ULTRAVIOLET AND PHASE MICROSCOPY OF SPORULATING *SACCHAROMYCES*

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ABSTRACT

MILLER, GLENDON R. (Southern Illinois University, Carbondale), DAN O. McCLARY, AND WILBERT D. BOWERS, JR. Ultraviolet and phase microscopy of sporulating *Saccharomyces*. J. Bacteriol. **85**:725–731. 1963.—During active growth, the cytoplasm of yeast cells is densely absorbent to ultraviolet light at 260 mμ, whereas the nucleus is only faintly so, and the vacuole is nonabsorbent. After 24 hr on presporulation medium (about the age of transfer to acetate sporulation medium), the cells manifest many characteristics of starvation. The cytoplasm is weakly absorbent to ultraviolet light except for a dense zone immediately surrounding the vacuole and one or two groups of highly refractile granules clustered on one or both sides of the juncture of the nucleus and the vacuole. After several hours on the acetate sporulation medium, the cells undergo a progressive vacuolation until four or more large vacuoles appear, separated by granular cytoplasm. The nucleus is obscured to ultraviolet light during the vacuolation stage, at which time previous studies have shown its division to occur, and remains so during the rest of the cycle. Photographs of densely granulated cells, at various wavelengths of ultraviolet and visible light, indicated several different types of granules with respect to their absorption spectra. With continued development of the ascus, the granules increase in number until they fill the entire cell and obscure the vacuoles, after which they condense into a compact mass, leaving much of the cell empty. Spores emerge from the granular mass as separate, ultraviolet-absorbent regions without distinguishable cell walls, which seem to be the last structures formed. The rudimentary spores contain a central cluster of dense granules and are separated within the ascus by optically dense, granular partitions which diminish as spore walls are laid down. With the maturing of the ascus, the granules and epiplasm disappear, and the ascus wall is drawn tightly around the more or less homogeneously absorbent spores.

Concepts of cellular morphology and fine structure depend upon the interpretation of data obtained by a variety of chemical and physical methods. Light microscopy has provided much of the information upon which cytological concepts are based, but, due to the transparent nature of protoplasm, it reveals little detailed structure unless the cells are differentially stained (a procedure requiring various physical or chemical fixing agents as well as dyes, the results of which are uncertain and highly controversial). The electron microscope, with its ultrahigh resolution, is unquestionably the only instrument generally available that can reveal the minute details of cellular structure but interpretation of data obtained by this instrument is complicated, not only by the necessity of fixation, but also by the requirement of ultrathin sectioning. The interpretation of data obtained by the above methods is aided by studying unfixed, living cells with phase, interference, and ultraviolet microscopy. The phase microscope has not been particularly revealing to the yeast cytologist, and results obtained by interference microscopy have not been generally reported. Ultraviolet microscopy provides perspectives not obtainable by other methods, which should be very helpful in the general interpretation of cytological data: (i) its resolution is approximately twice that of the light and phase microscopes; (ii) different degress of absorption of ultraviolet light by various chemical components of the cell make staining for many of its structures unnecessary; (iii) the gross morphological effects of fixation

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and staining, the subjects of greatest contention among cytologists, can be more precisely determined by comparing fixed specimens with living, untreated subjects.

Caspersson and Brandt (1941), using the ultraviolet microscope, observed the accumulation of cytoplasmic granules in starved yeast cells, and their dissolution in well-nourished cells, and postulated the relationship of ribonucleic acid (RNA) to protein synthesis. Svihla and Schlenk (1959, 1960) demonstrated the use of the ultraviolet microscope in determining the precise location of the accumulation of *S*-adenosylmethionine in living yeast cells. McClary, Bowers, and Miller (1962) studied the budding cycle of *Saccharomyces*, correlating observations made by ultraviolet microscopy on living cells with fixed and stained preparations. More recently, the results obtained by ultraviolet and phase microscopy on the sporulation phase of *Saccharomyces* were reported (Miller, McClary, and Bowers, 1962). More precise details and an extension of that study are presented in this report.

MATERIALS AND METHODS

Carbondale tetraploid *Saccharomyces* culture 11294 × 11296 was grown for 24 hr on presporulation agar slants containing: potassium chloride, 2 g; ammonium sulfate, 2 g; potassium phosphate (monobasic), 2 g; magnesium sulfate (anhydrous), 0.5 g; glucose, 20 g; yeast extract, 10 g; agar, 20 g; and distilled water to 1,000 ml. A large loopful of this culture was transferred to a 250-ml Erlenmeyer flask containing 10 ml of acetate sporulation broth containing: potassium acetate, 9.5 g; glucose, 1 g; yeast extract, 2.5 g; and distilled water to 1,000 ml. When solid medium was desired, 20 g of agar were added. Several such inoculated flasks were placed on a shaker at 30 C, and individual flasks were removed at desired intervals of time. Cells were removed and washed by centrifugation and decanting.

For ultraviolet microscopy, cells were suspended in water on a quartz slide, covered with a cover slip, carefully blotted, and sealed with Lubriseal (Arthur H. Thomas Co., Philadelphia, Pa.). Focusing with visible light and adjusting for ultraviolet photography, as well as staining and photomicrographic methods for yeast cells and onion-root-tip controls, were carried out as previously described (McClary et al., 1962).

For phase microscopy, cells in various stages of sporulation were suspended in 20% gelatin, or mounted on sporulation agar films in slide cultures. The latter technique permitted direct observation of the various stages of sporulation in individual cells, but the thickness of the preparations prevented satisfactory photography.

RESULTS

Cells actively growing on presporulation medium contained a high concentration of substance, probably RNA, that was absorbent to ultraviolet light at 260 mμ, and that obscured all other parts of the cell except the vacuole and occasionally the nucleus (Fig. 1). After vegetative growth for 24 hr, the cytoplasm had lost much of its optical density, and the nucleus was observed as a weakly absorbent body adjacent to the vacuole and toward the budding end of the cells (Fig. 2). Highly absorptive masses were found near the juncture of the nucleus and the vacuole, much as previously shown in cells starved in water, although their granular nature was not so distinct (McClary et al., 1962). When such cells were subjected to sporulation medium for a few hours (10 to 18 hr), rather extensive vacuolation occurred, along with the formation of immense numbers of ultraviolet-absorptive and -refractive granules which surrounded the vacuoles to produce a granular reticulum (Fig. 3 to 9). Photographs of these cells at different wavelengths of ultraviolet and visible light suggested several different chemical types of granules (Fig. 4 to 6). With continued maturation of the ascus (16 to 20 hr), the granules increased greatly in numbers, as seen in Fig. 10 and 11, until the vacuoles were obscured or apparently ceased to exist. The granules aggregated into compact masses (Fig. 10 and 11) in the central regions of the cells, leaving much of the cell optically empty, with thin threads of protoplasm extending from the central aggregates out to the peripheries. The granular masses divided into four recognizable rudimentary spores (Fig. 10 to 14). Some granules were included within the spores, but most of them aggregated into dense partitions separating the immature asci into four parts (Fig. 15). With the maturing of the ascus, the density of the partitions diminished (Fig. 16) until only a few granules appeared within the spores (Fig. 17). Finally, mature asci were only weakly absorbent to ultraviolet light (Fig. 18).

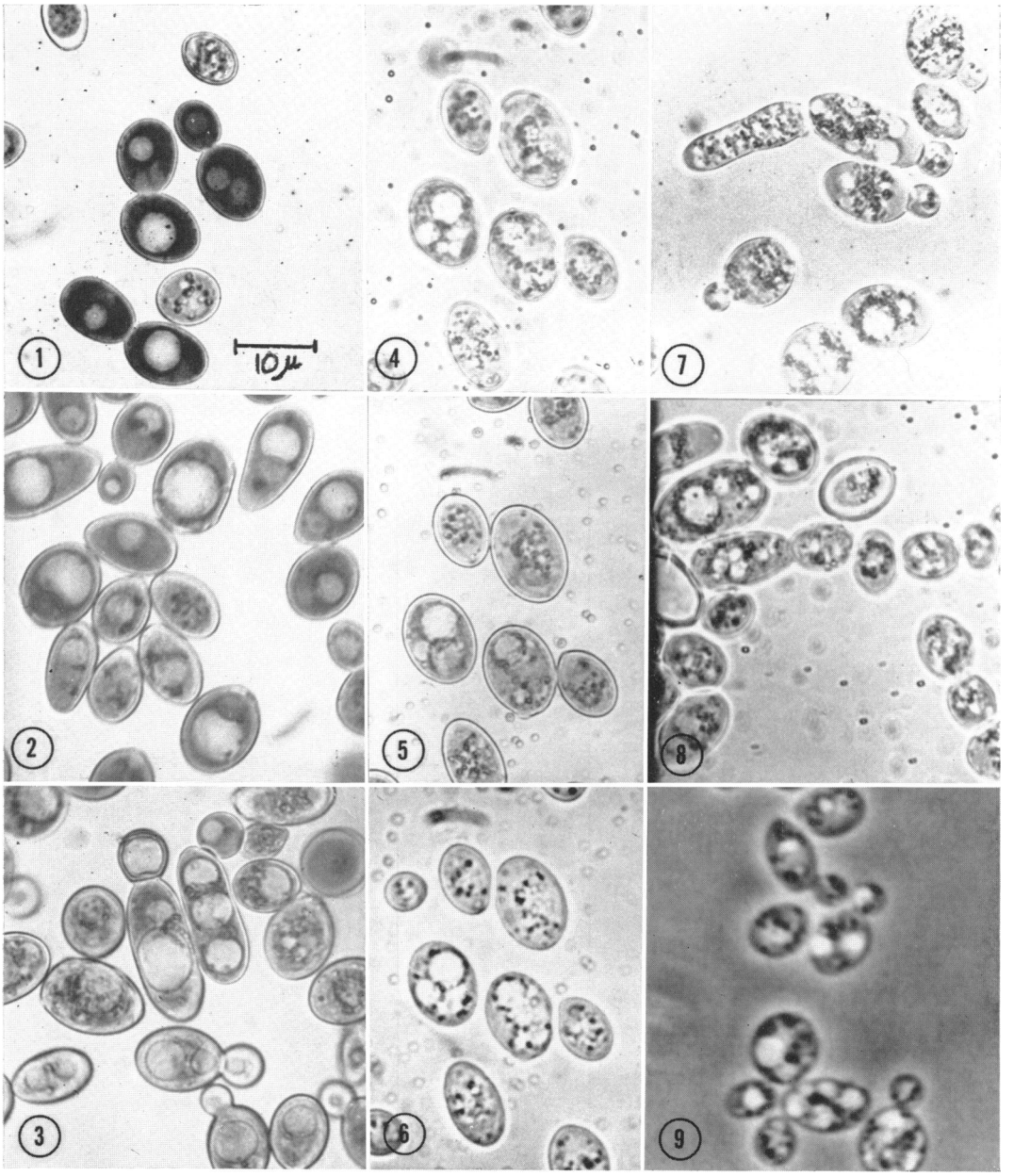

FIG. 1. *Actively budding cells from a 2-hr culture photographed at 260 mμ. The ultraviolet density suggests a high concentration of ribonucleic acid.*

FIG. 2. *Cells from a 16-hr presporulation agar slant photographed at 260 mμ. The light region toward the budding end of the cell is the nucleus. Note the aggregates of granules near the junctures of the vacuoles and the nucleus and the lower ultraviolet density, as compared with cells of Fig. 1, which are features of starvation.*

FIG. 3. *Yeast cells undergoing vacuolation and granulation after 10 hr on sporulation medium. Photographed at 260 mμ.*

FIG. 4, 5, and 6. *Group of vacuolated cells photographed at 260, 300, and 490 mμ. respectively. Note numerous granules in ultraviolet photomicrographs not visible in photomicrographs at 490 mμ. Cells after 16 hr on sporulation medium.*

FIG. 7. *Cells after 18 hr on sporulation medium photographed at 286 mμ. Granulation and vacuolation are more extensive.*

FIG. 8. *Cells in the same phase as in Fig. 7, photographed at 300 mμ.*

FIG. 9. *Phase photomicrographs of cells in approximately the same phase as those shown in Fig. 8. Individual granules are less obvious, but vacuolation is very clearly revealed.*

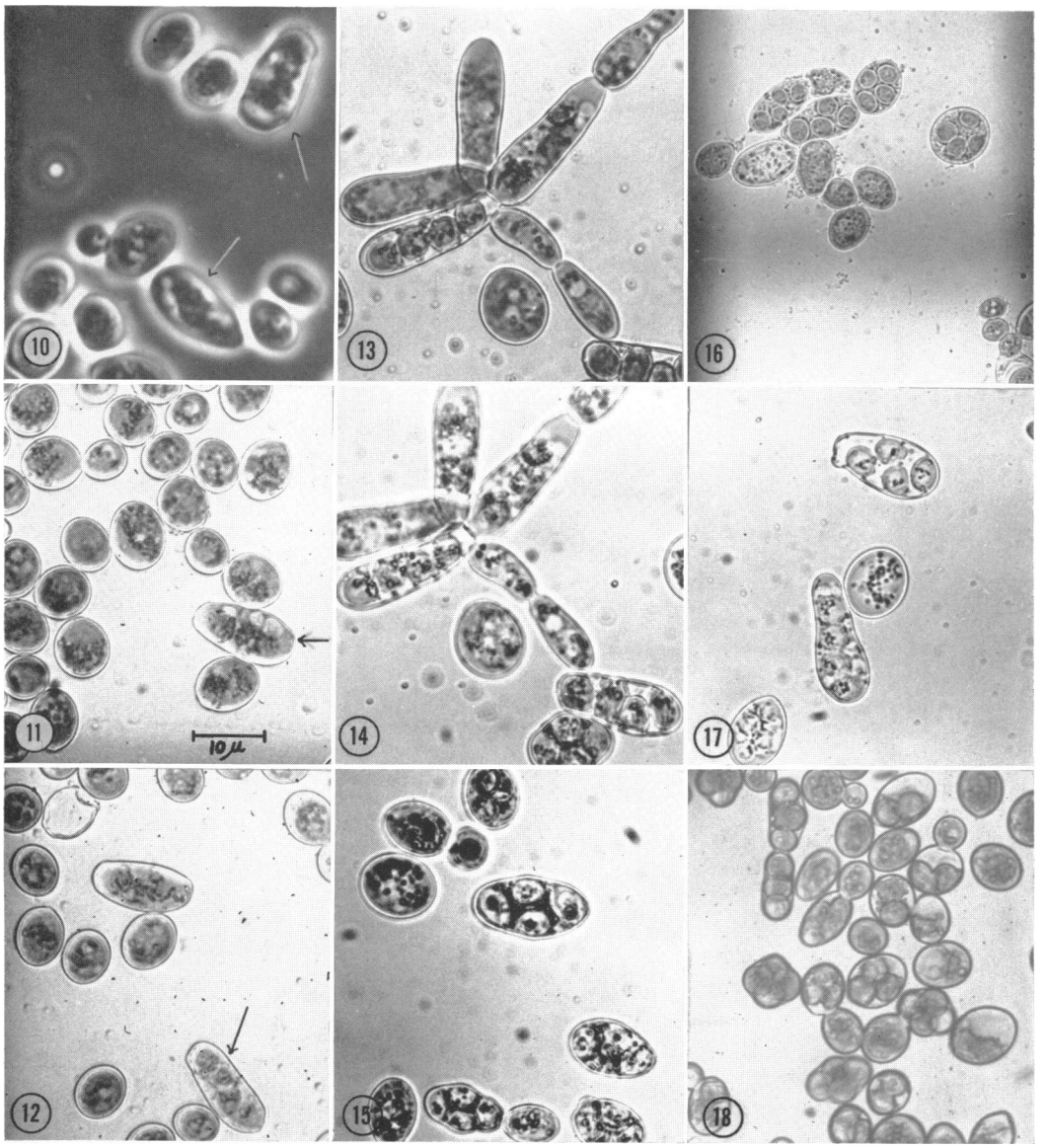

FIG. 10. *Phase photomicrograph of more advanced stages of sporulation (20 hr). Note the extensive granulation and the disappearance of definite vacuoles. Note* (lower arrow) *the aggregation of granular protoplasm along the central region of the cell with radiating strands toward the periphery. In the upper right* (arrow), *the protoplasm is dividing into the rudimentary spores.*

FIG. 11 and 12. *Ultraviolet photomicrographs (260 mμ) of the dense granular phase (20 hr of sporulation). Note the absence of discrete vacuoles and the aggregation of the granules in the middle of the cell* (arrow) *with the radiating strands of protoplasm and the beginning of division into rudimentary spores.*

FIG. 13 and 14. *Cells in a slightly more advanced stage of sporulation (22 hr) photographed at 260 and 300 mμ, respectively. Note the aggregations of granules within and among the spores.*

FIG. 15. *More advanced stage of ascus formation photographed at 300 mμ. Note the highly absorbent granular partitions of the young asci and the absorptive granules in the centers of the spores.*

FIG. 16. *Young asci in a 30-hr sporulating culture (260 mμ). Note ultraviolet-dense regions around the spores, the transparent inner wall, and the ultraviolet-absorbent protoplasm of the spores. An interesting comparison may be made with the Giemsa-stained ascus (Fig. 24) and the electron micrographs of other authors.*

FIG. 17 and 18. *More advanced stages of sporulation (48 hr), with the loss of absorption and disappearance of the epiplasm with the collapse of the ascus walls around the spore clusters.*

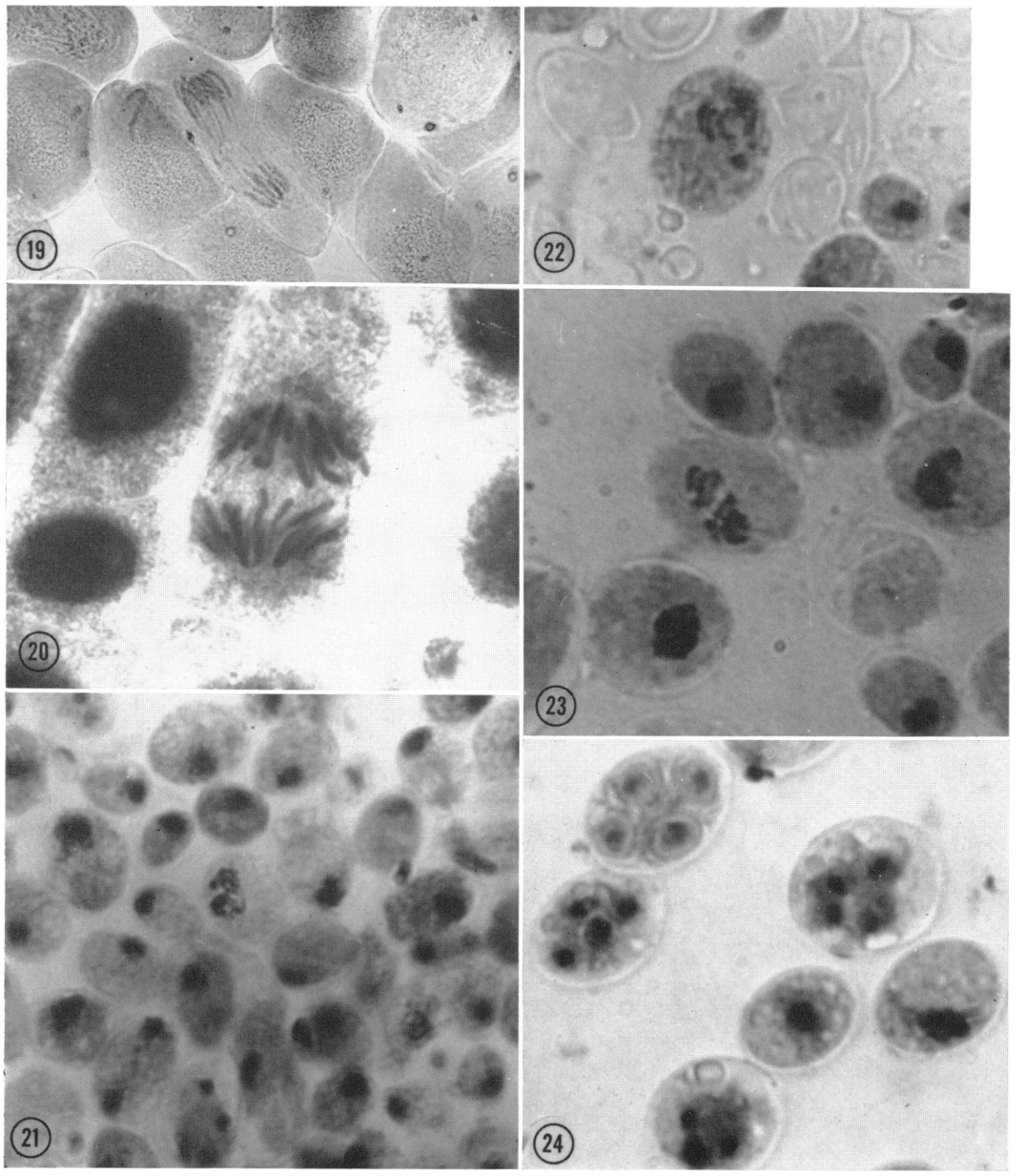

FIG. 19. *Onion-root-tip cells photographed at 300 mμ. Chromosomes exhibit uneven density suggestive of spirals. Note the granular, interphase nuclei with the nonabsorbent nucleolus. Although it is perhaps nothing more than coincidence, there is a remarkable morphological similarity between these interphase nuclei and Mundkur's "peripheral clusters" shown in electron micrographs of sporulating yeast cells.*

FIG. 20. *Onion-root-tip cells stained according to the modified Carnoy-perchloric acid-Giemsa technique.*

FIG. 21, 22, and 23. *Sporulating yeast cells (18 hr) stained by the modified Carnoy-perchloric acid-Giemsa technique. Previous reports of countable chromosomes were based upon similar figures.*

FIG. 24. *Various stages in the maturation of rudimentary spores. Note dense protoplast containing dark-staining nuclei at the bottom of the figure. Division of the protoplast is evident in the two cells nearer the center. The walls have formed around the spores in the ascus in the upper left-hand corner of the figure, leaving an exterior epiplasm. Note the similarity of this ascus to the ultraviolet photographs of those shown in Fig. 16.*

It was possible to study the nucleus only in fixed and stained preparations (Fig. 21 to 24) and, therefore, not possible to correlate its two divisions precisely with the observations made on living cells. From a time sequence, its divisions were found to occur most frequently between 16 and 20 hr, during the period of extensive vacuolation and granulation (Fig. 7 to 12). Previous workers (Nagel, 1946; McClary, Williams, and Lindegren, 1957) reported discrete chromosomes during the first division ("meiosis I") of the nucleus of sporulating *Saccharomyces*. Figures 21 to 24 depict yeast cells stained by the modified Carnoy-perchloric acid-Giemsa technique like those upon which the previous report from this laboratory was based. Figure 20 shows onion root-tip cells stained in the same manner, the results of which are very comparable with similar unstained specimens photographed at 300 mμ (Fig. 19). Not all of the yeast cells reveal chromosomes, but neither do most of those of the onion-root tip. In Fig. 24, the sequence of spore-wall formation can be seen to be similar to that depicted by ultraviolet microscopy (Fig. 12 to 16) and electron microscopy (Hashimoto et al., 1960; Mundkur, 1961).

DISCUSSION

The two most distinctive features of the sporulating yeast cell are its extensive vacuolation and its abundant accumulation of ultraviolet-absorbent and -refractile granules which form first around the peripheries of four or more prominent vacuoles, but which later aggregate into a dense mass, almost filling the cell. The granules then segregate into four groups from which the spores emerge, first without walls, followed by the laying down of an ultraviolet-dense outer wall and a transparent inner layer, presenting figures very similar to the electron micrographs of Hashimoto et al. (1960) and those of Mundkur (1961). The modified Carnoy-perchloric acid-Giemsa technique (McClary et al., 1957) reveals the same sequence of events.

McClary and Lindegren (1959), using phase microscopy, pointed out these distinguishing features of sporulation in *Saccharomyces*. Hashimoto et al. (1960) were not particularly concerned with these features, but noted the accumulation of numerous lipid granules. Mundkur (1961) noted both the extensive vacuolation and the immense accumulation of granules, but his figures do not reveal the precise patterns of granule aggregations and their subsequent partitioning among the spores. Although Mundkur (1961) observed the persistence of the vacuole throughout the sporulation cycle in his electron micrographic studies of frozen-dried yeast cells, ultraviolet and phase microscopic studies of living cells indicate that the vacuole (or vacuoles) disappears just before visible spores emerge from the dense, tetrakaryotic protoplast. Much the same granular configurations observed with ultraviolet microscopy are seen under the light microscope when cells in these phases are stained with Sudan IV, indicating that many of the granules consist of, or contain, lipid. This is in accord with the finding of Hashimoto, Conti, and Naylor (1958) and Hashimoto et al. (1960). The high accumulation of lipid during this cycle suggests that the stimulating effect of acetate upon the sporulation of yeasts may be similar to the role suggested for acetate with the bacteria (Slepecky and Law, 1961; Nakata, 1962)

Although the ultraviolet microscope is not directly applicable to the study of the yeast nucleus, it is very applicable to that of higher forms of life, such as meristematic cells of onion root tips, which may serve as very useful cytological controls. Cytological techniques used in the study of yeasts may be tested on onion root tip or similar controls, as shown by Yotsuyanagi (1960) in his electron microscopic studies of yeasts; morphological changes imposed by the procedures can readily be detected by comparing them with unstained material under the ultraviolet microscope. McClary et al. (1962) have previously shown that onion-root-tip cells stained by the modified Carnoy-perchloric acid-Giemsa technique are very comparable with unstained cells seen in ultraviolet photomicrographs. Sporulating yeast cells stained by the same technique reveal chromatinic bodies undergoing various recognizable phases of meiosis comparable with and in about the same proportion as those of onion-root-tip controls (Fig. 21 to 23).

ACKNOWLEDGMENT

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