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Ultraviolet Microscopy of Purines and Amino Acids in the Vacuole of *Candida albicans*

EDWARD BALISH[1] AND GEORGE SVIHLA

Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439

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Ultraviolet (UV) microscopy was used to study the capacity of yeast (ATCC 10231 and 10261) and filamentous (ATCC 10259) strains of *Candida albicans* to accumulate UV-absorbing materials from a medium supplemented with purines, pyrimidines, amino acids, or related compounds as the main nitrogen source. All strains accumulated UV-absorbing compounds when adenine, adenosine, isoguanine, xanthine, or uric acid was supplied as a nitrogen source, but they did not accumulate UV-absorbing compounds when pyrimidines were supplied. The filamentous strain accumulated UV-absorbing material from medium supplemented with hypoxanthine, but the yeast strains did not. In contrast, the yeast strains accumulated more UV-absorbing material than did the filamentous strain when guanine was the nitrogen source. Yeast strain 10231 not only accumulated UV-absorbing material from tyrosine-supplemented medium, but it became filamentous in form as well. Yeast strain 10261 and filamentous strain 10259 did not accumulate detectable amounts of UV-absorbing material, nor was their morphology noticeably affected by the supplement. The two yeast strains accumulated more lipid than the filamentous strain when they were incubated in a nitrogen-deficient medium.

The ability of *Candida albicans* to grow in filamentous (mycelial) as well as in yeast-like form and the difference in the pathogenicity of these two types have stimulated interest in the nutrition and metabolism of this organism in relation to its morphology (1). The extent of assimilation of nucleic acid constituents and their effects on *C. albicans*, especially when used as the principal or sole source of nitrogen, are not known in detail. Yeasts vary widely in their capacity to take up, metabolize, and store purines and pyrimidines. A study of the effect of these bases on *C. albicans* was encouraged by earlier observations on the involvement of the vacuole of certain yeasts in the accumulation of purines and related compounds (1, 7–9, 11); an influence on the properties of the yeast cell wall has been noted in some instances (1, 12).

This report describes experiments on the assimilation of purines and pyrimidines by *C. albicans*, with ultraviolet (UV) microscopy as the principal analytical method. Incidental observations on the cellular uptake of the UV-absorbing aromatic amino acids and the resultant distribution of UV-absorbing materials are also reported.

[1] Present address: Oak Ridge Associated Universities, Medical Division, Oak Ridge, Tenn. 37830.

MATERIALS AND METHODS

Organisms and culture media. Three strains of *C. albicans*, ATCC 10231, 10261, and 10259, were used. Strain 10259 is a filamentous strain derived from 10261 by MacKinnon (6). The methods used to maintain stock and experimental cultures have been previously described (1). A nitrogen-free medium, obtained by omitting $(NH_4)_2SO_4$ from the medium used in earlier, related experiments (1), consisted of: KH_2PO_4, 10 g; K_2HPO_4, 5 g; trisodium citrate, 1.0 g; $MgCl_2 \cdot 6H_2O$, 0.1 g; $MnSO_4 \cdot 7H_2O$, 0.1 g; $CaCl_2$, 0.1 g; $ZnSO_4 \cdot 7H_2O$, 0.1 g; biotin, 5 μg; glucose (sterilized separately), 15 g; distilled water to 1 liter. Purines, pyrimidines, adenosine, and UV-absorbing amino acids, all of commercial origin, were added to the nitrogen-free medium at a concentration of 1 to 2 μmoles/ml of medium when used as the source of nitrogen. About 5 mg of moist cells per ml, in a total volume of 40 to 50 ml of medium, was used to study the accumulation of UV-absorbing compounds from the medium. Experimental cultures were incubated at 30 C on a rotatory shaker. The cells were incubated for 15 min in the nitrogen-free medium before the UV-absorbing compounds were added. Samples were withdrawn from the culture medium just prior to the addition of UV-absorbing compounds and at various times thereafter. All cell samples were washed three times in cold distilled water and kept at 4 C until UV microscopy was used to demonstrate the location and relative, subjectively evaluated concentrations of UV-absorb-

ing materials in the cells. The equipment and methods used to obtain the UV micrographs have been described (1). In an experiment designed to observe the effects of culturing a yeast-phase organism (ATCC 10231) in a mammalian tissue culture, the yeast cells were introduced into a culture of pig kidney cells maintained on 95% Morgan's medium (Medium 199, Hanks' base, BBL) and 5% calf serum.

RESULTS

Cells cultured in nitrogen-deficient medium. When the yeast-phase strains of *C. albicans*, ATCC 10231 and 10261, were cultured in a nitrogen-deficient medium with 5% glucose, the cells gradually accumulated large amounts of lipid. The difference in appearance of these cells at the beginning of an experiment and after culture for 36 hr is illustrated in Fig. 1a and b. After only 24 hr in the nitrogen-deficient medium, an intermediate amount of lipid was present and the cytoplasm showed moderate UV absorption, similar to that in the (unrelated) experiment illustrated in Fig. 1f. Lipid accumulation was not as great when the mycelial mutant ATCC 10259 was cultured under nitrogen-deficient conditions (Fig. 1c and d). The darker vacuoles, indicative of greater UV absorption, are due to the presence of a larger amount of *S*-adenosylmethionine in this strain than in the other two strains (1).

Purine supplement. Cells of the yeast-phase strain 10231 accumulated large quantities of UV-absorbing material in their vacuoles in a very short time when uric acid was the main source of nitrogen in the culture medium. Regions of high UV absorption were apparent within many cells after 30 min in the uric acid medium. An increase in the number of cells with accumulations of UV-absorbing material in their vacuoles continued until a maximum was reached at about 2 hr (Fig 1e). After that, the number of cells containing UV-absorbing material decreased. At 5 hr, less than 50% contained UV-absorbing crystals; at 6 hr, about 1% and at 24 hr only an occasional cell could be found with crystals in its vacuole. At 24 hr, the cytoplasm lacked the moderate UV absorption at 265 nm that is characteristic of actively metabolizing cells, and a large amount of lipid in the form of globules was present in the cytoplasm, an indication of nitrogen deficiency (Fig. 1f).

With mycelial strain 10259 (Figure 1g and h), the results were similar to those obtained with yeast-phase strain 10231. There was no apparent difference between the uptake of uric acid by mycelial, pseudomycelial, or single cells in either strain. A slight difference in the shape of the crystalline masses within the vacuoles of the two strains may indicate a difference in the composition or distribution of their vacuolar contents. Although the rates of accumulation and utilization of uric acid were about the same for the two strains (10231 and 10259), the results varied with other purines or related compounds. Adenine was taken up rapidly (1 to 2 hr) by the yeast-phase strain (10231), and large quantities of UV-absorbing material were still present in the vacuoles at 24 hr (Fig. 2a). With the mycelial strain, however, only a slight accumulation was evident at 4 hr and, at 24 hr, only a few cells contained a large amount of UV-absorbing material (Figure 2b). The vacuole of the large round cell in Fig. 2b had an absorbance of 0.8, suggesting supersaturation or the presence of something other than adenine in the vacuole; a saturated solution of adenine in the vacuole would have an absorbance of about 0.05 (11) for the estimated light path of 3 μ. Xanthine was accumulated and utilized by both yeast and filamentous strains at about the same rate, but accumulation of UV-absorbing compounds did not begin until about 1 hr later than with uric acid (Fig. 2c and d). Hypoxanthine was not taken up readily by the yeast-phase strain at any time, whereas heavy accumulations of UV-absorbing material appeared in the mycelial strain late (24 hr) in the experiment (Fig. 2e and f). Guanine stimulated the earlier and more concentrated appearance of UV-absorbing material in the yeast-phase strain than in the mycelial strain (Fig. 2g and h). Isoguanine was present both early (2 hr) and late (24 hr) in the yeast-phase strain, and late in the mycelial strain (Fig. 3, a and b). A summary of these results is presented in Table 1.

Adenosine supplement. Because of the difference observed in the accumulation of UV-absorbing compounds in the vacuole of yeast and mycelial strains of *C. albicans* when both were cultured in a medium with adenine as a nitrogen source, a similar experiment was carried out with adenosine. Both yeast and filamentous forms manifested a lag period before UV-absorbing compounds accumulated in the vacuole (Table 1). The lag was more pronounced with the filamentous form than with the yeast strain. These results are in contrast to those reported previously for *C. utilis*. Although adenosine enters cells of *C. utilis* (3), no increase in UV-absorbing compounds in the vacuoles was detected, even when the organism was cultured in the presence of 25 μmoles of adenosine per ml of medium (11).

Pyrimidine supplement. Contrary to the results obtained with purines and adenosine, the vacuoles of yeast and mycelial forms of *C. albicans* did

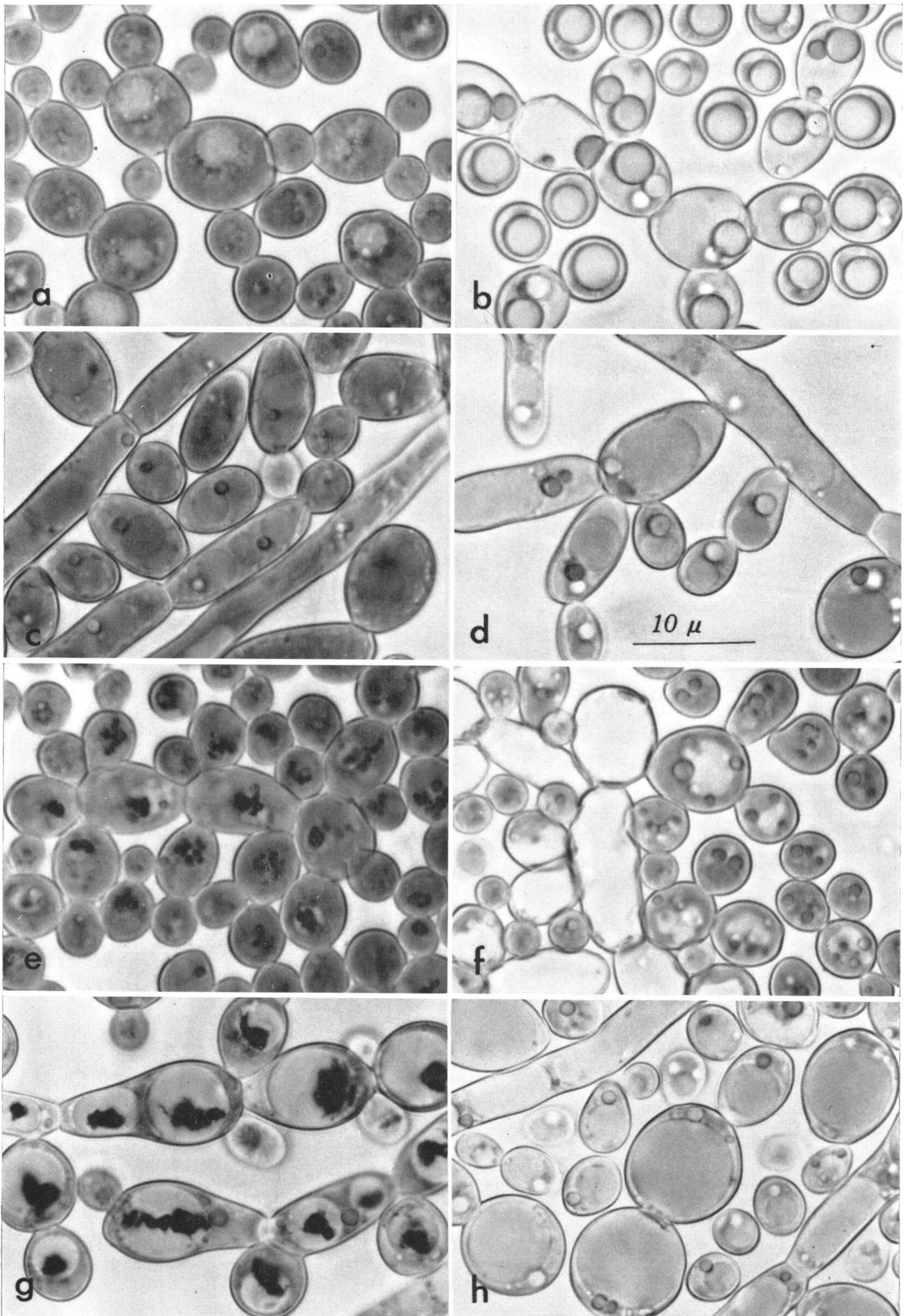

FIG. 1. *Accumulation of lipid and the effect of uric acid on the accumulation of UV-absorbing materials in Candida albicans. UV micrographs made at 265 nm. ATCC 10231: (a) control, 0 hr; (b) nitrogen deficient, 36 hr. ATCC 10259: (c) control, 0 hr; (d) nitrogen deficient, 36 hr. ATCC 10231: (e) uric acid, 2 hr; (f) uric acid, 24 hr. ATCC 10259: (g) uric acid, 2 hr; (h) uric acid, 24 hr.*

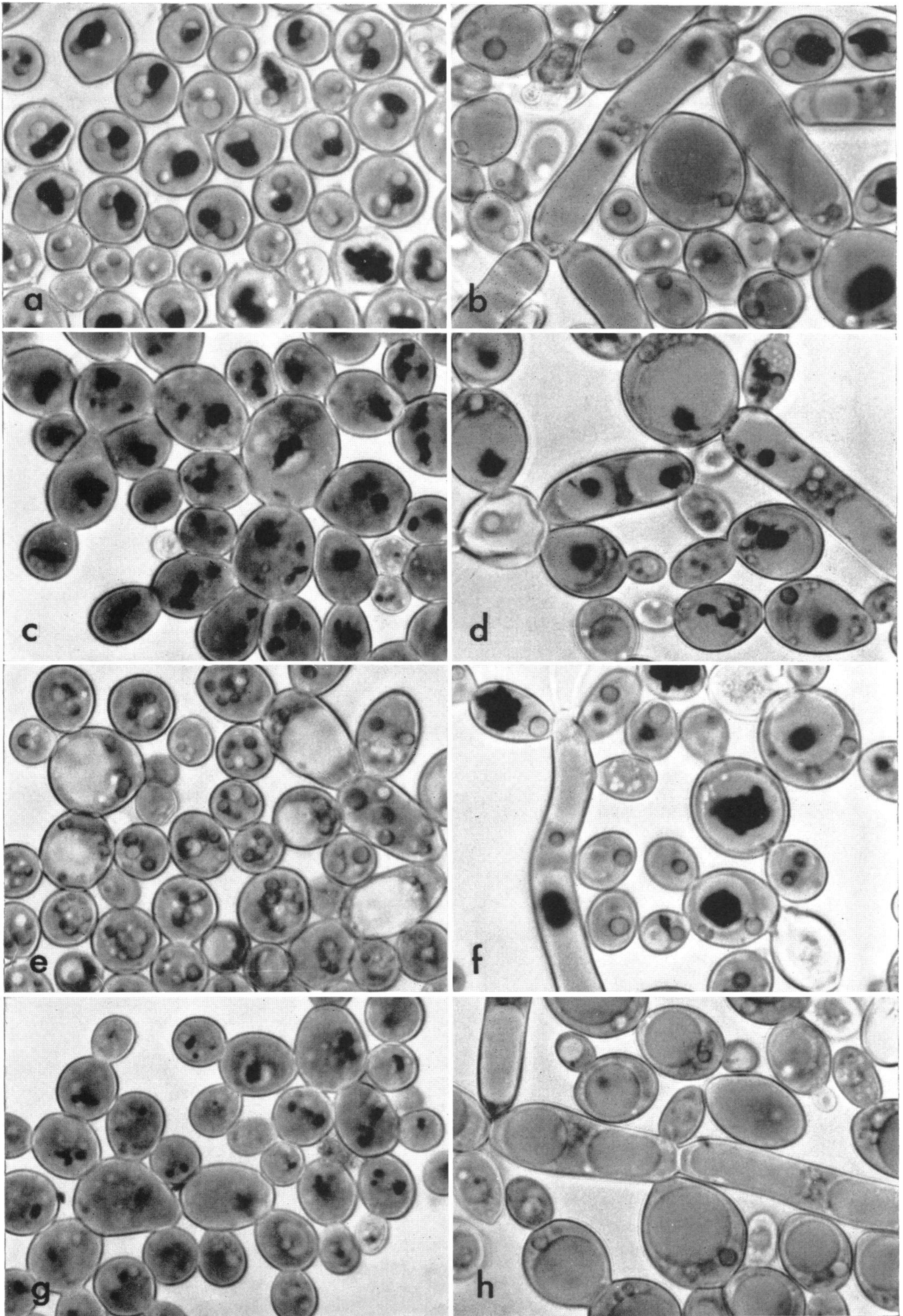

FIG. 2. *Effect of purines on the accumulation of UV-absorbing materials in Candida albicans. UV micrographs made at 265 nm. Adenine, 24 hr: (a) ATCC 10231; (b) ATCC 10259. Xanthine, 2 hr: (c) ATCC 10231; (d) ATCC 10259. Hypoxanthine, 24 hr: (e) ATCC 10231; (f) ATCC 10259. Guanine, 2 hr: (g) ATCC 10231; (h) ATCC 10259.*

TABLE 1. *Compounds which caused the accumulation of UV-absorbing material in the vacuole of yeast and filamentous strains of Candida albicans*[a]

Compound	ATCC 10231			ATCC 10259		
	2 hr	4 hr	24 hr	2 hr	4 hr	24 hr
Uric acid	+++	+++	−	+++	+++	−
Adenine	+	+	+++	−	+	++
Adenosine	−	+	++	−	−	+++
Guanine	++	++	+	−	+	−
Isoguanine	+	+	++	−	+	++
Xanthine	+	++	−	+	++	−
Hypoxanthine	−	−	−	−	+	+++
Tyrosine	+++	+++	−	−	−	−

[a] No accumulation of UV-absorbing compounds was observed in the vacuole of yeast and filamentous forms of *C. albicans* when the following compounds were used as the main nitrogen source: cytosine, thymine, uracil, *n*-acetyl tyrosine, 3,5-diiodotyrosine, tryptophan, phenylalanine. Yeast strain 10261 gave the same results as strain 10231 except tyrosine was without effect.

not show any increase in UV absorption when cytosine, uracil, or thymine was present in the medium.

Amino acids. Since the amino acids tyrosine and tryptophan absorb UV energy, it was possible to test whether culture in a medium that contained these amino acids might result in accumulation of UV-absorbing materials in the vacuoles of the yeast and mycelial forms of *C. albicans*. Cells of the yeast-phase strain 10231 showed a rapid accumulation of UV-absorbing material in a tyrosine-supplemented medium (Fig. 3c, e, and g). Of particular interest was the formation of a great number of hyphal cells after 8 hr of incubation in the presence of tyrosine (Fig. 3g). Comparison with earlier samples revealed a trend toward mycelial growth as early as 5 hr (Fig. 3e). Although the mycelial strain 10259 did not show an accumulation of material in the vacuoles, the UV-absorption in general was a little stronger than in other experiments and a greater incidence of mycelial growth was noted (Fig. 3d and f). When another yeast-phase strain, ATCC 10261, was cultured under similar conditions, no vacuolar accumulation of UV-absorbing material or hyphal growth was observed. Culture of the three strains of *C. albicans* with tryptophan, 3,5 diiodotyrosine, *N*-acetyltyrosine, or phenylalanine showed no accumulation of UV-absorbing materials or change in morphology. An accumulation of lipid in these cells after 24 hr indicated that the cells were in a nitrogen-deficient state. The accumulation of lipid was most pronounced in the yeast-phase strains.

Bovine serum albumin and mammalian tissue culture medium. To test whether *C. albicans* could accumulate UV-absorbing material present in serum or in tissue culture, yeast-phase *C. albicans* cells were inoculated into 5% bovine serum albumin and into a growing pig kidney tissue culture. No additional supplements of purines, pyrimidines, or amino acids were added. No accumulation of UV-absorbing material in the vacuoles of *C. albicans* was detected by UV micrography. Yeast-phase strains did show some mycelial growth under these conditions (Fig. 3h).

DISCUSSION

The discovery by Roush (7) that certain yeasts take up large quantities of purines from the culture medium and accumulate them in the vacuole gave hope that a better understanding of the function of this cellular compartment could be achieved. Subsequent studies, however, have shown significant differences in this respect among various species (1, 9, 11). This cast doubt that a general theory of the vacuole as a storage compartment could be formulated at this time. The present study has revealed that there is a lack of uniformity even among various strains of one organism, *C. albicans*. This organism was selected for this study because it permitted a comparison of the yeast and filamentous forms. Assimilation and storage of purines by *C. albicans* was observed in some instances, but no significant differences between yeast and mycelial form were evident. The inability of this organism to accumulate pyrimidines in quantity is shared with other yeasts. It must be remembered, however, that UV micrography reveals only massive concentrations of UV-absorbing compounds. The path of the UV energy traversing the cells or vacuoles is too short to result in significant absorbance by low concentrations of the compounds studied here.

A novel observation is the capacity of the yeast-

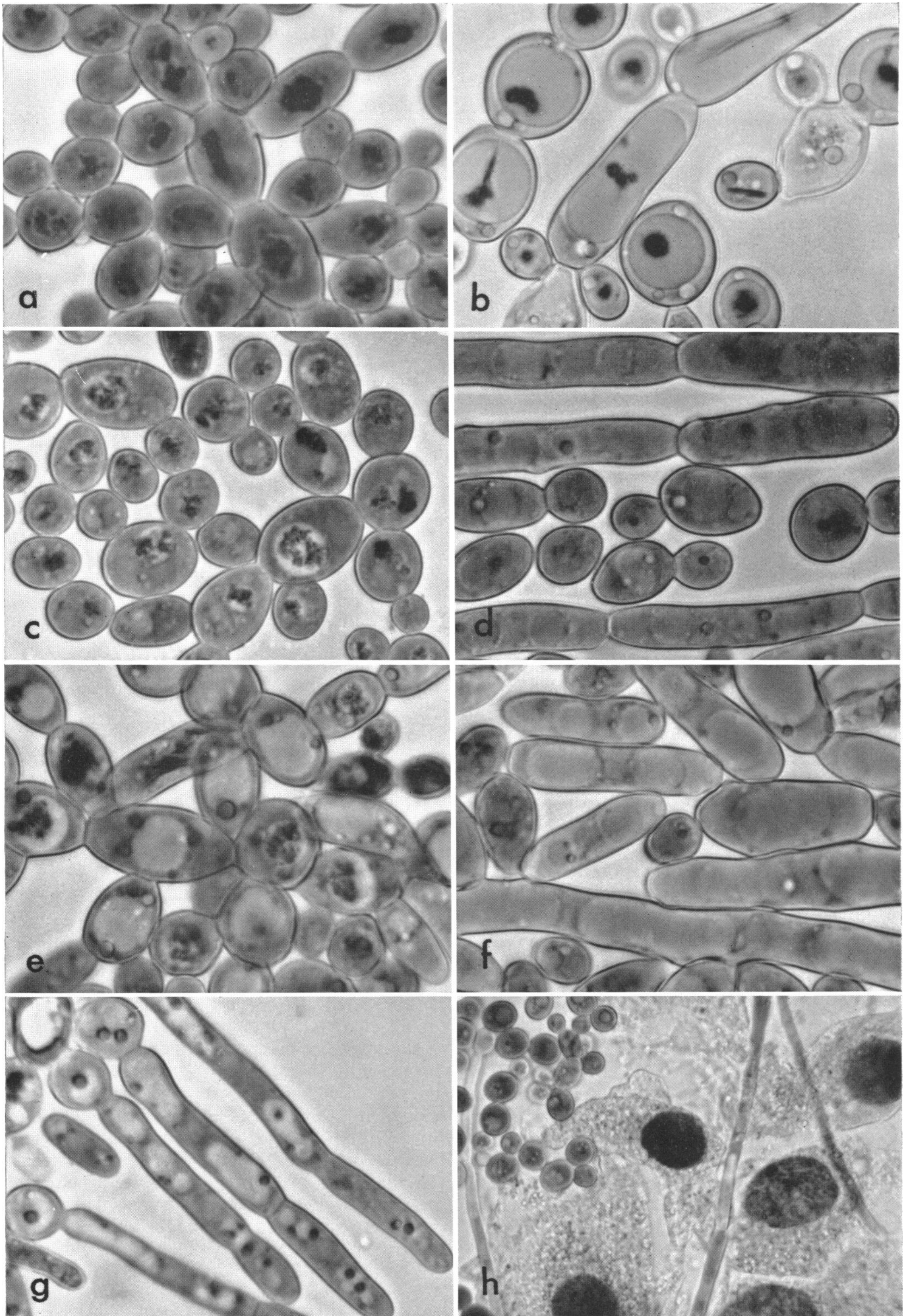

FIG. 3. *Effects of purines and amino acids on the accumulation of UV-absorbing materials in and on the morphology of Candida albicans. UV micrographs made at 265 nm. Isoguanine: (a) ATCC 10231, 2 hr; (b) ATCC 10259, 24 hr. Tyrosine, 1 hr: (c) ATCC 10231; (d) ATCC 10259. Tyrosine, 5 hr: (e) ATCC 10231; (f) ATCC 10259. Tyrosine, 8 hr: (g) ATCC 10231; (h) ATCC 10231 cells grown in a pig kidney cell tissue culture to show development of a mycelium under these conditions.*

phase strain of *C. albicans* ATCC 10231 to concentrate detectable amounts of UV-absorbing compounds in the vacuole as a result of feeding tyrosine. A specific membrane transport system appears to function because UV-absorbing compounds were not found in the vacuole by UV optical techniques when phenylalanine, tryptophan, and some derivatives of tyrosine were supplied. The high level of lipids in cells whose nitrogen supply is derived from purines resembles a state of nitrogen deficiency. Some yeasts cannot assimilate the nitrogen of heterocyclic compounds (2, 5). In others, induced enzymes (7, 8, 9) gradually provide nitrogen for metabolic purposes by breakdown of the purine ring system, and a temporary nitrogen deficiency may prevail in the early phases of the culture.

The dimorphism phenomenon in yeasts has been reviewed by Scherr and Weaver (10) and more recently by Hayes (4). The ramifications are numerous. In the present investigation, we utilized UV micrography as another tool in the continued morphological study of *C. albicans*.

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