LOCALIZATION OF *S*-ADENOSYLMETHIONINE IN *CANDIDA UTILIS* BY ULTRAVIOLET MICROSCOPY[1]

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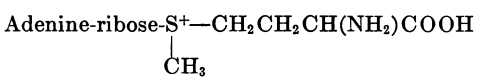
Supplementation of the culture medium of *Candida utilis* (*Torulopsis utilis*) and of *Saccharomyces cerevisiae* with L-methionine results in greatly increased formation and accumulation of *S*-adenosylmethionine (Schlenk and DePalma, 1957*a*), a sulfonium compound whose structure comprises adenosine and methionine in the following way:



Under suitable conditions, a concentration of 40 to 60 μmoles per gram of yeast cells on a dry weight basis may be obtained. Because the molecular weight of the sulfonium ion is 399, this corresponds to a concentration of 1.6 to 2.4 per cent. *S*-Adenosylmethionine has the typical absorption spectrum of an adenosine compound with E_m 15,400 at 260 mμ. Its concentration remains essentially constant after growth and metabolism have ceased and the quantity of nucleic acids has declined. It appeared possible, therefore, to determine the location of *S*-adenosylmethionine within the cells by ultraviolet microscopy.

As a preliminary to the optical studies and for correlation, the earlier chemical studies had to be amplified. The present report is a detailed account of our observations which have been summarized earlier (Svihla and Schlenk, 1958).

MATERIALS AND METHODS

Candida utilis (*Torulopsis utilis*) strain ATCC 9950 was used in the present experiments. The composition of the growth medium, culture conditions, and harvesting procedure have been described earlier (Schlenk and DePalma, 1957*b*). L-Methionine in a concentration of 4 to 6 μmoles per ml, or a combination of *S*-methyl-L-methionine and L-homocysteine (4 μmoles of each) was used as organic sulfur supplement. For maximal accumulation of *S*-adenosylmethionine in the cells, the medium, except for the sulfur amino acids, should contain nitrogen in the form of NH_4^+ only. After centrifugation at 1000 × G for 15 min, a slurry of cells was obtained which contained 83 ± 2 per cent moisture as determined by drying of samples at 110 C to constant weight. All data in this report are computed to a dry weight basis.

Assay of *S*-adenosylmethionine was accomplished by extraction with cold 1.5 N perchloric acid, chromatography on Dowex 50, H^+ resin, and ultraviolet spectrophotometry (Schlenk and DePalma, 1957*a*). The procedure of Ogur *et al.* (1952) for extraction and measurement of nucleic acids and nucleotides was used, but it was found impossible to extract *S*-adenosylmethionine with 2 per cent perchloric acid at low temperature in the nucleotide fraction. Instead, treatment with 0.5 N perchloric acid for 20 min at 70 C was used to determine the total ultraviolet absorption at 260 mμ. This comprises the nucleic acids, nucleotides, *S*-adenosylmethionine, and their split products. We were able to confirm that the conditions specified by Ogur *et al.* (1952) provide complete extraction; higher acid concentration, higher temperature, or prolonged extraction did not result in improvement. In view of the disparity of the molar extinction of nucleic acid components and *S*-adenosylmethionine, it was considered preferable to list the optical density derived from 1 mg of dry cell material per ml whenever heterogeneous extracts were analyzed. The data thus are listed as $E_{(260m\mu)}$ 0.1 per cent, 1.0 cm.

For adenine and guanine analysis the directions of Loring (1955), Cohn (1955), and Magasanik (1955), summarized in Chargaff and Davidson's (1955) monograph were followed.

Microscopy was carried out with the aid of a color-translating ultraviolet microscope, model UV 91, of the Scientific Specialties Corp., Boston Massachusetts (Land *et al.*, 1949; Land, 1952; Shurcliff, 1952). The machine makes automati-

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cally controlled exposures on 35-mm spectrum-analysis film at each of three selected wave lengths, processes the film, and then projects the three images through red, green, and blue filters in superposition upon a translucent screen to produce a color-translated image. The resulting colors are due to differences in absorption of the three wave lengths by the material being examined. With the wave lengths used, exposure time is about 30 sec.

Washed and centrifuged cells were suspended in distilled water and placed on Vycor slides under Vycor cover glasses ringed with paraffin oil. A Bausch and Lomb UV reflecting objective, 2.8 mm, 0.72 N.A., 53×, was used with a 6× ocular.

Selection of the area to be photographed and focussing were done with light of the mercury green line (546 mμ). New fields were selected after each set of exposures to avoid ultraviolet irradiation effects.

The wave lengths usually employed were 260, 350, and 280 mμ, with a bandwidth of about 4 mμ. Absorption at 260 mμ was translated as blue, 350 mμ as green, and 280 mμ as red.

RESULTS

An example of the effect of organic sulfur supplements on the concentration of *S*-adenosylmethionine is given in table 1. A slight inhibitory effect on the yield of cells occurs often, and the cell centrifugate occasionally appears somewhat slimy, but no differences between *S*-adenosylmethionine enriched cells and control cells are discernible by ordinary microscopy. While methionine is utilized for the biosynthesis directly

TABLE 1

S-Adenosylmethionine in Candida utilis (ATCC 9950)

Sulfur Amino Acid Supplement in Culture Medium	Yield of Dry Cell Material	Conc of *S*-Adenosylmethionine
	g/100 ml medium	*μmoles/g dry cells*
None	0.76	5.5
L-Methionine (8.0 μmoles/ml)	0.66	31.0
S-Methyl-L-methionine + L-homocysteine (4.0 μmoles of each/ml)	0.56	60.0

The culture period was 48 hr at 30 C in a medium containing salts, NH_4^+, and glucose.

TABLE 2

Influence of methionine on the concentration of adenine and guanine in Candida utilis

Organic Sulfur Supplement	Yield of *S*-Adenosylmethionine	Total Adenine	Total Guanine
	μmoles/g	*μmoles/g*	*μmoles/g*
None	4.5	38.5	35.4
L-Methionine (5.0 μmoles/ml)	57.5	87.5	38.0

Washed cells of a 48-hr culture were examined by acid extraction, hydrolysis, chromatography, and spectrophotometry. Details are given in the text.

(Cantoni and Durell, 1957), *S*-methyl-L-methionine and L-homocysteine undergo transmethylation to yield methionine (Shapiro, 1955) which, in turn, is incorporated into *S*-adenosylmethionine (Schlenk and DePalma, 1957*a*). The increased cellular concentration of this compound indicates a substantial investment of adenine. It appeared important to learn whether this occurs by depletion of other purine-containing compounds or by synthesis of adenine. Thus, *S*-adenosylmethionine containing cells and control cells were exhaustively extracted with cold 1.5 N perchloric acid, and the extracts were hydrolyzed at 100 C for 2 hr. Separation of adenine and guanine was accomplished by ion exchange chromatography on Dowex-50-H^+ resin. From the data listed in table 2 it is apparent that after enrichment of the cells, the amount of adenine bound in *S*-adenosylmethionine is greater than that in all other adenine-containing compounds including the nucleic acids. There is no drastic change in the total guanine content nor in the concentration of other adenine compounds. As a further preliminary to ultraviolet microscopy it was desirable to compare the optical density at 260 mμ of exhaustive extracts of the two types of cells. The results are listed in table 3. The values obtained with extracts from sulfur-supplemented cells are higher by ample margin to account for the *S*-adenosylmethionine present. In both instances the amount of total ultraviolet-absorbing material goes through a maximum with progressive age of the culture. The accumulation of *S*-adenosylmethionine is rapid, and there is only a slight decline in its concentration with advancing age of the culture. Most of the optical obser-

TABLE 3

Ultraviolet-absorbing material extractable from Candida utilis (ATCC 9950)

Culture without Organic Sulfur Supplement				Culture with Organic Sulfur Supplement		
Culture time	Yield of cells	Optical density of extract at 260 mμ	Adenosylmethionine	Yield of cells	Optical density of extract at 260 mμ	Adenosylmethionine
hr	*g/100 ml*		*μmoles/g*	*g/100 ml*		*μmoles/g*
0	0.1	2.4	10.0	0.1	2.4	10.0
4	0.15	3.0		0.12	3.0	30.6
7	0.22	2.9		0.25	3.7	44.8
18	0.47	2.1		0.46	3.9	45.0
28	0.51	1.9		0.47	3.1	43.1
48	0.53	2.0	11.1	0.51	2.9	41.8

The cultures with organic sulfur supplement contained 4.0 μmoles of *S*-methyl-L-methionine and 4.0 μmoles of L-homocysteine per ml. The optical density value at 260 mμ represents the material extracted from 1.0 mg of dry cells per ml, measured over a light path of 1.0 cm. The zero time values refer to the starter yeast which was cultivated in the absence of organic sulfur supplement.

vations were made, therefore, with 40- to 48-hr cultures. At this time ultraviolet-absorbing matter other than *S*-adenosylmethionine is at a minimum, and the optical difference between the two types of cell extracts is most nearly accounted for by the adenine sulfur compound.

In figure 1, the absorption spectrum (molar extinction) of highly purified *S*-adenosylmethionine is compared with the optical density of a perchloric acid extract from Candida cells which had not been enriched with the adenine sulfonium compound. Since the absorption maximum is at 260 mμ in both instances, *S*-adenosylmethionine cannot be distinguished *in vivo* from cellular nucleic acids. Its localization appeared possible, however, on the basis of the increment in optical density at 260 mμ, or by its occurrence in discrete sites apart from the nucleic acids of the cells.

Examination of the color-translated images of *S*-adenosylmethionine containing cells showed the vacuoles to be blue whereas the vacuoles of control cells were the gray of the background. The blue color of the *S*-adenosylmethionine-containing vacuole represents absorption at 260 mμ while the gray of the control vacuole repre-

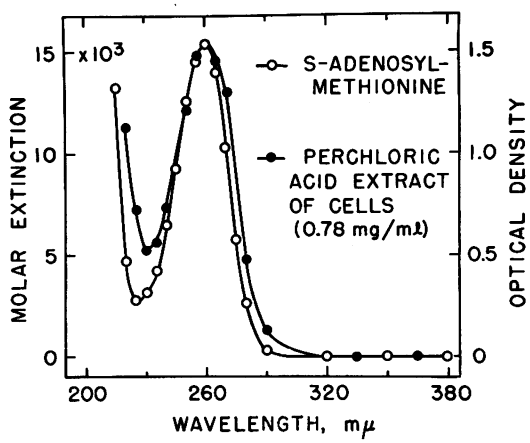


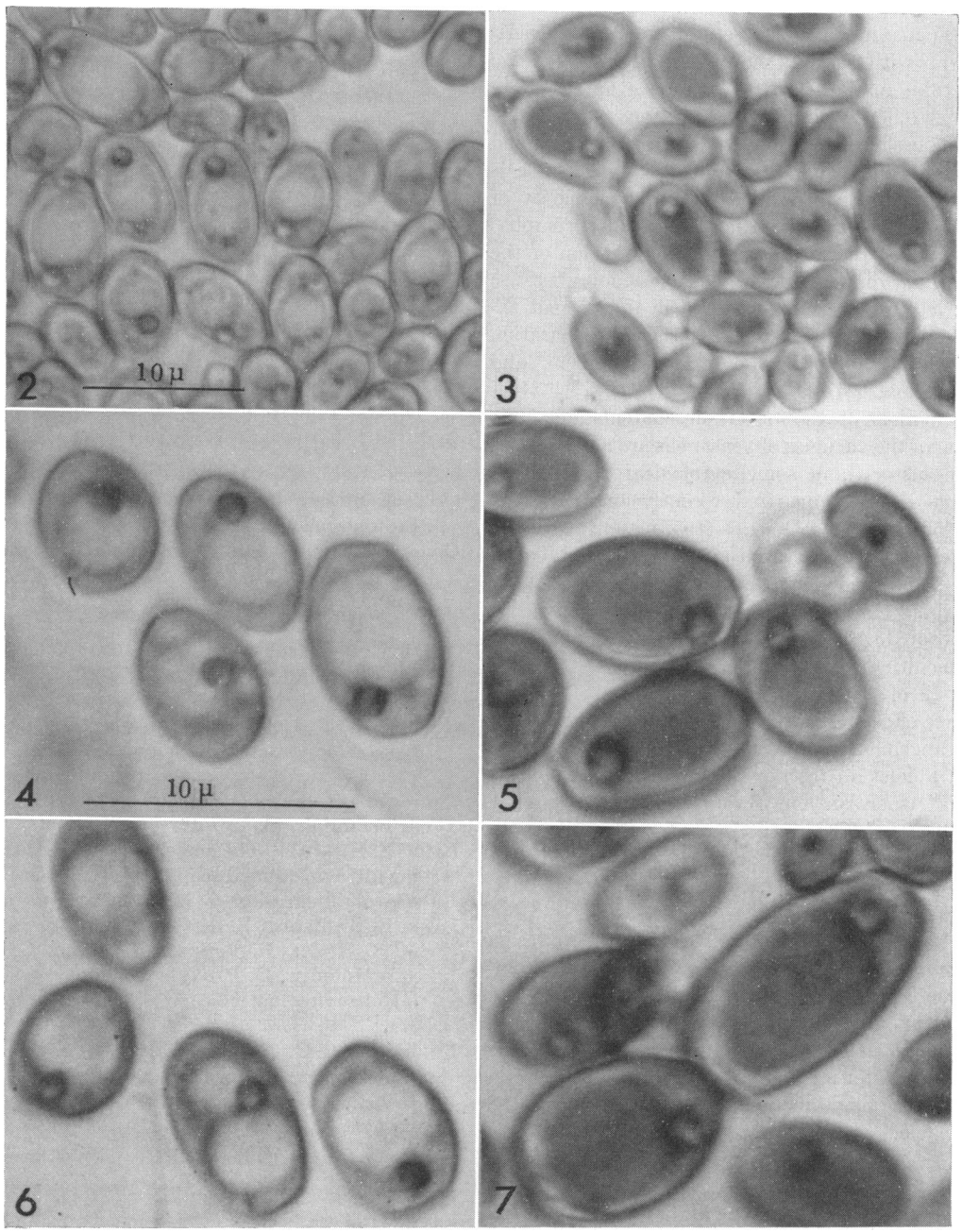
Figure 1. Ultraviolet absorption of yeast cell constituents.

sents lack of absorption at all three of the wave lengths used. Although the color translation is useful when making observations with the microscope (Graham and Crozier, 1956), it is simpler to use black and white prints for purposes of illustration. Figures 2 to 7 are reproductions of prints made from negatives exposed at 260 mμ, the degree of blackening being roughly proportional to the amount of absorption. Exposures made at 280 mμ showed similar results but with less contrast. The 350 mμ wave length, to which the cells are quite transparent, was used as a reference. Observations were made on numerous samples over a two year period with identical results.

It appears rather obvious that the vacuoles of cells containing *S*-adenosylmethionine absorb more ultraviolet light than those of the controls. It is interesting to compare these figures with those of Caspersson (1950) showing localization of ribose nucleotides.

DISCUSSION

Although *S*-adenosylmethionine has multiple function as a biochemical intermediate and group donor (Cantoni, 1952; Schlenk *et al.*, 1958), there is no explanation for the fact that yeasts, in comparison with other cells, in the presence of methionine can produce and accumulate such unusual amounts of *S*-adenosylmethionine. The increase in adenine concentration under the influence of methionine has been emphasized by Schmidt *et al.* (1956) who noted a concomitant increase in the



Figures 2 to 7. Ultraviolet photographs of *Candida utilis* at 260 mμ (*2*) Control, and (*3*) cells containing *S*-adenosylmethionine, 24-hr culture at 30 C; (*4*) control, and (*5*) cells containing *S*-adenosylmethionine, 48-hr culture at 30 C; (*6*) control and (*7*) cells containing *S*-adenosylmethionine, 48-hr culture at 25 C.

All cells were obtained from identical cultures except that the control cultures lacked the methionine supplement.

total guanine in bakers yeast. The present experiments with Candida showed only a slight increase in total guanine, but the cultures examined were much older. *C. utilis* stores *S*-adenosylmethionine and does not resort to this material when depleted of nutrients. Our observation that the vacuole is the site of deposit may explain the tenacity of retention of this material. It should be emphasized that there can be no decomposition of the material during storage in the vacuole, because old cultures of the type used in the present experiments are the source material for preparation of the compound in pure form (Schlenk and DePalma, 1957*b*).

Opinions on the formation, contents and function of the vacuoles of yeast cells are not uniform; the history and some cytological observations have been summarized recently by Narayana (1956), Agar and Douglas (1957), and Hashimoto *et al.* (1958). Lindegren (1949, 1952) and Townsend and Lindegren (1953) report that the vacuole harbors chromosomes, the nucleolus, and metaphosphate. These authors point out numerous difficulties encountered in the study of structural detail of yeast cells, mainly because of the adverse effects of fixing and staining on the interior arrangement of the cellular constituents. The ultraviolet microscopy applied to yeast cells first by Caspersson and Brandt (1941) avoids such difficulties. The methods used in this investigation involve short exposures of the cells without pretreatment and thus give assurance that artifacts are avoided.

It may be that *S*-adenosylmethionine shares its confinement to the vacuole with many other compounds which are not as readily detectible. The present observations entail many questions concerning transfer of *S*-adenosylmethionine into the vacuole after its formation in the cytoplasm, and its metabolic function and availability in the vacuole.

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SUMMARY

Candida utilis cultivated in the presence of methionine produces and stores large amounts of *S*-adenosylmethionine. Ultraviolet microscopy reveals that this compound is located in the vacuole.

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