 1. Electron micrograph of a longitudinal section through a budding yeast cell. 14,000 \times . A, bud with cytoplasm continuous with that of mother cell; B, mature bud with developing cross wall between mother and daughter cell; C, bud scar; D, extension of cell wall material into cytoplasm.

development is shown in figure 1. The cytoplasm of bud A is still continuous with that of the mother cell, while in bud B the cytoplasmic continuity no longer exists. The extension of cell wall material into the cytoplasm of the mother cell and bud B is evident at D and, as will be

pointed out below, this phenomenon appears to be characteristic of the later stages of the budding process. A well-defined bud scar is present at C. In whole mounts of isolated cell walls, a bud scar appears as a circular, raised rim on the surface of the cell wall (Northcote and Horne, 1952;



Figures 2-6

Figure 2 (left). Initiation of bud formation as a small bulge in cell wall. 8,000 ×.

Figure 3 (center). Enlargement of cell wall bulge to a small bud. 9,000 ×.

Figure 4. (right). Further enlargement of bud with cytoplasm of mother and daughter cells continuous. 8,000 ×.

Figure 5. Formation of cell wall material by mother and daughter cells. 11,000 ×.

Figure 6. Double cross wall between mother and daughter cells near completion. 9,000 ×.

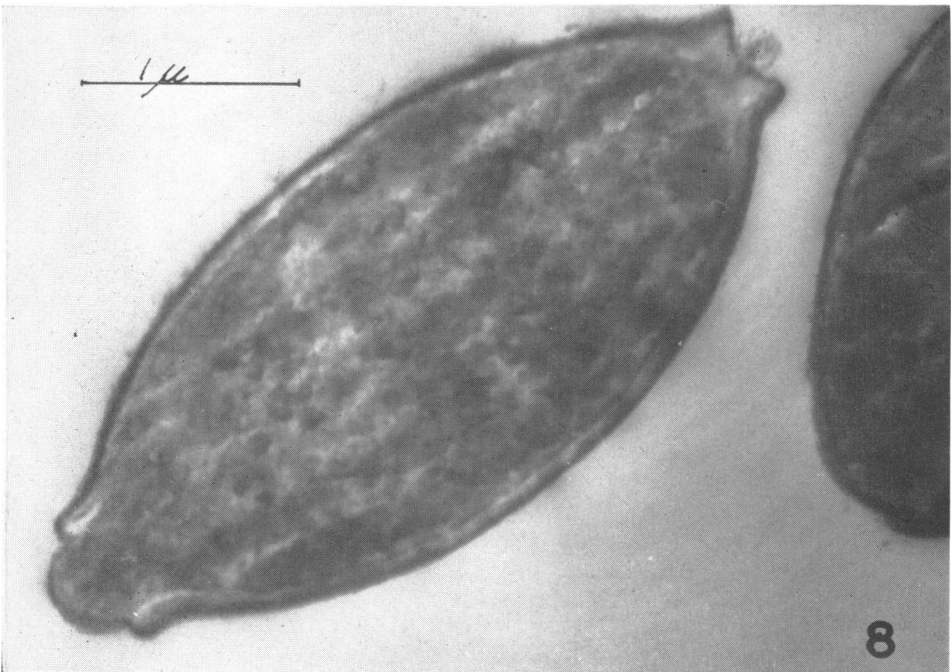
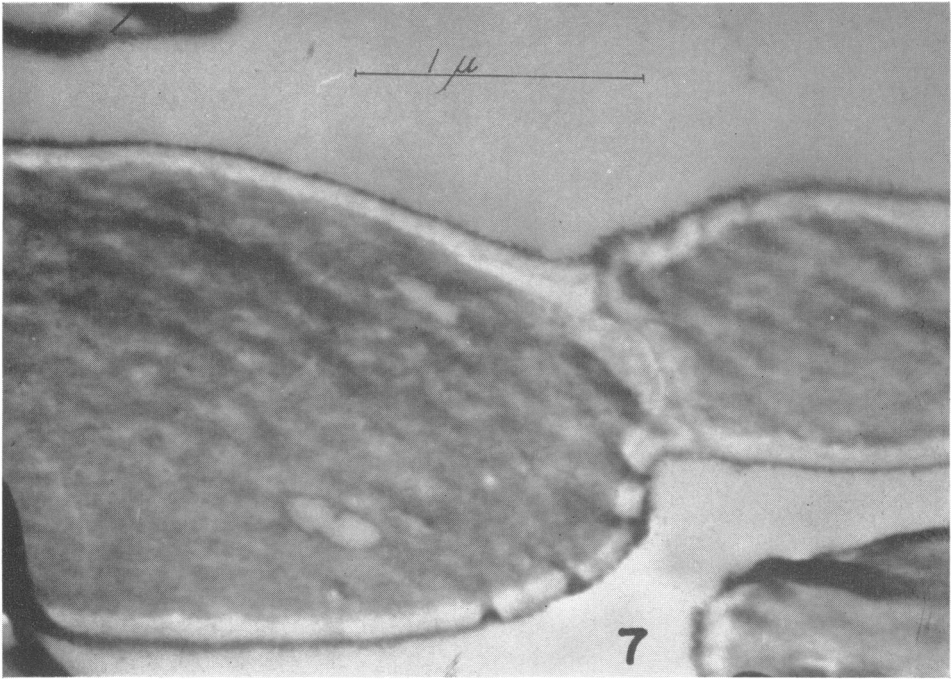


Figure 7. Double cross wall completely formed showing the geometric fit of bud scar and birth scar. 38,000 \times .

Figure 8. Single yeast cell with birth scar at upper right, and bud scar at lower left. 29,000 \times .

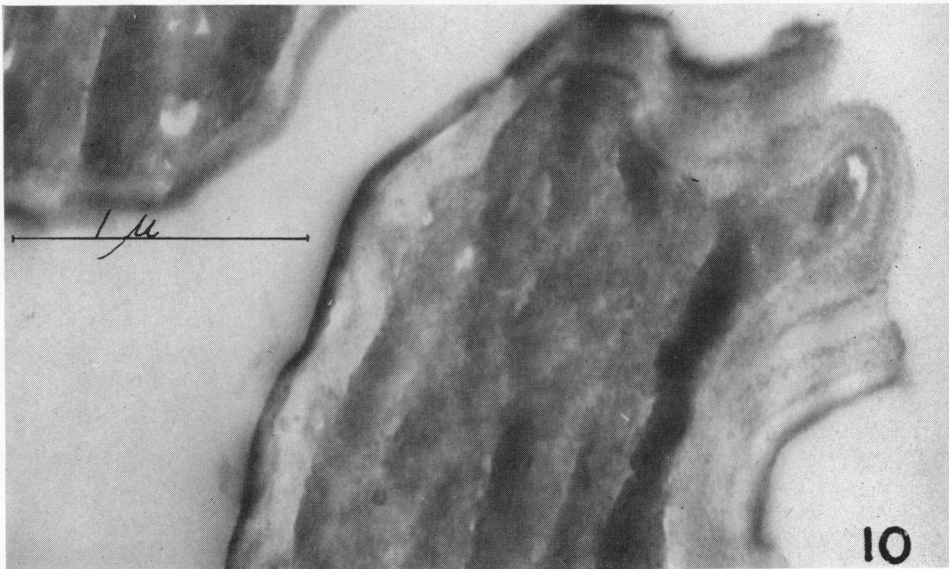
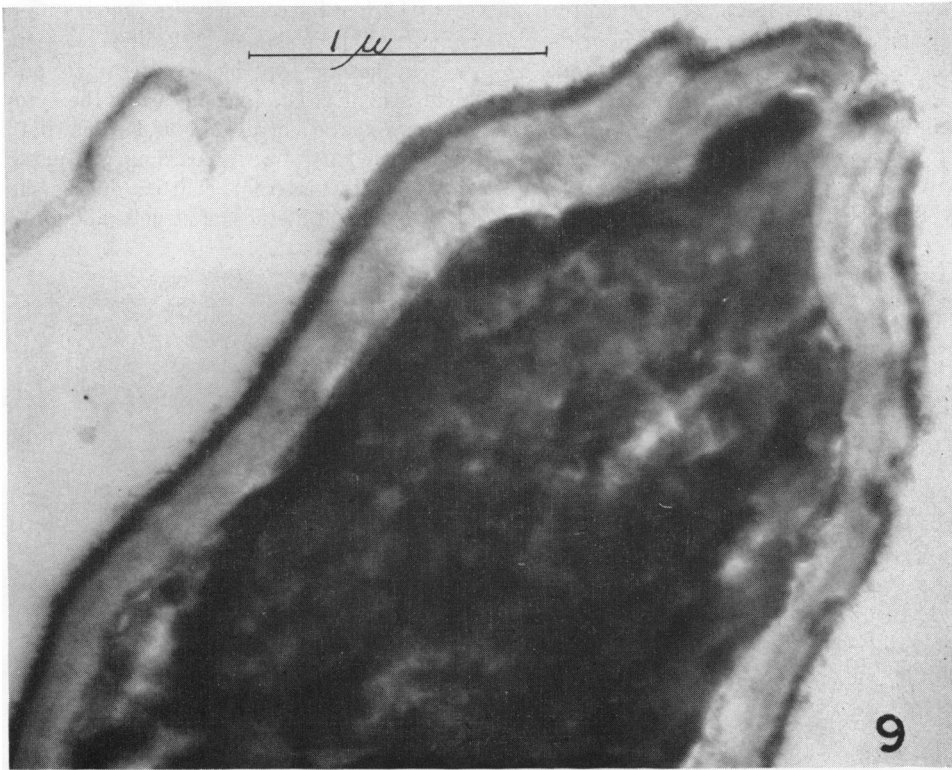


Figure 9. Longitudinal section, shows lamellae of inner layer of cell wall.
Figure 10. Longitudinal section, shows lamellae of the bud scar.

Houwink and Kreger, 1953). The section through the scar at C shows the elevated edge of the rim and the enclosed, rounded plug of cell wall material which forms the surface of the scar. The discontinuity in wall structure at the point of contact of the plug and rim of the bud scar is also apparent.

Very little structural detail is evident in the

cytoplasm of the cells in figure 1 or the succeeding figures. This is due to difficulties in sectioning which, in our experience, appear to be characteristic of yeast. In many cases the cytoplasm appears to be corrugated and somewhat compressed. This "wash-boarding," caused apparently by internal chatter during cutting, makes difficult a study of structural detail in the

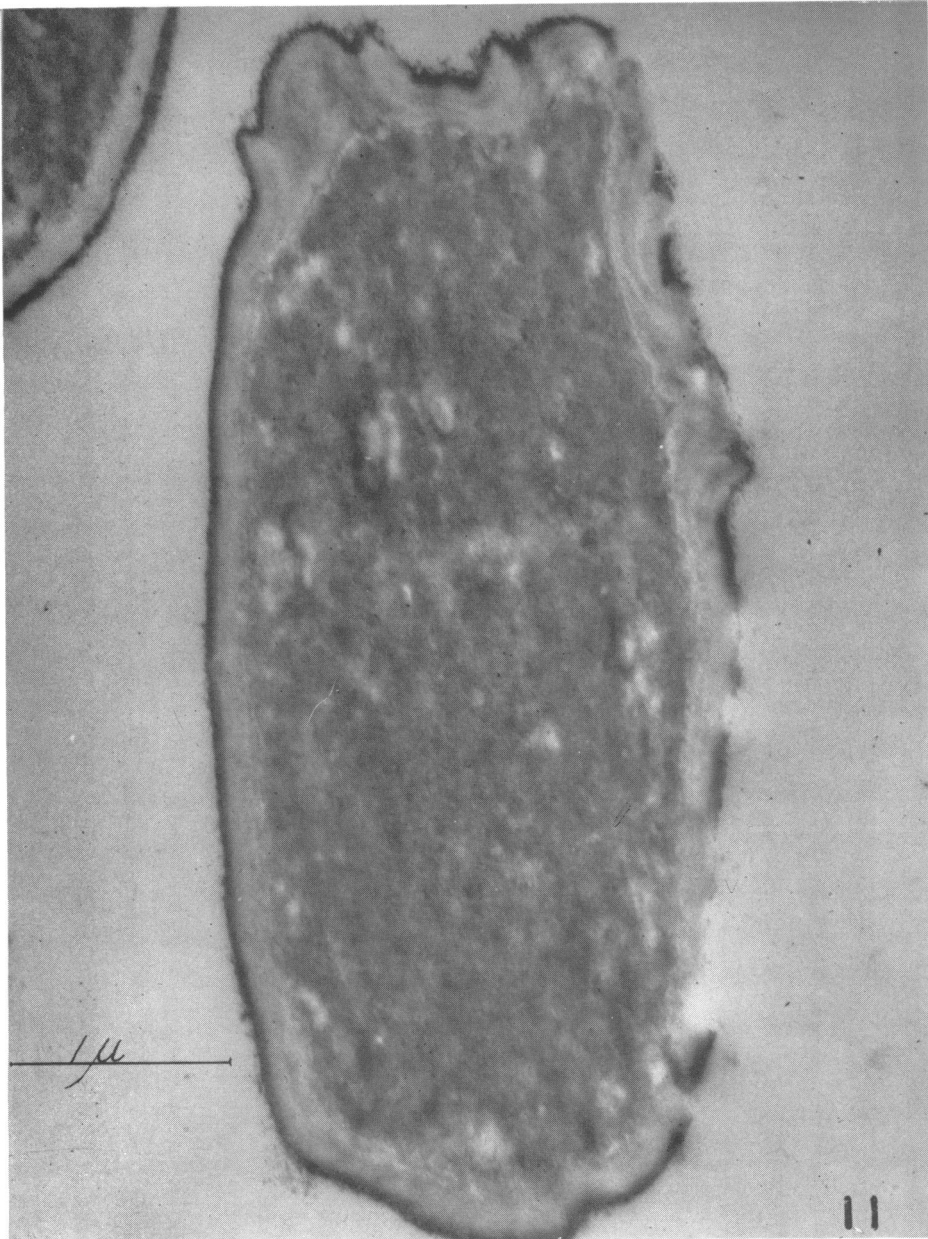


Figure 11. Electron micrograph of a longitudinal section through a yeast cell showing the electron dense fibrils lining inner surface of cell wall. 29,000 X.

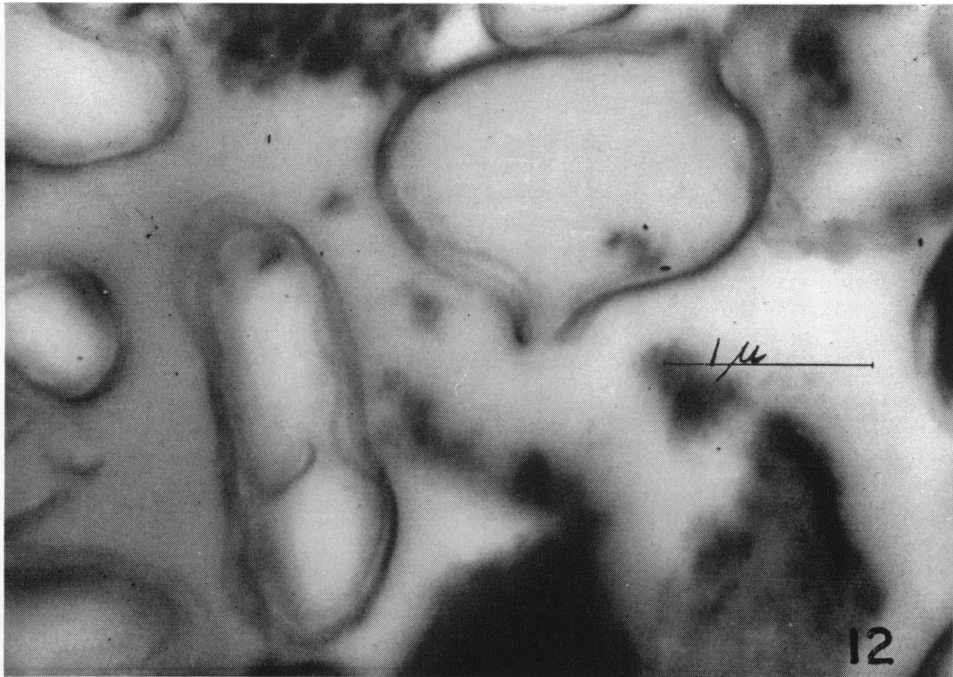


Fig. 12. Electron micrographs of sectioned cell walls showing laminated structure of the wall. 28,000 \times .

cytoplasm. None of our sections have shown structures which could be identified as nuclei, vacuoles, or cytoplasmic granules, although their presence in the cells of strain IF-5 can easily be demonstrated by other techniques.

Figures 2 to 8 represent cells in different stages of bud formation. An early stage is seen in figure 2, where the bud is represented by a small bulge in the wall of the mother cell. A somewhat later stage is presented in figure 3. In figure 4 the bud is quite large but the cytoplasm of the two cells is still continuous. The formation of the wall which separates the cells is shown in figures 5 and 6. In both figures there appears the characteristic extension of the cell wall material into the cytoplasm which was noted previously in figure 1. While it is difficult to interpret the significance of this phenomenon it appears to us to represent a stage of active synthesis of the cell wall material, which eventually forms the convex and concave plugs of cell wall sealing the constricted opening between the cells. A cleavage line can be seen in the new cell wall material in figure 6, while in figure 7 cleavage appears to be complete and the geometric fit of the concave bud scar and the convex birth scar is clearly demonstrated. Figure 8 shows a single cell with a concave birth scar at one pole and a convex bud scar at the other.

The precise manner in which the cell wall separating mother and daughter cells is laid down is not known, but our micrographs indicate that its formation might well resemble the pattern of centripetal extension of the external cell wall that occurs in bacteria (Knaysi, 1941; Chapman and Hillier, 1953), with the essential difference being that new cell wall material may originate over a much wider area. This is suggested by the appearance of figures 5 and 6 described above, and also by the structure of the bud scars. The plug of cell wall material of the bud scar appears to be attached to the inner side of the external cell wall over a considerable area (figures 1 and 10), and it may well be that the cell wall material of the plug originated over the entire area where plug and external wall are contiguous.

With regard to the structure of bud and birth scars, our observations are in agreement with those of Barton (1950), who first pointed out that the two could be distinguished by their respective convex and concave appearances. This was questioned, however, by Bartholomew and Mittwer (1953), who found no difference in structure of bud and birth scars of cells that had been treated with ultraviolet light to make them more transparent to the electron beam.

The cell wall of *S. cerevisiae* is known to be a complex structure which consists principally of

glucan and mannan, and smaller amounts of protein, lipid and chitin (Northcote and Horne, 1952; Houwink and Kreger, 1953). Northcote and Horne also presented chemical and microscopic evidence which indicated that the cell wall consists of at least 2 distinct layers, one of which is composed exclusively of glucan. Their observations also suggested a role for lipids as cementing substance in the laminated cell wall, for when purified cell walls were extracted with lipid solvents separation of the wall into two membranes was observed.

Our observations are in agreement with the conclusion of Northcote and Horne that the cell wall of *Saccharomyces* consists of distinct layers. This is evident in the section shown in figure 9. The outer layer of the cell wall consists of electron dense material, about 0.05μ in average thickness, which has a somewhat filamentous appearance in most sections. Beneath this is a layer of lesser electron density which has an average thickness of about 0.2μ , and which is subdivided by thin, electron dense lamellae that are particularly prominent at or near the bud scars (figure 10). Lining this layer is what appears to be a network of electron-dense fibrils which are quite sinuate in form (figure 11). Whether these structures are part of the cell wall or the cytoplasm is difficult to say, but according to Houwink and Kreger (1953) the innerside of the cell wall of *S. cerevisiae* is lined with a network of fibrils.

When sections of isolated cell walls were observed, the laminated nature of the cell wall was quite evident, particularly in areas near scars where the cell wall had separated. This is shown in figure 12, where the wall has separated into three distinct layers. In none of the cell wall preparations, however, was there any suggestion of the presence of the electron dense outer layer which was so prominent in the sections of whole cells. It is possible that this layer is lost during the manipulations required for the preparation of the cell walls, although extensive washing of whole cells both before and after fixation did not alter the appearance of this outer layer.

Study of this yeast is being continued with the hope of obtaining sections satisfactory for the observation of cytoplasmic detail.

SUMMARY

Thin sections of yeast cells and "purified" yeast cell walls have been prepared and examined

with the electron microscope. Some of the stages of the budding process have been described.

The yeast cell wall consists of several distinct layers. Observations of cell wall structure made on sections of whole cells and isolated cell walls were in general agreement, with the exception that an outer layer of electron dense material was consistently present in whole cells but not in isolated cell walls.

Details of the internal structure of the cells were obscured due to difficulties of fixation and sectioning.

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