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Comparative Study of the Cell Walls of the Yeastlike and Mycelial Phases of *Histoplasma capsulatum*

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Cell walls were prepared from the yeastlike and mycelial phases (YP and MP) of *Histoplasma capsulatum* and from *Saccharomyces cerevisiae* by mechanical disruption and washing. Lipids were extracted with methanol-ether, chloroform, and acidified methanol:ether; a final extraction was made with ethylenediamine. The lipid contents of *H. capsulatum* YP and MP walls were about the same. Qualitative and quantitative analyses were made of the products obtained from treatment of the cell walls, or fractions from them, with weak acid or with enzymatic preparations containing glucanase and chitinase activities. YP walls contained much larger quantities of chitin and smaller quantities of mannose and amino acids than the MP walls. *H. capsulatum* MP was shown to resemble *S. cerevisiae* by low chitin content and by the presence of a mannose polymer, soluble in ethylenediamine and water. *H. capsulatum* MP chitin appeared to be intimately associated with glucose in the wall, since enzymatic hydrolysis of the residue after mild acid hydrolysis of cell walls or fractions from them resulted in the release of glucose and acetylglucosamine; only acetylglucosamine was released from YP walls with such treatment. By electron microscopic observations, the unextracted MP cell walls were much thinner than the YP, and neither wall appeared laminated.

Little is known of the chemical composition of the cell walls of either the yeastlike (YP) or mycelial (MP) phases of *Histoplasma capsulatum*. The presence of chitin, a polymer of *N*-acetyl-D-glucosamine, in the cell walls of both phases was indicated by the X-ray diffraction studies of Blank (5). Pine, Boone, and McLaughlin (23) detected glucosamine, neutral carbohydrates, and amino acids in the YP walls. McNall et al. (18), however, did not find glucosamine in either YP or MP walls, although glucose, mannose, and glucuronic acid were present in an insoluble cell-wall polysaccharide obtained from both phases.

We have isolated YP and MP cell walls and have analyzed them qualitatively and quantitatively for their chitin, monosaccharide, and amino acid constituents. To ascertain structural differences between YP and MP cell walls, as well as the relative purity of various preparations, electron microscopic examinations were made. *Saccharomyces cerevisiae*, for which data on cell-wall composition were already available (16), was carried through the same procedures as *H. capsulatum* for comparative purposes.

MATERIALS AND METHODS

Cultural methods. H. capsulatum Sweany B, isolated from a human infection in 1964, was obtained from H. C. Sweany, Missouri State Sanitorium, Mt. Vernon. *S. cerevisiae* 18.29 was obtained from W. J. Nickerson, Rutgers University, New Brunswick, N.J. *H. capsulatum* YP and *S. cerevisiae* were maintained by weekly transfer on Brain Heart Infusion (BHI) agar slants at 37 and 26 C, respectively.

The medium in which the fungi were grown just prior to cell-wall isolation was a glucose-yeast extract (GYE) broth (2 and 1%, respectively), *p*H 6.9 (12); 100-ml (for YP) or 200-ml (for MP) amounts were distributed in 500-ml Erlenmeyer flasks.

H. capsulatum cells from a 5-day-old culture on BHI agar were transferred to GYE and incubated at 37 C for 3 days on a Gyrotory shaker operating at 165 rev/min. Of this culture, 3 ml was transferred to 100 ml of GYE to obtain large quantities of YP cells. The cells were harvested by centrifugation, washed three times with distilled water, and, if not used immediately, stored at −20 C. *S. cerevisiae* was grown at 26 C and was otherwise handled in the same manner.

To obtain conversion of *H. capsulatum* YP to MP, GYE was inoculated with YP and incubated at 26 C with intermittent Gyrotory shaking (about 4 hr per day). Each time mechanical shaking was stopped, the

"tide line" was washed from the sides of the flask by manual swirling. Four successive, weekly transfers resulted in complete conversion of YP to MP. The cells consisted solely of long branching hyphae; pellets or spores were absent. Amounts of 10 to 20 ml of MP suspension were used to inoculate fresh medium, which was incubated as described for 7 days. The mycelia were harvested by filtration and were stored at −20 C.

Preparation and isolation of cell walls. Cells of *S. cerevisiae* and *H. capsulatum* YP and MP were agitated with glass beads (0.25 to 0.30 mm in diameter) in a model MSK Braun cell homogenizer (Bronwill Scientific Co., Rochester, N.Y.), at 4,000 cycles per min (20). While in operation, the homogenizer chamber was maintained at about 4 C by frequent blasts of carbon dioxide. The ratio of slurry components was 30 g of beads to 10 g (wet weight) of the fungus suspended in 5 to 10 ml of distilled water.

The degree of breakage of *S. cerevisiae* and *H. capsulatum* YP was followed by light microscopy, by use of classical and modified Gram stains (4). Lactophenol cotton blue mounts were prepared of MP.

Cell walls were separated from cytoplasmic debris in an International Refrigerated Centrifuge at 450 × *g*. They were washed 10 times with 0.2 M NaCl, once with 1 M NaCl, and 20 to 30 times with distilled water, and were lyophilized and stored in vacuo at 26 C.

Extraction and degradation of cell walls. Lipids were extracted from the cell walls by the method of Kessler and Nickerson (16) with methanol-ether, chloroform, and acidified methanol-ether. The lipid-extracted cell walls were washed extensively with methanol and water, lyophilized, then further extracted with anhydrous ethylenediamine (17). Three fractions were obtained: fraction C was insoluble in ethylenediamine, and fractions A and B were both soluble in ethylenediamine and insoluble in methanol. Fraction A was soluble whereas fraction B was insoluble in water.

Preparation and assay of the crude enzyme systems. Streptomyces sp. ATCC 11238 was incubated 8 days at 30 C on a Gyrotory shaker in a basal salts medium (25) to which colloidal crustacean chitin (3) or unextracted cell walls of *H. capsulatum* YP were added in 1% concentrations (w/v). The crude enzymes were then prepared from the culture filtrates (3), and their actions on the various substrates were determined (29). The quantity of enzyme preparation required to yield the maximal release of acetylglucosamine was ascertained beforehand by varying the amounts of the crude enzymes with a standard quantity of substrate. Colloidal crustacean chitin and laminarin (no. 1L23), a β-1,3-glucan obtained from the Institute of Seaweed Research, Midlothian, Scotland, were used as controls in the enzymatic experiments. The acetylglucosamine and glucose released by enzymatic action were detected and determined quantitatively as the *O*-trimethylsilyl ether derivatives (9, 31) by use of a Barber-Coleman gas-liquid chromatograph apparatus with a 3% SE-30 column at a column temperature of 160 C.

Acid hydrolysis for monosaccharides and amino acids. After hydrolysis of 10-mg samples in 2 ml of 1 N HCl in methanol at 80 C for 40 hr in sealed tubes (32, modified by E. Barbosa, *personal communication*), the monosaccharides were determined by gas chromatography as above, but at a column temperature of 145 C. A second 10-mg sample of each cell-wall preparation was hydrolyzed and analyzed as above, but, prior to silylation, the hydrolysate was acetylated to facilitate the separation of any glucosamine or galactosamine from other monosaccharides (13).

The amino acids of *H. capsulatum* YP and MP were determined by Roland Coulson and Thomas Hernandez, Louisiana State University School of Medicine, New Orleans, in a Modified Technicon Amino Acid analyzer (7) after hydrolysis of 10-mg samples in 2 ml of 6 N HCl for 18 hr at 110 C in evacuated sealed tubes.

Electron microscopic methods. The preparations of *H. capsulatum* were fixed in 3% glutaraldehyde and postfixed with 1% osmium tetroxide (14, 28), washed twice with 70% alcohol, and embedded in 1.5% agar (15). Small pieces of agar containing the fungal material were dehydrated and embedded in Maraglas epoxy resin (11, 30), from which thin sections were cut on a Servall Porter-Blum Ultramicrotome. The sections were stained with lead citrate (26) and examined in a Siemens Elmiskop I electron microscope.

RESULTS

Lipid content and solubility characteristics of the cell walls. The YP walls of *H. capsulatum* contained 6.8% and the MP walls 6.2% total lipid. The lipid content of the cell walls of *S. cerevisiae* was not determined.

The weight recoveries of the fractions obtained from lipid-extracted cell walls by ethylenediamine extraction are shown in Table 1. Each preparation reacted differently; 50% of the *S. cerevisiae* cell wall was soluble in ethylenediamine but only 4 and 15%, respectively, for the YP and MP walls of *H. capsulatum*. The extraction was repeated on wall preparations from different culture lots of each organism; solubility characteristics were constant with the stated conditions of growth and with the method of cell-wall preparation and extraction described.

Enzymatic action on the cell-wall preparations. From the enzymatic studies, it was deduced that the YP walls of *H. capsulatum* contained approximately 25% chitin, the MP walls, approximately 4%, and *S. cerevisiae* walls, somewhat less than 0.5% (Table 2). The chitin content for *H. capsulatum* was calculated on the basis of the quantities of acetylglucosamine obtained from either the lipid-extracted cell walls or fraction C. Much more acetylglucosamine could be released from these than from the cell walls which contained lipid. β-1,3-Glucanase activity was shown to be present in both crude enzyme preparations by their action on laminarin. Since both glucose and acetylglucosamine were released dur-

TABLE 1. *Solubility characteristics of lipid-extracted cell walls of Histoplasma capsulatum and Saccharomyces cerevisiae*

Cell wall prepn	Lipid-free cell walls extracted	Ethylenediamine-soluble				Resistant to ethylenediamine (C)	
		Water-soluble (A)[a]		Water-insoluble (B)			
		Amt	Per cent	Amt	Per cent	Amt	Per cent
	mg	*mg*		*mg*		*mg*	
H. capsulatum							
Yeast phase	1,000	27.5	2.8	15.0	1.5	937.8	93.8
Mycelial phase	500	68.0	13.6	3.0	0.6	428.5	85.7
S. cerevisiae	300	75.2	25.1	61.7	20.6	146.5	48.9

[a] Letter represents fraction of cell wall with particular solubility characteristics.

TABLE 2. *Enzymatic action on various preparations of Saccharomyces cerevisiae and Histoplasma capsulatum*

Prepn	Chitinase[a]		Cell wall enzyme[b]	
	Glucose	Acetylglucosamine	Glucose	Acetylglucosamine
	%	%	%	%
H. capsulatum yeast phase				
Unextracted cell walls	9.9[c]	10.7	8.5	7.5
Lipid-extracted cell walls	3.1	25.6	—	—
Ethylenediamine-resistant fraction (C)	1.8	31.5	5.6	29.0
H. capsulatum mycelial phase				
Unextracted cell walls	20.5	3.1	18.6	3.0
Lipid-extracted cell walls	3.5	2.0	—	—
Ethylenediamine-resistant fraction (C)	6.5	6.0	—	—
S. cerevisiae				
Unextracted cell walls	19.2	0.4	—	—
Lipid-extracted cell walls	16.4	0.3	—	—
Ethylenediamine-resistant fraction (C)	20.6	0.5	—	—

[a] The enzyme system resulting from incubation of the *Streptomyces* sp. with chitin.
[b] The enzyme system resulting from incubation of the *Streptomyces* sp. with cell walls prepared from the yeast phase of *H. capsulatum*.
[c] Calculated on the basis of milligrams of compound released per 10 mg of substrate hydrolyzed.

ing enzymatic hydrolysis, Table 2 includes the calculated values for both.

When MP cell walls were first subjected to mild acid hydrolysis followed by enzymatic hydrolysis, the chitin value was higher, 6% versus 4%, and both glucose and acetylglucosamine were released. Only acetylglucosamine was released from the YP cells under similar conditions.

Monosaccharide constituents. Glucose and mannose were the only non-nitrogenous sugars found in cell walls of *S. cerevisiae* and *H. capsulatum*. Gross differences in the quantities released from the two phases of *H. capsulatum* by mild acid hydrolysis were observed (Table 3). The decrease in the glucose content of the YP cell walls may have been accounted for by the fact that glucose was found in the hydrolysates of the tightly bound lipids of the YP wall. Corresponding hydrolysates of the lipids of the MP did not contain glucose.

Mild acid hydrolysis of fractions A, B, and C revealed that, in the cell walls of *S. cerevisiae* and of the MP of *H. capsulatum*, fraction A contained predominantly mannose, whereas fractions B and C contained predominantly glucose. In contrast, all fractions of the YP of *H. capsulatum* showed glucose as the predominant monosaccharide.

Larger quantities of monosaccharides were released by mild acid hydrolysis from fractions

TABLE 3. *Monosaccharide determinations on various cell wall preparations of Saccharomyces cerevisiae and Histoplasma capsulatum*

Prepn	Mannose (%)[a]	Glucose (%)	Total (%)
Unextracted cell walls			
H. capsulatum yeast phase	1.2	21.1	22.3
H. capsulatum mycelial phase	5.1	5.5	10.6
S. cerevisiae	21.4	8.0	29.4
Lipid-extracted cell walls			
H. capsulatum yeast phase	1.1	16.2	17.3
H. capsulatum mycelial phase	5.2	5.4	10.6
S. cerevisiae	18.4	15.4	33.8
Fractions[b]			
A			
Yeast phase	2.4	17.3	19.7
Mycelial phase	16.7	3.7	20.4
S. cerevisiae	42.8	9.7	52.5
B			
Yeast phase	0.6	3.9	4.5
Mycelial phase	2.2	11.2	13.4
S. cerevisiae	5.3	39.3	44.6
C			
Yeast phase	1.0	21.3	22.3
Mycelial phase	2.6	9.6	12.2
S. cerevisiae	4.7	53.7	58.4

[a] Calculated on the basis of milligrams of sugar released per 10 mg of substrate.

[b] These were fractions of the lipid-extracted walls: A was soluble in ethylenediamine and in water; B was soluble in ethylenediamine and insoluble in water; and C was insoluble in ethylenediamine and in water.

A, B, and C of *S. cerevisiae* than from the corresponding lipid-extracted or unextracted cell walls (Table 3). By examination of the fractions, it was deduced that the predominant sugar in the cell walls of *S. cerevisiae* was glucose and that the walls contained about 50% monosaccharides. Examination limited to the unextracted or lipid-extracted walls would make it appear that *S. cerevisiae* contained only 30 to 40% monosaccharides. In contrast, increased quantities of monosaccharides were not released from the corresponding fractions of *H. capsulatum* YP or MP.

Amino acid constituents. The amino acid analyses (Table 4) indicated that there was a higher concentration of amino acids in the cell walls of the MP than of the YP; differences were also revealed in distribution and quantities among the fractions from the walls. Ethylenediamine extraction of the YP walls released 80% of the amino acids which were then split between fractions A and B. Similar extraction procedures with the MP walls removed only 40% of the amino acids, the majority of which appeared in the water-soluble fraction A. Therefore, the amino acids in the YP appeared largely in fractions A and B, and those of the MP, in A and C. The major amino acids, in order of decreasing concentration in the YP, were glycine, glutamic acid, lysine, leucine, alanine, and phenylalanine, whereas those of the MP were glycine, glutamic acid, threonine, serine, valine, aspartic acid, and alanine.

Electron microscopic observations. The cell wall of the intact YP had an average thickness of 0.07 μ (range of 0.05 to 0.08 μ), and that of the MP, 0.01 to 0.02 μ. Isolated, unextracted cell walls of either phase (Fig. 1 and 2) appeared the same as those of intact cells of the respective phase. A prominent, electron-dense cell membrane appeared between the cell wall and the cytoplasm of both YP and MP cells, and the absence of this membrane was used as a criterion to indicate the purity of cell-wall preparations. Occasionally, as seen in Fig. 1, isolated cell-wall preparations had small pieces of membrane contaminating them. Neither the cell wall of the YP nor of the MP appeared laminated. Electron microscopy of the lipid-extracted cell walls and of fraction C (Fig. 3) revealed that a definite structure still remained after lipid-extraction or extraction with ethylenediamine. Fraction C, however, was narrower and irregular along the length of a fragment, and was less electron-dense than isolated, unextracted cell walls.

DISCUSSION

The chemical and enzymatic degradation of isolated cell walls or fractions from them have revealed differences between *H. capsulatum* YP and MP and have shown some unanticipated similarities between *H. capsulatum* MP and *S. cerevisiae*. One of the major differences between *H. capsulatum* YP and MP centered around chitin, its content and its structural relationship with glucose in the walls. As expected, the chitin content of *S. cerevisiae* was very low; unexpectedly, the chitin content of *H. capsulatum* MP was quite low compared to that of the YP, contrary to what one would have anticipated from previous work with yeasts and mycelial forms of other fungi (2, 6, 21, 22, 24, 27). In the MP, in contrast to the YP, part of the glucose was intimately associated with chitin, since enzymatic hydrolysis of residues after acid hydrolysis caused the release of glucose as well as of acetylglucosamine. Mild acid hydrolysis of the YP walls released all of the glucose that might otherwise have been available to enzymatic action; subsequent enzymatic attack

TABLE 4. *Quantitative amino acid determinations on untreated cell walls and fractions derived from Histoplasma capsulatum*

Amino acid	Yeastlike phase[a]				Mycelial phase[a]			
	Whole[b]	A	B	C	Whole[b]	A	B	C
Aspartic acid	0.1	3.8	3.6	trace[c]	0.7	6.3	trace	0.2
Threonine	0.2			trace	1.1		trace	0.8
Serine	0.2	1.2	1.6	trace	1.0		trace	0.5
Glutamic acid	0.5	2.6	2.0	trace	1.2	3.5	trace	0.7
Proline	trace	trace	trace	trace	trace	0.5	—	0.1
Glycine	0.6	2.9	2.7	0.1	1.7	4.0	trace	1.2
Alanine	0.3	1.1	1.6	trace	0.7	1.4	trace	0.3
Valine	G[d]	1.1	1.7	G[d]	0.7	1.6	trace	0.3
Methionine	trace	trace	trace	trace	trace	—	trace	—
Isoleucine	0.3	0.9	1.2	trace	0.3	0.9	trace	0.1
Leucine	0.4	1.6	2.7	0.1	0.4	1.0	trace	0.1
Tyrosine	0.1	0.4	0.3	trace	trace	0.5	trace	0.1
Phenylalanine	0.3	1.5	1.4	trace	0.1	0.8	trace	0.1
Lysine	0.4	1.2	1.3	trace	0.6	1.3	trace	0.3
Histidine	0.1	0.7	0.5	trace	0.2	0.7	trace	0.1
Arginine	0.3	1.0	1.1	trace	0.2	0.9	trace	0.2
Ornithine	—	0.1	0.1	trace	trace	0.1	trace	trace
Ethanolamine	0.8	0.3	0.3	—	—	0.3	—	—
Total (mg/10 mg)	0.5	5.2	7.0	trace	1.0	2.9	trace	0.6
Amino acids(%)	5.0	52.0	70.0	trace	10.0	29.0	trace	6.0

[a] Expressed in micromoles per 10 mg.

[b] The whole cell walls were unextracted. A, B, and C were fractions of the cell wall; A was soluble in ethylenediamine and in water; B was soluble in ethylenediamine and insoluble in water; and C was insoluble in ethylenediamine and in water.

[c] Less than 0.1 μmole.

[d] Overlapped by glucosamine.

of the residue released only acetylglucosamine. This observation suggests the presence of a chitin which is different from the classical crustacean chitin. Although the MP chitin of *H. capsulatum* differs from crustacean chitin, the following evidence suggests that both YP and MP do contain a chitin. Acetylglucosamine, not glucosamine, was detected and quantitated as the product of the enzymatic action. Chitosan, the only other polymer on which chitinase is known to act (33), can be ruled out since it is soluble in weak acid (10), and the polymer containing acetylglucosamine in the cell walls of YP and MP was not soluble in weak acid. In fact, equal or greater quantities of acetylglucosamine were released from walls which had first been subjected to mild acid hydrolysis.

In *H. capsulatum* MP walls, glucose might serve to shield chitin from enzymatic attack, because larger quantities of acetylglucosamine were released from the walls which had first been hydrolyzed with weak acid. Perhaps, the glucanase present in the enzyme preparations was only specific for some of the glucose linkages present in the wall, so that preliminary acid hydrolysis was necessary to remove this barrier and expose more chitin to enzymatic attack. Lipid in the walls of the YP also appeared to protect the chitin from attack, since much larger quantities of acetylglucosamine were released from the lipid-extracted cell walls than from unextracted walls. This lipid component could be phosphatidylethanolamine or a similar compound which is poorly soluble in methanol alone but which could be extracted with methanol-ether.

Extraction with ethylenediamine and subsequent mild acid hydrolysis of the resultant fractions supported the view that the walls of *H. capsulatum* YP and MP differ, and it is here that a similarity between *S. cerevisiae* and MP was apparent. The fact that *H. capsulatum*, like *S. cerevisiae*, contained mannose, presumably as a polymer, was surprising, since there is only one other report in the literature of a mannose polymer in a mycelial form, namely *Mucor rouxii* (2). In that organism, however, a mannose polymer was present in greater concentration in the YP walls.

The results obtained here for *S. cerevisiae* are

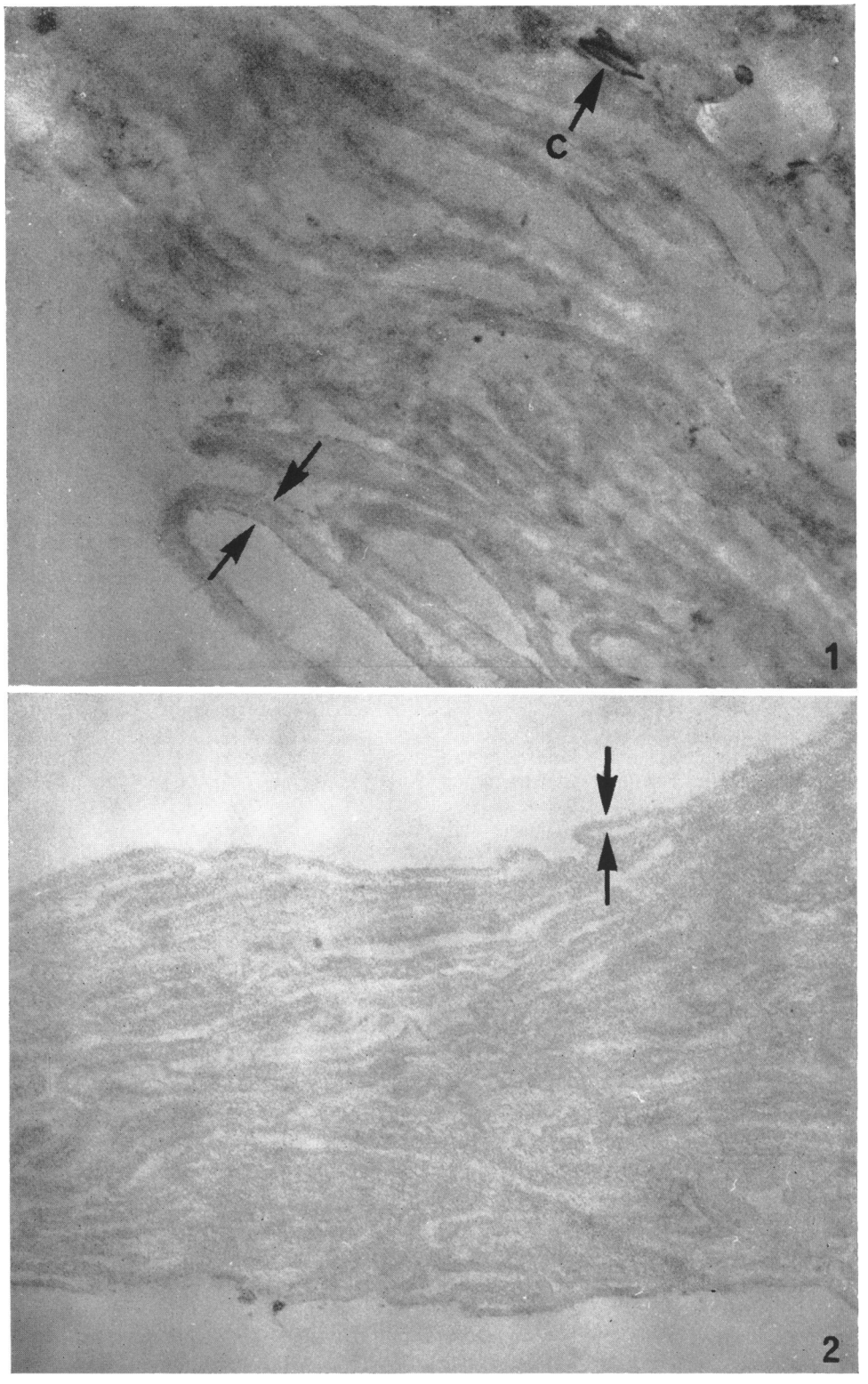

FIG. 1. *Electron micrograph of isolated, unextracted cell walls of the yeastlike phase of Histoplasma capsulatum as seen in a thin section. One arrow, labeled C, points to a fragment which could be a piece of contaminating cell membrane. The distance between the points of the other two arrows is the thickness of one cell wall.* × *50,000.*

FIG. 2. *Electron micrograph of a thin section of isolated, unextracted cell walls of the mycelial phase of Histoplasma capsulatum. The distance between the points of the two arrows is the thickness of one cell wall.* × *64,000.*

not directly comparable to those obtained for the same strain by Kessler and Nickerson (16), because their methods of culture and chemical analysis were different. However, it can be deduced from their data, and from ours as well, that glucose and mannose were both found in large quantities in the walls. The glucose to mannose ratio found by Kessler and Nickerson was 1.4:1; our value was 1.7:1. Our calculations were based on the mannose content of the unextracted cell walls and the glucose contents of the fractions. Mannose was probably lost during the lipid extraction and subsequent washings; thus, the data obtained with unextracted walls present a more accurate picture of the actual mannose content. In contrast, however, mannan probably shielded the glucan in the unextracted or lipid-extracted walls, so that, in this case, the fractions were considered to be more truly representative.

The amino acid analyses also showed differences between the two phases of *H. capsulatum*, in that the total amino acid concentration in the MP was greater than in the YP, and they were distributed differently among the cell-wall fractions after ethylenediamine extraction.

Of the many cytological studies of yeasts and other fungi that have been published since the advent of the electron microscope, few have attempted correlative ultrastructural and chemical studies. Northcote and Horne (22) were able to discern in the *S. cerevisiae* cell wall two distinct layers correlated with the polymers glucan and mannan. Layering of the *H. capsulatum* YP cell wall has also been reported by Edwards, Hazen, and Edwards (8). We were not able to discern layering of the cell wall for either MP or YP; in the case of the YP at least, this might be due to the high chitin content. Studies of the structure of some fungal walls have indicated a fibrous structure, with the chitin present as a fibrillar network throughout the wall (1). It is conceivable then that the chitin in *H. capsulatum* might act as a supporting mesh for the other wall constituents, the extraction of which would do little to the actual integrity of the wall.

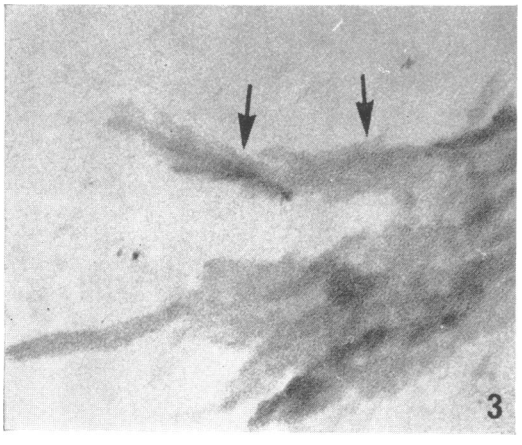

FIG. 3. *Electron micrograph of the ethylenediamine-resistant fraction from the cell walls of the yeastlike phase of Histoplasma capsulatum. The arrows point to places where pieces seem to be missing from the cell wall.* × *54,000.*

It is interesting to compare the overall results obtained for the YP and MP of *M. rouxii* (2) with those obtained here for *H. capsulatum*. The chitin contents of the former are not as strikingly different as those of *H. capsulatum*; cantrary to *H. capsulatum*, *M. rouxii* MP contained larger quantities of chitin and smaller quantities of mannose and protein than the YP.

The fact that such a large quantity of chitin was found in the YP of *H. capsulatum* leads one to speculate briefly on its possible role in pathogenicity. Presumably, the cells in the human body do not produce a chitinase. Unable to break down chitin, the body tissues appear to treat the chitinous wall as a foreign body, evoking cellular tissue reaction and dystrophic calcification.

The data presented here account for about 60% of the *H. capsulatum* YP wall and for something less than 50% of the MP wall as complexes of chitin, carbohydrate, protein, and lipid. These low recoveries might be explained in several ways: (i) the protein values may have been low owing to the destruction of amino acids in the presence of carbohydrates during the hydrolysis (19); (ii) the mild acid hydrolysis may not have released all the sugars from the cell walls, as suggested by the fact that almost four times as much glucose was released from the MP wall by enzymztic action as by hydrolysis with weak acid; (iii) the values for the mannose content of the walls may be low because of its highly soluble character; and (iv) the possibility exists also that large quantities of phosphate may be bonded to the cell wall and contributing to its weight. Bartnicki-Garcia and Nickerson (2) found that the cell walls of *M. rouxii*, both YP and MP, contained more than 20% phosphate.

We feel that structural studies of cell walls through use of the solubility scheme of Korn and Northcote (17) for *S. cerevisiae* cannot be directly correlated for structural characterization of the cell walls of other organisms. For example, fraction A of Korn and Northcote was shown to be essentially a mannan-protein polymer, whereas the same fraction of the YP contained mostly glucose and small amounts of protein and man-

nose. The finding of predominantly glucose and protein in fraction B of the YP suggests the possibility that the glucan-mannan-protein polymer of fraction B of *S. cerevisiae* is, as Korn and Northcote (17) had suggested, mannan and glucan polymers linked by protein.

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