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Authors
Birnbaum, Walter
Hayes, Thomas L.

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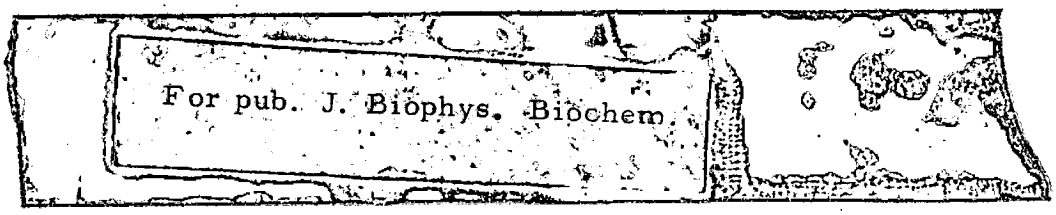

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Walter Birnbaum, Jr., and Thomas L. Hayes

December 14, 1959

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Introduction

The physical and biochemical properties of uranyl nitrate suggest that it may be applied to advantage as an electron stain. Uranyl ion, containing a heavy metal, may be expected to act as an effective electron stain when incorporated into a cell or tissue to be observed with the electron microscope. Investigations into the metabolism of uranyl nitrate by yeast cells, revealed that yeast cells have a strong affinity for uranium.[1] These investigations also pointed out that a very stable complex of uranium is formed within yeast cells when the cells are suspended in a solution of uranyl nitrate. The only other substances studied that form uranyl complexes of strength equal to that of the yeast complex are high molecular-weight polymers of phosphate such as nucleic acids and adenosine triphosphate. Uranyl ion also forms complexes with other biological substances such as proteins, but these complexes do not possess the stability of those with the phosphate compounds. These biochemical data indicate that uranyl nitrate may be of use as an electron stain which is relatively specific for high-molecular-weight polyphosphate compounds such as nucleic acids.

The structure of the yeast cell nucleus has been the object of several recent investigations; however, the results of these studies have not been wholly satisfactory. Osmic acid has not been shown to be a very satisfactory electron stain for yeast; potassium permanganate as applied by Hashimoto, Conti, and Naylor[2] has been the most satisfactory fixative and stain for yeast yet applied for electron microscopy. It has not yet been possible to demonstrate chromosomes or chromosomal material in the yeast nucleus by means of electron microscopy. The application of uranyl nitrate appears to offer some solution to this problem.

Methods and Materials

Cells of Saccharomyces cerevisiae were employed in this study. These were diploid cells of the X901 strain, grown in yeast extract and dextrose for 3 days preceding fixation. The cells were fixed and stained by being placed in aqueous solutions of uranyl nitrate ranging in concentration from 2% to 50%. The duration of fixation and staining was varied from 3 minutes to 18 hours. Best results were obtained with a 40% aqueous solution of uranyl nitrate in which the cells were suspended for 1 hour at room temperature. Yeast cells of the same species and strain were also stained and fixed in a 1.5% aqueous solution of potassium permanganate for 30 minutes at room temperature--the method of Hashimoto, Conti, and Naylor[2], and of Luft[3] to provide a comparison of the results obtained by the two methods. The cells were embedded in a mixture of butyl and methyl methacrylate at a 3:1 ratio. They were sectioned with a Porter-Blum ultramicrotome equipped with a diamond knife, and the specimens were observed with an RCA EMU-2E electron microscope.

Results and Discussion

Uranyl nitrate was incorporated into the yeast cells and appeared to be an effective electron-scattering agent. The cells appeared to be adversely affected by the process of fixation and staining with the uranium compound, and distortion of the cells was observed. Whether this was the result of the acidity of the uranyl nitrate solution (pH 3) or of the biochemical effects of the uranyl ion itself is not known. The cytoplasm exhibited a granular constitution, and no vacuoles, mitochondria, or endoplasmic reticulum could be observed.

However, a more lightly staining area was found in almost every cell examined; this area appeared to be the nucleus of the yeast cells. Within this nuclear area were observed, in most cases, very darkly stained structures (Fig. 1). These structures were in every case found within the lighter stained area, which was thought to be the nucleus, and were paired and symmetrical. The appearance and location of these darkly stained inclusions suggested that they might represent the chromosomal material of the yeast cell. These structures, if chromosomes or chromatin aggregations, would indicate that the yeast nucleus has material within it analogous to the chromosomal material of the cells of other organisms. Since the sections of the cells were cut approximately 400 Å thick, and the yeast

cells are about 4 or 5 micra in thickness, the sections represent only about 1/100 of the cell diameter. It was therefore not possible to estimate the number of these structures in the nucleus from single micrographs of cells. There was also some lack of good resolution. Refinement of the technique of staining cells for electron microscopy with uranyl nitrate is now in progress. The testing of various concentrations of uranyl nitrate, buffers, and solvents other than water is being attempted in order to provide better preservation of the cells and improved resolution of cellular structures in the nucleus and in the cytoplasm. The use of other uranium compounds is also under investigation in an attempt to better resolve these structures. Uranyl nitrate is being tested as a stain for electron microscopy of other cells than yeast, and the results of these tests, we hope, will verify the assumption that the structures observed in the yeast nucleus with this stain are chromosomal structures.

Staining of yeast cells by the use of potassium permanganate by the method of Hashimoto, Conti, and Naylor[2] provided much better resolution of the morphology of the yeast cell as a whole. Hashimoto et al., who used $KMnO_4$ as an electron stain in their studies of yeast, stated that the nucleus appeared to be devoid of internal differentiation in resting cells and in ascospores, and that only some irregular staining was found in the nucleus of budding cells. In our study, we observed definite accumulations of chromatin particles in the nucleus (Fig. 2). These aggregations of chromatin bear close resemblance to chromosome-like structures seen by means of osmic acid staining in the cells of higher organisms. It seems reasonable to assume that these dense formations of nuclear material represent the chromosomes of the yeast cell.

Summary

The nuclei of yeast cells were examined by electron microscopy by using uranyl nitrate and potassium permanganate as stains. The cells stained with uranyl nitrate exhibited dense structures in the nucleus which may be the chromosomal material; those stained with $KMnO_4$ also showed some indication of similar material.

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2. Hashimoto, T., Conti, S.F., and Naylor, H.B., Studies of the Fine Structure of Microorganisms. III. Electron Microscopy of Resting and Germinating Ascospores of Saccharomyces cerevisiae, J. Bacteriology, 1958, 76, 406-416.
3. Luft, J.H., Permanganate-a New Fixative for Electron Microscopy, J. Biophysical and Biochemical Cytology, 1956, 2, 799-801.

Figure Legends

Fig. 1. Cells of Saccharomyces cerevisiae stained with uranyl nitrate. Plate 1 - ×32,000.

Fig. 2. Cells of Saccharomyces cerevisiae stained with potassium permanganate. Plate 2 - ×30,000.

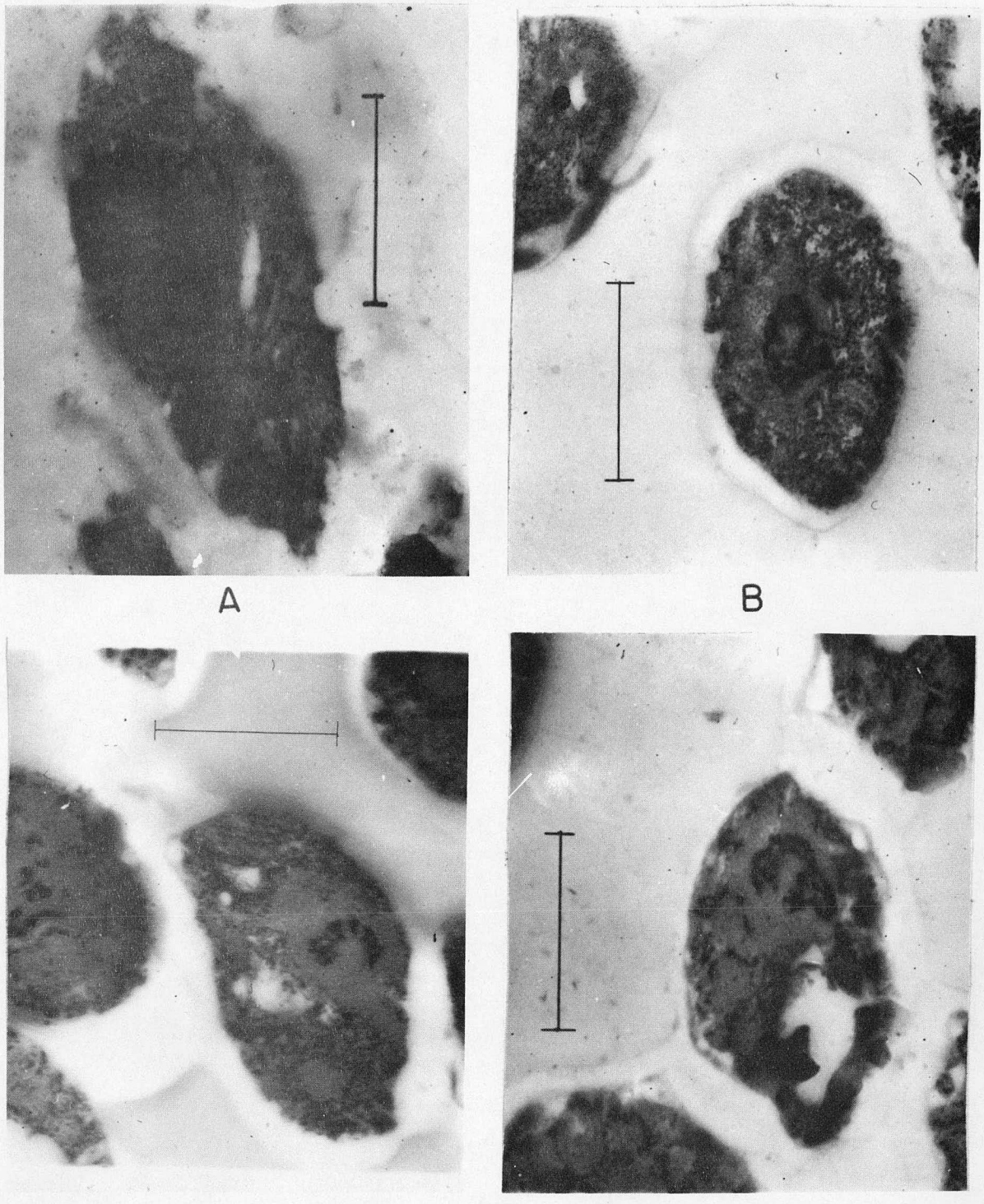

Plate I

ZN-2299

Fig. 1

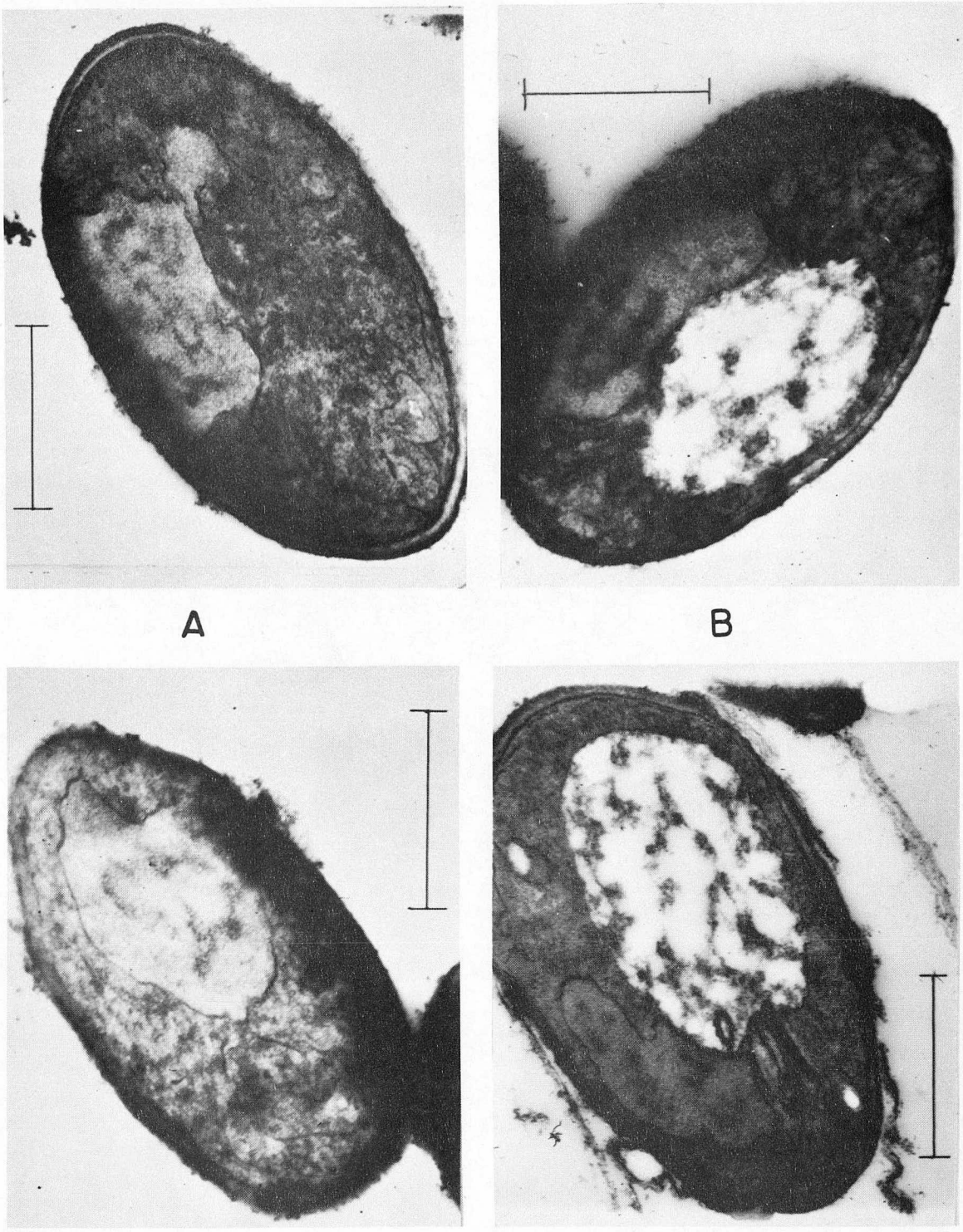

Plate 2

ZN-2300

Fig. 2