REDUCTION OF SELENITE BY INTACT YEAST CELLS AND CELL-FREE PREPARATIONS

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ABSTRACT

FALCONE, GIUSEPPE (Rutgers, The State University, New Brunswick, N.J.), AND WALTER J. NICKERSON. Reduction of selenite by intact yeast cells and cell-free preparations. J. Bacteriol. **85**:754–762. 1963.—Nonproliferating cell suspensions of *Candida albicans* rapidly reduced selenite to red, metallic selenium in the absence of added substrate. Cell suspensions reduced selenite optimally at pH 4.2. No added metabolite was found to be stimulatory; reduction was inhibited by methionine and formate as well as by fluoride, dinitrophenol, and certain sulfhydryl poisons. Cell-free preparations capable of reducing selenite were obtained from *C. albicans* and from baker's yeast disintegrated in a Hughes press. The enzymatic system had optimal activity at pH 7 with 10^{-2} M selenite. Activity of the system was lost on dialysis but was restored upon the addition of dialyzable substances or of boiled, undialyzed extract.

A selenite-resistant strain of *Candida albicans* (RM806) grew readily in media containing as much as 10^{-2} M sodium selenite. During growth of this yeast, uptake of selenite was both rapid and extensive. The major part of the selenite accumulated was reduced and retained within the cell as red, metallic selenium. Reduction of selenite was also accomplished by intact, nonmultiplying cells. Enzymatic reduction of selenite to selenium has been studied with cell-free preparations obtained from this strain as well as from baker's yeast. A preliminary report on this work has appeared (Falcone and Nickerson, 1960*a*).

Examination of conditions promoting selenite reduction by intact cells and cell-free preparations showed that the process operates at the expense of endogenous cellular metabolism. Through the use of radioactive isotopes, phosphate ions ($H_2PO_4^-$) were shown to compete with selenite ions ($HSeO_3^-$) for uptake by intact cells; dinitrophenol inhibited uptake of phosphate and selenite ions alike.

Selenite-reducing activity in cell-free preparations was found to reside in a soluble, nonparticulate fraction and to be complex in that activity was lost upon dialysis but was restored by the addition of heat-stable, dialyzable components.

MATERIALS AND METHODS

Microbiological procedures. C. albicans RM806 (previously described by Nickerson, Taber, and Falcone, 1956) and *Saccharomyces cerevisiae* (baker's yeast) were used in this study. The strain of *C. albicans* was maintained by frequent subcultures at 28 C on agar slants containing 10^{-3} M sodium selenite in a nutrient medium (GGY medium) comprised of: glycine, 10 g; glucose, 20 g; yeast extract, 1 g; agar, 20 g; and distilled water, 1,000 ml. Cultures were grown in GGY liquid medium at 28 C with continuous agitation on a reciprocal shaker. Baker's yeast was obtained as pressed cakes, free from starch or other additives, from Anheuser-Busch, Inc., Old Bridge, N.J. The cakes, wrapped in parafilm sheets and refrigerated at 2 C, were used within a few days of receipt.

Cell-free preparations. Several methods were employed to obtain cell-free extracts: plasmolysis of cells with toluene; grinding with alumina or with glass powder; freezing and thawing; and disintegration in a Hughes press. The most active enzyme preparations were obtained by the latter method.

For plasmolysis with toluene, 1 lb of pressed baker's yeast was broken into fragments and mixed in a beaker with 120 ml of warm toluene. The beaker was placed in a water bath at about 60 C, and its contents were occasionally mixed with a rigid spatula. When the yeast suspension

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reached a temperature of 38 C, it liquefied and gas was evolved. Water (200 ml) was added, the top layer of toluene was eliminated, and the yeast suspension was centrifuged at 18,000 × *g* for 10 min at 2 C.

In the freezing and thawing procedure, portions of yeast cake, broken by hand into small fragments, or washed packed cells of *C. albicans*, were placed in a Lusteroid centrifuge tube and alternately frozen and thawed three or four times in liquid nitrogen, followed by warming under running water. The cell suspension, diluted with water, was centrifuged at 18,000 × *g* for 10 min at 2 C.

A press (Hughes, 1951) with a cylindrical inner chamber (38 × 70 mm) and a capacity of ca. 30 ml of cell paste was employed; the piston was driven by a guillotine device (Gest and Nordstrom, 1956) in which a weight of 38.5 kg was dropped a distance of 185 cm onto the piston. Portions of pound cakes (about 25 g) were reduced by hand to small fragments, frozen in liquid nitrogen, packed into a cold press (maintained at −20 C), and crushed without addition of abrasive. Liquid nitrogen was poured liberally over the press after it was assembled and after the plunger had been compressed in the guillotine. The mass extruded from the press was extracted three times with a total of 100 ml of 8.5% sucrose solution and centrifuged in the cold at 3,000 × *g*. The supernatant liquid portions were pooled and centrifuged at 18,000 × *g* for 20 min at 2 C; the resulting supernatant liquid was filtered through a Millipore filter (pore size 0.45 μ) to eliminate any residual particulate matter. The extracts were stored at −20 C until required.

Washed-cell preparations. *C. albicans* RM806, grown with continuous agitation for 48 hr at 28 C in GGY broth, was harvested by centrifugation, washed three times with water, and resuspended in water. Suspension densities were determined, after appropriate dilution, at 420 mμ in a Klett-Summerson colorimeter. The cells were incubated at 28 or 37 C for 150 min in 0.02 M phthalate-NaOH buffer (pH 4.2) with sodium selenite (0.01 M).

Cell-free extracts. After cellular disruption and centrifugation, preparations were dialyzed at 2 C in 50-ml portions against 6 liters of 0.9% NaCl solution or tris(hydroxymethyl)aminomethane (tris)-maleate buffer (0.01 M, pH 7.2), with continuous agitation in an Oxford multiple dialyzer for 24 hr; external solutions were renewed after 12 hr of dialysis. The extracts were transparent after dialysis against tris buffer. Extracts were slightly turbid after dialysis against NaCl solution, and some protein precipitated during storage at −20 C; however, activity of the extract was only very slightly impaired. Extracts were incubated in tris-maleate buffer (0.01 M, pH 7.0) with sodium selenite (0.01 M) for 60 min at 37 C.

Estimation of selenite reduction. Extent of reduction of selenite to red, metallic selenium by nonproliferating cell suspensions, or by cell-free preparations, was estimated by the method of Falcone and Nickerson (1960*b*). This method involves determination of increase in optical density (OD) at 420 mμ (due chiefly to formation of red selenium) and correction for nonspecific increase in turbidity as measured at 660 mμ. The corrected OD readings at 420 mμ were converted to μg of selenite reduced (Table 1) or to μg of selenium by reference to a calibration curve (Fig. 1) based on selenite reduced with hydrazine sulfate according to the method of

TABLE 1. *Spectrophotometric determination of elemental amorphous selenium*

Optical density at 420 mμ	Optical density at 660 mμ	Change in OD	Selenium produced	Selenite reduced
			μg/ml	*μg/ml*
0.054	0.012	0.042	2.4	5.3
0.124	0.020	0.104	6	13.2
0.258	0.032	0.225	12	26.5
0.452	0.068	0.374	20	43.9
0.544	0.082	0.462	24	52.7

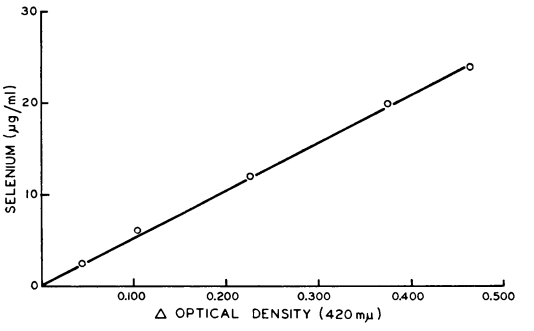

FIG. 1. *Calibration curve for estimation of selenite reduced to metallic selenium from change in OD at 420 mμ, calculated as described in the text.*

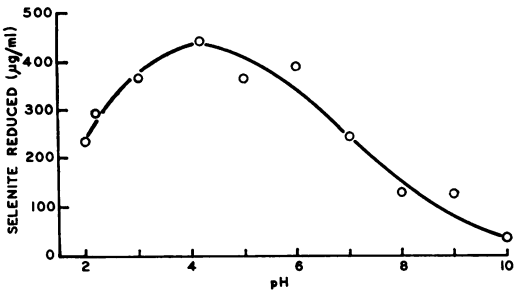

FIG. 2. *Effect of pH of suspending medium on selenite reduction by nonmultiplying cells of Candida albicans RM806. Incubation at 37 C for 150 min with 10^{-2} M Na_2SeO_3 in 0.01 M buffers of the following compositions: pH 2, HCl-KCl; pH 3 to 5, phthalate-HCl or phthalate-NaOH; pH 6 to 8, tris-maleate; pH 9, tris-HCl; pH 10, glycine-NaOH. Note optimum at pH 4.2. Cells (dry weight)/ml = 11 mg.*

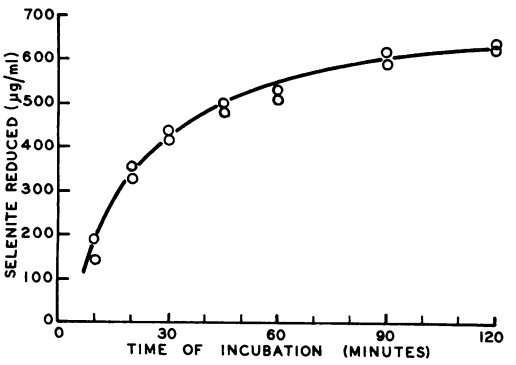

FIG. 3. *Increase in amount of selenite reduced by nonmultiplying cells of Candida albicans RM806 with time of incubation. Phthalate-NaOH buffer (pH 4.2, 0.01 M) containing 10^{-2} M Na_2SeO_3; incubation at 37 C for times noted. Cells (dry weight)/ml = 15 mg.*

Tuller (1954), and estimated spectrophotometrically at 420 and 660 mμ.

RESULTS

Selenite reduction by nonproliferating cells of C. albicans. Intact cells of *C. albicans* RM806 suspended in an aqueous solution of sodium selenite reduced selenite rapidly. Reduction of selenite was markedly influenced by environmental conditions, such as selenite concentration, pH, temperature, and time of incubation. The effect of pH on selenite reduction by resting cells is shown in Fig. 2; an optimum lies in the range from pH 4.2 to 4.7. As will be presented subsequently in more detail, the existence of an optimal pH for reduction of selenite by intact cells reflects the effect of pH on selenite permeation into intact cells. The dissociation constant for the first H of selenious acid is 3×10^{-3} (pKa = 2.52) and for the second H is 5×10^{-8} (pKa = 7.30). A maximal concentration of the biselenite ion ($HSeO_3^-$) is attained at pH 4.9; this form may be assumed to be the species of selenite permeating intact cells.

TABLE 2. *Effect of selenite concentration on selenite reduction by nonproliferating cells of Candida albicans*

Selenite concn	Selenite reduced*
M	μg/ml
10^{-1}	44–54
10^{-2}	420–480
10^{-3}	88–108

* Determined after 150 min of incubation at 37 C, with a cell suspension of approximately 13 mg (dry wt)/ml.

On exposure of washed cells to a solution of selenite, uptake and reduction of selenite commenced promptly, without lag or induction period; the amount reduced increased on continued incubation (Fig. 3), reaching a plateau after about 60 min. With nonmultiplying cells, selenite reduction was extensive at a selenite concentration of 10^{-2} M (Table 2), a concentration ten times higher than that optimal for selenite reduction by multiplying cells.

In Table 3, values are reported for selenite reduction in the presence of a variety of substrates incorporated into the incubation mixture. It is obvious that none of the substances tested exerted any significant stimulatory influence on selenite reduction. Thus, metabolism of endogenous reserve materials suffices to supply reductants for the conversion of selenite to selenium. It is noteworthy that compounds which have been implicated in methylation of selenium (Challenger, 1945) were without stimulatory effect (Table 3). On the contrary, of this group, methionine consistently caused a slight inhibition of selenite reduction; formate, either alone or in association with methionine, homocystine, or betaine, strongly inhibited selenite reduction; formaldehyde had an even more pronounced inhibitory effect. No evidence for the formation of volatile selenium compounds was obtained in

TABLE 3. *Effect of methylating agents and substrates on selenite reduction by nonproliferating cells of Candida albicans*

Additions[a]	Selenite reduction[b]
Methylating agents[c]	
Choline	95
Inositol	105
Betaine	98
DL-Homocystine[d]	82
DL-Methionine	74
Na-formate	41
Na-formate + DL-methionine	45
Na-formate + DL-homocystine[d]	38
Na-formate + betaine	39
Choline + inositol	98
Formaldehyde[e]	22
Carbon substrates[e]	
Glucose	105
Succinate	95
Ethanol	92
Pyruvate	90
Citrate	92
Acetate	89
Lactate	92

[a] The compounds listed were added to cell suspensions at zero time and incubation continued for 150 min at 37 C.

[b] Activity expressed as percentage of endogenous control.

[c] Added at a concentration of 0.02 M.

[d] Added at 0.004 M.

[e] Added at 0.01 M.

TABLE 4. *Effect of metabolic inhibitors on reduction of selenite by intact cells of Candida albicans**

Addition†	Concn of test substance	
	10^{-3} M	10^{-4} M
2,4-Dinitrophenol	57	27
KCN	50	7
NaN_3	82	63
Cu^{++}	100	56
Hg^{++}	100	85
$AsO_3^{=}$	25	0
Ag^{+}	91	73
p-Chloromercuribenzoate	49	18
Iodoacetate	75	15
Iodoacetamide	55	0
Fluoroacetate	0	0
Fluoride	15	0
Ethylenediaminetetraacetate	6	0
Atabrine	0	0
Aminopterin	0	
Sulfathiazole	0	

* Results are expressed as per cent inhibition in comparison with the control.

† Test substances were added to the cell suspension at zero time. Incubation was for 150 min at 37 C.

studies with growing cultures of *C. albicans*. In cultures held for many days, only traces of alkyl selenide formation were detected, beginning many days after both multiplication and selenite reduction had ceased (Falcone and Nickerson, 1960*b*). In the short-term experiments with nonmultiplying cells and cell-free preparations, no evidence for the formation of volatile selenium compounds was obtained. Thus, inhibition of selenite reduction by methylating agents does not result from diversion of selenium into volatile organic combination.

The influence of various well-known metabolic poisons on selenite reduction by intact cells is shown in Table 4. Some sulfhydryl inhibitors, such as Cu^{++}, Hg^{++}, Ag^{+}, and iodoacetate (at final concentrations of 10^{-4} M) strongly inhibited selenite reduction, whereas other sulfhydryl poisons, including arsenite, *p*-chloromercuribenzoate, and iodoacetamide, were only slightly inhibitory at concentrations of 10^{-3} M. Fluoride at 10^{-2} M, a concentration sufficient to block glycogen utilization in yeasts (Nickerson and Chung, 1952), almost completely inhibited selenite reduction. At 10^{-4} M, inhibition by cyanide was slight, but inhibition by sodium azide was quite extensive; the mechanism of action of azide in this instance has been examined and will be described later. Dinitrophenol (DNP) inhibition of selenite reduction by multiplying cells can be overcome by the addition of riboflavine 5′-phosphate (FMN; Falcone and Nickerson, 1960*b*); DNP also strongly inhibited selenite reduction by nonproliferating cells. The yellow color of DNP was discharged by acidification before determination of reduced selenium. However, the deep yellow color of riboflavine phosphate could not easily be eliminated, and the effect of FMN on DNP inhibition of selenite reduction was not investigated with nonproliferating cells.

The inhibitory effect of phosphate on selenite reduction in growing cultures of *C. albicans* has been reported (Falcone and Nickerson, 1960*b*). As shown in Fig. 4, this effect of phosphate is

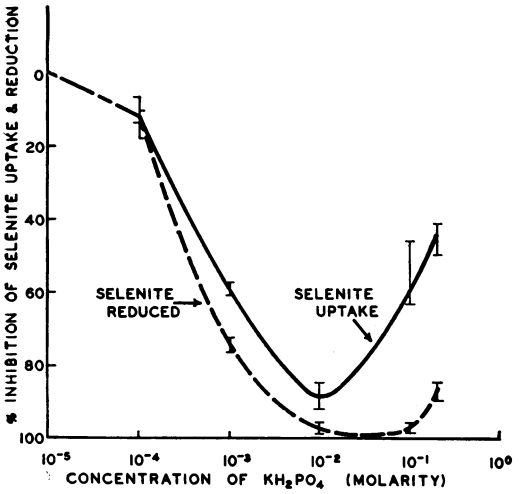

FIG. 4. *Effect of phosphate concentration on selenite uptake and selenite reduction by nonmultiplying cells of Candida albicans RM806. Incubation for 60 min at 37 C in 0.01 M phthalate-NaOH buffer (pH 4.2) containing 10^{-2} M Na_2SeO_3 and KH_2PO_4 in concentrations noted.*

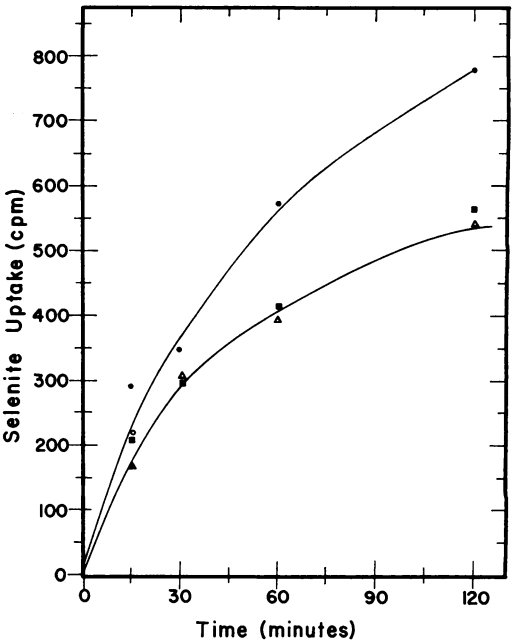

FIG. 5. *Effect of phosphate concentration on uptake of radioactive selenite by nonmultiplying cells of Candida albicans RM806. Incubation for times noted in 0.01 M phthalate-NaOH buffer, pH 4.3, in suspending medium containing $Na_2Se^{75}O_3$ and carrier Na_2SeO_3 to give 0.01 M final concentration and concentrations of KH_2PO_4 as follows: ●, zero; ■, 10^{-3} M; △, 10^{-2} M; ○, 10^{-1} M.*

very marked with non-proliferating cells; inhibition is maximal at phosphate concentrations equimolar with that of selenite. Determinations of the selenite content of the suspending medium at the beginning and at the end of an experiment showed that selenite uptake was inhibited by phosphate. From experiments with multiplying cells, the hypothesis was advanced that such inhibition of selenite reduction could be explained by a competition between phosphate and selenite for sites of absorption. Support for this hypothesis is given by the present demonstration of the parallel inhibition of selenite uptake and reduction in the presence of phosphate. The curious shape of the curves in Fig. 4 is to be noted. Maximal inhibition (90%) of selenite uptake was observed repeatedly at equimolar concentrations of phosphate and selenite (0.01 M), with markedly less inhibition (60%) at phosphate concentrations either one-tenth or ten times that of selenite.

Addition of radioactive selenite ($Na_2Se^{75}O_3$) to carrier selenite, to give a final concentration of 0.01 M, served to confirm the fact that phosphate caused inhibition of selenite uptake. In contrast to results obtained by chemical analyses for residual selenite in the suspending medium (see Fig. 4), little difference was noted in the extent to which uptake of labeled selenite was inhibited by phosphate over a concentration range from 10^{-3} to 10^{-1} M (Fig. 5). In view of competition between phosphate and selenite permeation into the cell, one might anticipate that a selective inhibitor of phosphate permeation would also interfere with uptake of selenite. Such, indeed, was found to be the case. Inhibition of phosphate uptake by DNP is well known (Hotchkiss, 1944). Marked inhibition of selenite uptake by DNP was also found in studies with Se^{75} (Fig. 6); 5×10^{-5} M DNP inhibited selenite uptake by 50%.

Effect of selenite on glucose assimilation. Sodium selenite strongly inhibited glucose oxidation by *C. albicans* 806; at the same time, carbon dioxide was produced in excess of the oxygen consumed. With a selenite concentration of 10^{-3} M, oxygen uptake was inhibited 84%, whereas CO_2 production was inhibited only 63%; the RQ, meanwhile, was elevated from 1.0 to 2.3. In the selenite-resistant strain RM806, on the other hand, respiration was considerably

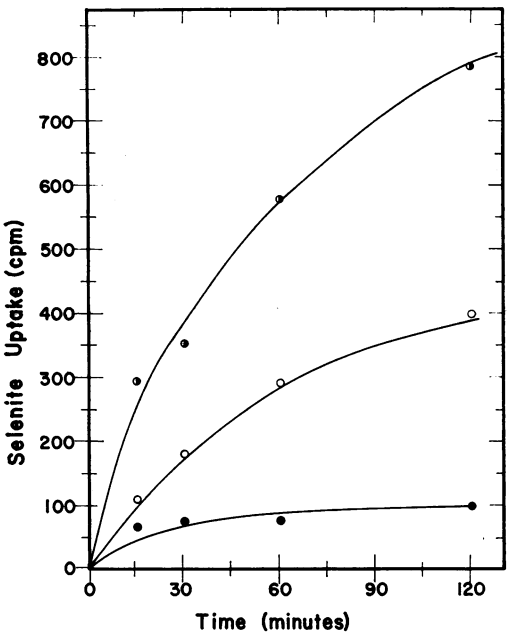

FIG. 6. *Effect of dinitrophenol on selenite uptake by nonmultiplying cells of Candida albicans RM806. Incubation at 37 C for times noted in 0.01 M phthalate-NaOH buffer (pH 4.3) containing 10^{-2} M Na_2SeO_3, and concentrations of 2,4-dinitrophenol as follows: ◑, zero; ○, 5×10^{-5}; ●, 5×10^{-4} M.*

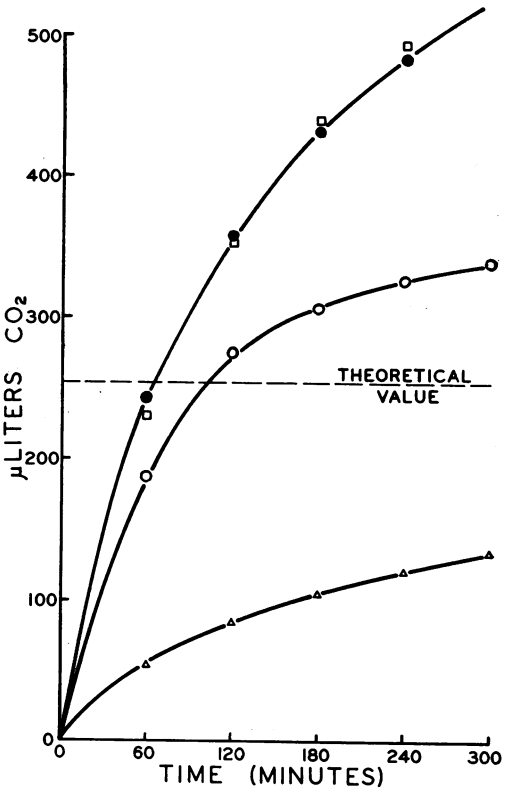

FIG. 7. *Suppression of anaerobic assimilation of glucose in presence of selenite. Nonmultiplying cells [7.8 mg (dry weight)/vessel] of Candida albicans 806 studied manometrically for time noted under N_2 atmosphere at 37 C with 1 mg of glucose and the following concentrations of Na_2SeO_3: ○, zero; ●, 10^{-3}; and □, 10^{-2} M. Endogenous fermentation in the absence of added glucose or selenite shown by △. Theoretical value for complete fermentation of 1 mg of glucose shown by dotted line. Note resemblance to typical "dinitrophenol effect" on assimilation.*

less sensitive to inhibition by selenite, and in no instance was an excess in CO_2 production observed (Falcone and Nickerson, 1960*b*).

Endogenous and exogenous (glucose) fermentation of *C. albicans* RM806 was insensitive to selenite. In contrast, endogenous fermentation of selenite-sensitive strain 806 was stimulated markedly by selenite, and fermentation of glucose went to completion in the presence of selenite; in other words, anaerobic assimilation of glucose in this strain was blocked by selenite (Fig. 7). The action of selenite on respiration and fermentation of strain 806 was remarkably similar to that observed after the addition of DNP.

Reduction of selenite by cell-free extracts. Undialyzed cell-free extracts of *C. albicans* RM806 and of baker's yeast were found to be capable of reducing sodium selenite to metallic selenium. Extracts prepared by autolysis with sodium acetate or by freezing and thawing showed very little activity, whereas extracts prepared by grinding or crushing cells and by autolysis with toluene were very active. Extracts of baker's yeast obtained with a Hughes press were used extensively, since fresh material was available in quantity, and special precautions against infection were not necessary.

Only the soluble part of the extract was active in reducing selenite. With extracts prepared by the different procedures described, and exercising the utmost care in preserving the integrity of mitochondrial particulates, removal of cellular particulates did not cause any impairment of the activity of the extract; the isolated mitochondrial particulates were inactive (Table 5). The cell-free extract was stable; its activity remained almost unchanged after 15 days of storage at −20 C, or

TABLE 5. *Selenite-reducing activity of various fractions of cell-free preparations**

Fraction	Selenite reduced
Cell-free extract	100
Supernatant (18,000 × *g*)	106
Mitochondrial particulate	1
Dialyzed extract	2
Dialyzed extract + boiled yeast extract	92

* Enzymatic assays were carried out in tris-maleate buffer (0.01 M, pH 7) with 0.01 M Na_2SeO_3. Activity was measured after 60 min of incubation at 37 C, and is expressed as per cent of value for cell-free extract.

TABLE 6. *Effect of phosphate and metals on selenite reduction by cell-free extract of Candida albicans RM806**

Additions	Concn	Activity
	M	
Control	—	100
Phosphate	5×10^{-2}	49
Cu^{++}	10^{-3}	10
	10^{-4}	18
p-Chloromercuribenzoate	10^{-4}	73
Mn^{++}	10^{-4}	73
Zn^{++}	10^{-4}	18

* Results are expressed as percentage of activity in comparison with the control. Incubation for 60 min at 37 C with 0.01 M $NaSeO_3$ in 0.01 M tris-maleate buffer (pH 7.0).

after 3 days at 2 C, but activity was lost after 5 min at 60 C. Enzymatic activity was strongly inhibited by copper or zinc ions and partially inhibited by *p*-chloromercuribenzoate, phosphate, and manganous ions (Table 6).

The effect of pH on selenite reduction by cell-free extracts is shown in Fig. 8; an optimum at about pH 7 is apparent, suggesting that the pH optimum observed with nonproliferating cells is not due to a direct effect of hydrogen ion concentration on the enzymatic activity of the cell, but probably reflects the concentration of the biselenite ion, $HSeO_3^-$. With tris-maleate buffer at pH 7, it was observed that high concentrations of buffer inhibited selenite reduction. With a final concentration of 5×10^{-2} M, 50% inhibition was observed; in all other experiments,

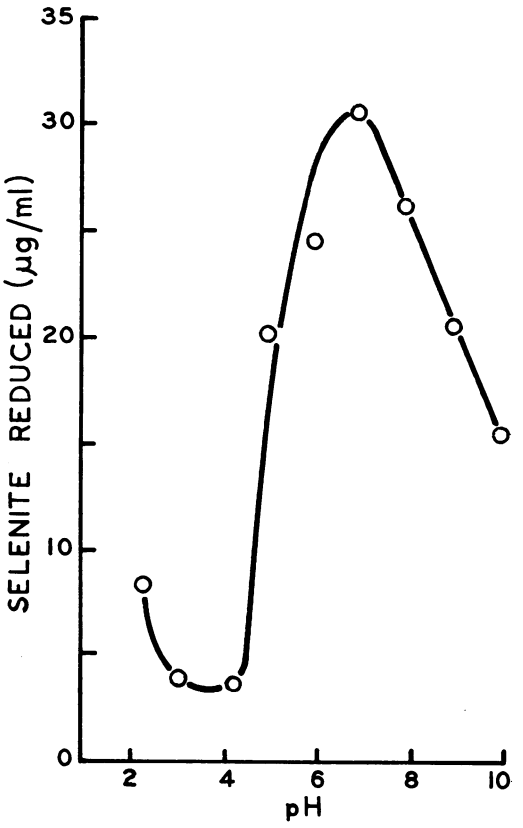

FIG. 8. *Effect of pH on selenite reduction by cell-free enzyme preparation from baker's yeast. Incubation for 60 min at 37 C with 0.01 M Na_2SeO_3 in 0.01 M buffer of composition as given in legend of Fig. 2. Note optimum at pH 7.*

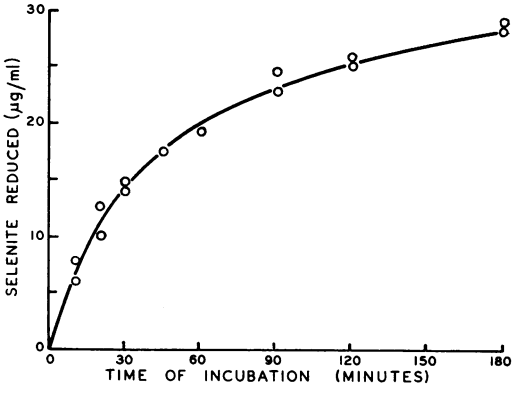

FIG. 9. *Increase in amount of selenite reduced by cell-free enzyme preparation from baker's yeast with time of incubation at 37 C in tris-maleate buffer (0.01 M, pH 7.0) containing 0.01 M Na_2SeO_3.*

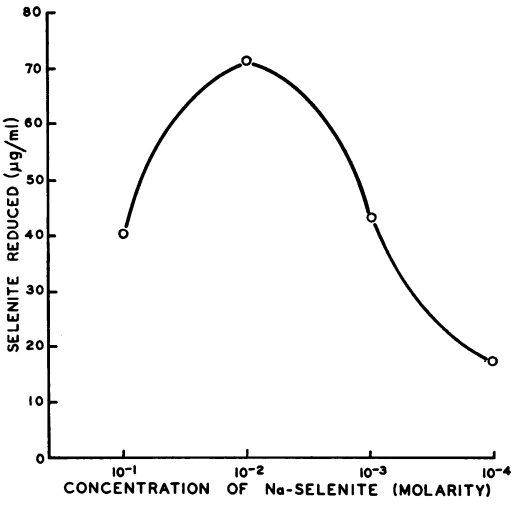

FIG. 10. *Effect of selenite concentration on selenite reduction by cell-free enzyme preparation from baker's yeast. Incubation for 60 min at 37 C in 0.01 M tris-maleate buffer (pH 7.0) with concentrations of Na_2SeO_3 noted.*

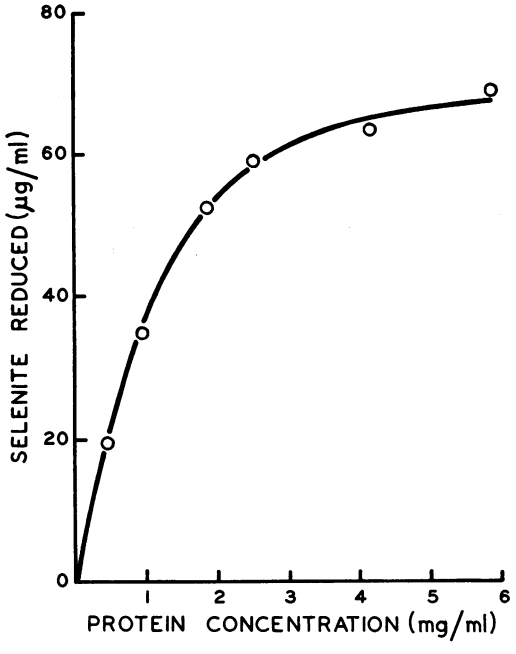

FIG. 11. *Relationship between amount of selenite reduced and amount of protein added in cell-free enzyme preparation from baker's yeast. Incubation for 60 min at 37 C in 0.01 M tris-maleate buffer (pH 7.0) containing 0.01 M Na_2SeO_3.*

the buffer concentration was kept constant at 10^{-2} M. The amount of selenite reduced increased with time; after an initial sharp rise, the rate declined to a plateau (Fig. 9). Enzymatic activity was maximal with 10^{-2} M buffer (Fig. 10) and varied directly with enzyme concentration (Fig. 11).

Dialyzed extract did not reduce selenite, whether dialysis was carried out against distilled or running water, NaCl solution, or tris-maleate buffer (pH 7.2). Activity of the dialyzed extract could be restored by the addition of boiled yeast extract (Table 5). Boiled extract, by itself, reduced selenite to a very limited extent, and a blank for evaluation of reduction from this source was always included in enzymatic assays. Various known cofactors added to the dialyzed extract neither restored activity nor enhanced the activity of a dialyzed preparation to which boiled extract had been added. The substances tested, singly and in combinations, included: FMN, flavin adenine dinucleotide, di- and triphosphopyridine nucleotides, folic acid, biotin, coenzyme A, lipoic acid, and thiamine pyrophosphate.

Discussion

Uptake of selenite by nonproliferating cells of a selenite-resistant strain of *C. albicans* ensues without lag on exposure to selenite. In contrast to uptake of $H_2PO_4^-$, for which exogenous glucose metabolism is essential (Mullins, 1942), uptake of $HSeO_3^-$ occurs in the absence of added substrate. In fact, no exogenously supplied substance has been found to promote $HSeO_3^-$ uptake, and, therefore, endogenous metabolism of some $(CH_2O)_n$ reserve is implicated as a source of energy for uptake and reduction of $HSeO_3^-$. Fluoride inhibition of such processes may be interpreted in this light. Nevertheless, uptake of both $H_2PO_4^-$ and $HSeO_3^-$ is inhibited by DNP. Uptake of the biselenite ion (HSO_3^-) is competitively antagonized by the phosphate ion ($H_2PO_4^-$). Conversely, selenite interferes with phosphate uptake and causes an inhibition of oxidative assimilation that is comparable to the effect of DNP on glucose metabolism.

Selenite taken up by intact cells of *C. albicans* is reduced to red, metallic selenium to such an extent that suspensions become bright red. Although no evidence for the production of volatile selenium compounds was obtained,

either with cultures of *C. albicans* or with non-proliferating suspensions, methylating agents and other substances involved in one-carbon transfer reactions were found to inhibit formation of elemental selenium. Whatever the mechanism of this inhibition may be, it does not involve diversion of selenite to form dimethyl selenide, or other volatile selenide.

Cell-free enzyme preparations capable of reducing selenite to elemental selenium were obtained from *C. albicans* and from baker's yeast. The particulate-free fraction obtained by high speed centrifugation comprised the activity of cell-free extracts; the mitochondrial particulate fraction was inactive. Formation of elemental selenium ensued promptly upon exposure of the enzyme system to selenite, even with extracts of baker's yeast which had not previously been exposed to selenite. Enzymatic activity was lost upon dialysis, but restored by the addition of dialyzed suspension or of boiled cell-free extract. No intermediate states of reduction were detected between Se^{+4} and Se°.

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LITERATURE CITED

CHALLENGER, F. 1945. Biological methylation. Chem. Rev. **36**:315–361.

FALCONE, G., AND W. J. NICKERSON. 1960*a*. Enzymatic reduction of selenite. Bacteriol. Proc., p. 152.

FALCONE, G., AND W. J. NICKERSON. 1960*b*. Metabolism of selenite and mechanism of inhibitory action of selenite on yeasts. Giorn. Microbiol. **8**:129–150.

GEST, H., AND A. NORDSTROM. 1956. A plunger-hammer for the Hughes microbial press. News Letter, Society of American Bacteriologists **22**(2): 15–16.

HOTCHKISS, R. D. 1944. Gramicidin, tyrocidine, and tyrothricin. Advan. Enzymol. **4**:153–200.

HUGHES, D. E. 1951. A press for disrupting bacteria and other microorganisms. Brit. J. Exptl. Pathol. **32**:97–109.

MULLINS, L. J. 1942. Permeability of yeast cells to radiophosphate. Biol. Bull. **83**:326–330.

NICKERSON, W. J., AND C. W. CHUNG. 1952. Reversal of fluoride inhibition of yeast growth with glucose-1-phosphate. Am. J. Botany **39**:669–679.

NICKERSON, W. J., W. A. TABER, AND G. FALCONE. 1956. Physiological bases of morphogenesis in fungi. 5. Effect of selenite and tellurite on cellular division of yeast-like fungi. Can. J. Microbiol. **2**:575–584.

TULLER, W. N. 1954. The sulphur data book. McGraw Hill Book Co., Inc., New York.