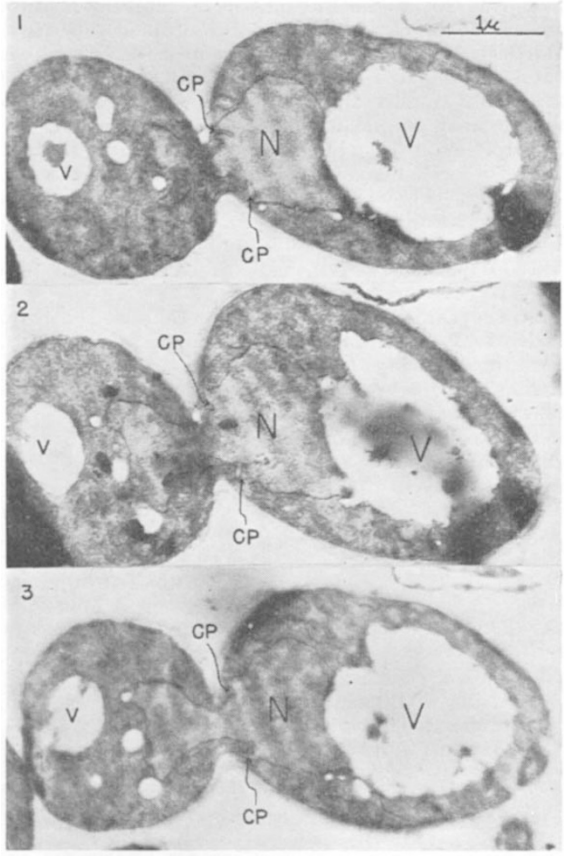
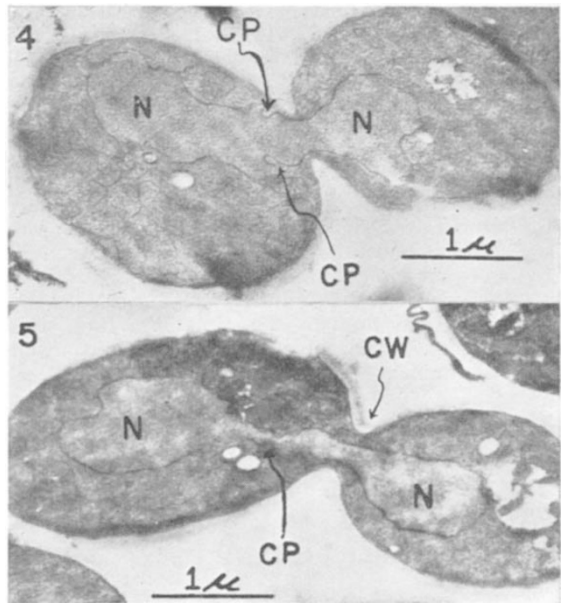
Nuclear Changes occurring during Bud-formation in *Saccharomyces cerevisiae* as revealed by Ultra-thin Sectioning

The lack of clarity prevailing among yeast cytologists as to the mode of budding in *Saccharomyces cerevisiae* tempted us to study this process by means of an improved ultra-thin sectioning technique. This technique has enabled us to observe nuclear behaviour during bud formation and subsequent division. Except for substitution of potassium permanganate for osmium tetroxide as the fixative, the method of preparing specimens for ultra-thin sections was exactly the same as previously reported[1,2]. A distillers yeast, *S. cerevisiae M*-1, was grown in a broth medium for several hours at 25° C. with aeration by means of vigorous shaking. The cells were collected by centrifugation and washed once with distilled water. The washed cells were then fixed in a 1·5 per cent aqueous solution of potassium permanganate at room temperature for 30 min. The time of fixation and concentration of permanganate were critical for preservation of internal cell structure. Explosion was minimized by prolonged treatment of the cells with cold partially polymerized *n*-butyl methacrylate. The sections were cut on a Porter–Blum microtome and observed in an *RCA* model *EMU-2b* electron microscope. Figs. 1, 2 and 3 show three parallel sections of a budding yeast cell. It is interesting that the conspicuous invagination of the dividing nucleus is situated a short distance away from the constricted cell wall of the bud. The difference in the



Figs. 1–3. A budding yeast cell. *N*, nucleus; *CP*, constriction point; *V*, vacuole; *CW*, cell wall



Figs. 4–5. Budding yeast cells at a later stage. Key as in Figs. 1–3

planes of constriction between the nucleus and the cell wall seems to be a routine occurrence and is considered characteristic of vegetative division in *S. cerevisiae*. This suggests that there is some mechanism which regulates the constriction of the nucleus at this point other than the simple pressure of the constricting cell wall. Close observation of these figures reveals that the constriction starts at certain points rather than being initiated uniformly around the site of constriction. The presence of the nuclear membrane throughout vegetative nuclear division implies that typical mitosis in *S. cerevisiae*, at least in the strict sense of the word, is lacking. Chromosomal figures which have been observed in the nuclei of the cells of higher organisms[3–5] have not been detected in either the nuclear vesicle or on the nuclear membrane during any stage of the nuclear division. Figs. 4 and 5 demonstrate somewhat later stages of nuclear division during bud-formation.

From the observations made in this study, we conclude:

(1) The nuclear membrane of *Saccharomyces cerevisiae* does not disappear during vegetative division. This favours the view that division of the nucleus during budding does not occur by a typical mitotic process.

(2) The nucleus of *Saccharomyces cerevisiae* undergoes an autonomous constriction during bud formation. Constriction of the nucleus does not coincide with constriction of the cell wall, but always occurs a little inside the mother cell.

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