The Effect of Various Substances on the Respiration[1] of Histoplasma capsulatum

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Histoplasma capsulatum, the etiological agent of histoplasmosis, exhibits both yeast-like and mycelial growth characteristics. Since the first

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phase is the one usually found in infected tissues and is difficult to maintain in prolonged culture, a great number of investigations have been concerned with the growth requirements of this phase. Salvin (1949) found biotin to be necessary for the development of yeast-like cells, and also demonstrated the need for sulfur compounds as cysteine or cystine. Pine (1954, 1955), and more recently Scherr (1957) have further emphasized the importance of sulfur containing compounds for the growth of the organism. Aspartic and glutamic acids have been found to stimulate growth of the fungus, particularly when used in combination with cysteine (Pine, 1954). Oleic acid in the presence of albumin or starch was required for growth when small inocula of yeast cells were used. Pine (1957) has also reported on the effect of various vitamins on the growth of the organism.

There has been no report on the oxidative metabolism of *H. capsulatum*. The objective of this work was to study the effect of various substances on the oxygen uptake of the fungus.

Materials and Methods

Initial studies were carried out using the Dillard isolate of H. *capsulatum*. In the major portion of this work, however, the Scritchfield isolate was used. Preliminary results obtained with the two were approximately the same. Both of these cultures were isolated from human cases of histoplasmosis and were obtained from Dr. Michael L. Furcolow, U. S. Public Health Service, Kansas City, Kansas.

A yeast-phase culture of this organism was grown for 4 days at 37 C. in 25x150 mm. culture tubes sealed with parafin. The medium used was that described by Cozad (1958), a modification of Salvin's medium (1950).

The organisms were suspended and washed 3 times in 0.067 M phosphate buffer, pH 7.0. After the last washing, the cells were centrifuged for 6 minutes at 1,800 r.p.m. and the packed cells diluted 1:10 in the buffer.

One-half ml. of the cell suspension was added to each Warburg flask and 0.5 ml. of substrate to the sidearm. The final volume of liquid in all flasks was q. s. to 2.0 ml. with the buffer. All substrates, except when otherwise stated, were added in final concentration of M/300.

Determinations were made in an atmosphere of air at 37 C., using standard manometric techniques as described by Umbreit *et al.* (1949).

Results were tabulated as the percent inhibition or stimulation of oxygen uptake in the experimental flasks as compared to that of the control flasks. These results represent the averages from at least two separate determinations. Because of the rather rough nature of the cell suspensions and the accuracy of the method, no results were considered significant unless the deviation from the control for each substrate used was more than 10 per cent.

Results

Tricarboxylic acid cycle intermediates: All of the Krebs cycle intermediates except citric, cis-aconitic, and isocitric acids appreciably stimulated the oxygen uptake of the fungus (Table I). The greatest amount of stimulation occurred with pyruvic acid. This was followed in amount of activity by succinic acid, oxaloacetic acid, and alpha-ketoglutaric acid.

Table I Effect of tricarboxylic acid cycle intermediates on the oxidative metabolism of *Histoplasma capsulatum*

Substrate (M/300)	Oxygen uptake (μl) with substrate	Oxygen uptake (μl) without substrate	Per cent stimulation or inhibition
Pyruvic acid	467.9	219.8	+112.9
Oxaloacetic acid	328.1	219.8	+ 49.3
Citric acid	201.5	204.4	— 1.4
Cis-aconitic acid	227.3	204.4	+ 11.2
Isocitric acid	210.2	204.4	+ 2.8
Oxalosuccinic acid	262.4	219.8	+ 19.4
Alpha-Ketoglutaric acid	278.2	204.4	+ 36.1
Succinic acid	309.3	204.4	+ 51.3
Fumaric acid	248.0	198.3	+ 25.1
Malic acid	253.5	204.4	+ 24.0

37 C., Ph 7.0

Totals for 5 hours

Amino acids: The presence of the L- or naturally occurring forms of all the amino acids tested increased oxygen uptake with the exception of L-arginine. Results of the active substrates are presented in Table II. Amino acids with the greatest amounts of activity were L-isoleucine, L-cystine, and L-threonine. The D-forms of alanine, valine, leucine, phenylalanine, methionine, and asparatic acid did not show a significant amount of activity. A comparison of the rates of oxygen uptake for representative D-and L-isomers is shown in Figure 1.

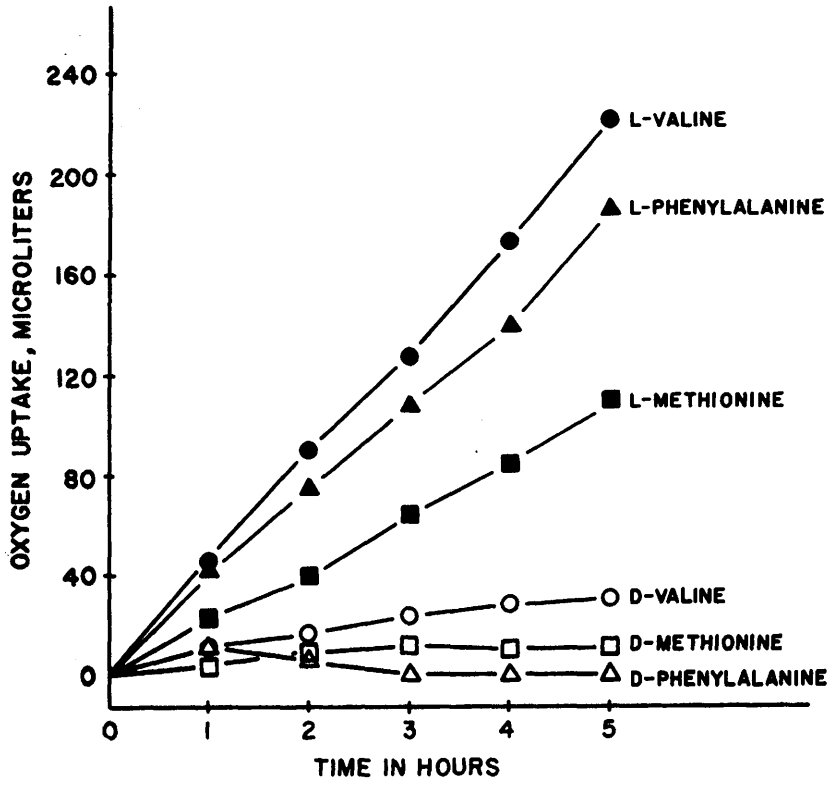

Figure 1—Effect of D-and L-isomers of various amino acids on oxygen uptake of *Histoplasma capsulatum*. (Endogenous values subtracted)

Table II Stimulatory effects of certain amino acids on the oxidative metabolism of *Histoplasma capsulatum*

Substrate (M/300)	Oxygen uptake (μl) with substrate	Oxygen uptake (μl) without substrate	Per cent stimulation
Phenyl glycine	212.4	183.0	16.1
L-Alanine	485.7	285.1	70.4
L-Valine	446.1	224.3	98.4
L-Leucine	405.1	266.4	52.1
L-Isoleucine	627.0	257.1	143.9
DL-Serine	437.3	251.9	73.6
L-Threonine	370.0	183.0	102.2
L-Phenyalanine	445.3	259.0	71.9
L-Tyrosine	318.1	166.0	91.6
L-Tryptophan	275.1	224.3	22.6
DL-Tryptophan	200.8	166.0	21.0
L-Cysteine	427.6	219.8	94.5
L-Cystine	488.5	219.8	122.2
L-Methionine	345.7	235.6	46.7
L-Aspartic acid	353.9	273.6	29.3
L-Glutamic acid	413.7	273.6	51.2
DL-Histidine	217.8	188.9	15.3
L-Proline	339.9	203.1	64.4
DL-Proline	358.0	224.4	59.5
DL-Citrulline	213.4	188.9	13.0

37 C., Ph 7.0

Totals for 5 hours

Fatty acids, organic acids, and alcohols: Incubation of yeast cells of *H. capsulatum* in the presence of sodium acetate, sodium propionate, and sodium butyrate, resulted in an immediate and continued increase in the oxygen uptake (Table III). Sodium oleate rapidly and completely inhibited respiration.

Sodium lactate stimulated oxygen uptake, while M/300 malonate caused 12.2 per cent inhibition of oxygen uptake and at M/18 concentration the inhibition was 67.4 per cent. Malonate at these two higher concentrations also inhibited the oxidation of succinic acid. Inhibition of 12.3 per cent occurred with M/100 malonate and 56.8 per cent in the presence of M/18 malonate.

Mannitol was used oxidatively by the organism. The other hexitols, sorbitol and dulcitol, had no effect (Table III).

Table III Effect of various fatty acids, organic acids, and alcohols on the oxidative metabolism of *Histoplasma capsulatum*

Substrate (M/300)	Oxygen uptake (μl) with substrate	Oxygen uptake (μl) without substrate	Per cent stimulation or inhibition
Na-acetate	470.1	293.5	+60.2
Na-propionate	438.5	240.0	+82.7
Na-butyrate	531.3	323.2	+64.4
Na-oleate	11.1	240.0	—95.4
Na-lactate	455.5	323.2	+40.9
Na-malonate	305.0	323.2	— 5.6
Dulcitol	289.9	299.4	+ 3.2
Sorbitol	254.7	240.0	+ 6.1
Mannitol	315.4	240.0	+31.4

37 C., Ph 7.0

Totals for 5 hours

Carbohydrates: The results of this study are presented in Table IV. Xylose was used oxidatively by the fungus, while arabinose, the other pentose tested, was without effect.

All of the hexoses tested caused a definite increase in the respiratory rate. Glucose was the most effective, with mannose and fructose following in the order named.

Maltose was the only disaccharide tested which affected oxygen uptake, lactose and sucrose were without effect.

Table IV. Effect of carbohydrates on the oxygen uptake of *Histoplasma capsulatum*

Substrate (M/300)	Oxygen uptake (μl) with substrate	Oxygen uptake (μl) without substrate	Per cent stimulation
Pentoses			
Xylose	283.6	240.0	18.2
Arabinose	253.7	240.0	5.7
Hexoses			
Glucose	617.6	291.3	112.0
Mannose	472.9	299.4	57.9
Fructose	195.1	150.8	29.4
Disaccharides			
Lactose	165.6	150.8	9.8
Maltose	397.2	323.2	23.1
Sucrose	286.2	272.0	5.2

37 C., Ph 7.0

Totals for 5 hours

Discussion

The respiration of *H. capsulatum* appears similar in some respects to that of *Blastomyces dermatitidis,* another diphasic fungus causing systemic mycotic infections in man. The lower fatty acids, from acetic through butyric, increase oxygen uptake by both organisms, while the higher fatty acids markedly inhibit respiration (Bernheim, 1942; Levine

and Novak, 1950) . Oxygen uptake by both organisms is stimulated by xylose, mannose, and glucose. Bernheim reported, however, that fructose has no effect on the respiration of *B. dermatitidis,* while *H. capsulatum* shows a marked increase in oxygen uptake in the presence of this carbohydrate.

The respiration of *H. capsulatum* was found to differ in other respects. Levine and Novak (1950) reported mannitol, dulcitol, and sorbitol to stimulate the respiratory rate of *B. dermatitidis,* but only the first stimulated oxygen uptake of *H. capsulatum.* These same investigators found succinate at M/300 and malonate at M/18 concentrations to have no effect on oxygen uptake of *B. dermatitidis.* In the present study, M/300 succinate was found to stimulate and M/18 malonate to inhibit the oxygen uptake of *H. capsulatum.* Bernheim (1942) reported that both the natural and non-natural isomers of the amino acids increase the oxygen uptake of *B. dermatitidis.* With yeast cells of *H. capsulatum* all of the naturally occurring isomers except one appreciably stimulated respiration, however, the non-natural isomers had no effect.

The results of this study suggest that at least part of the tricarboxylic acid cycle is involved in the metabolism of *H. capsulatum.* The lack of citrate, cis-aconitate, and isocitrate utilization has been shown in some instances to be due to a cell wall or membrane permeability factor. Cell free homogenates prepared from other microorganisms have been found to actively oxidize these substrates in the absence of activity by whole cells (Pan, *et al.,* 1957). This we have attempted to clarify by using cell-free homogenates and acetone-dried cells in place of whole yeast cells. However, neither of these preparations showed activity in the presence of added substrate.

Summary

The effects of various substances on the respiration of the yeast phase of *Histoplasma capsulatum* were determined, using conventional manometric techniques.

All of the tricarboxylic acid cycle intermediates except citric, cis-aconitic, and isocitric acids appreciably stimulated the oxygen uptake of the fungus.

The presence of the L- forms of all the amino acids tested increased the oxygen uptake, with the exception of L-arginine. The greatest amounts of activity occurred with L-isoleucine, L-cystine, and L-threonine. None of the D-forms tested showed any effect upon the respiration of the fungus.

The lower fatty acids, from acetate through butyrate, stimulated respiration. Sodium oleate, one of the higher fatty acids, inhibited oxygen uptake.

Malonate in M/18 concentration inhibited endogenous respiration and also the oxidative utilization of succinic acid by the cells.

Mannitol stimulated oxygen uptake, and dulcitol and sorbitol were not used oxidatively by this organism.

Of the carbohydrates tested glucose, mannose, fructose, xylose, and maltose stimulated respiration, while lactose, sucrose, and arabinose were without effect.

Literature Cited

Bernheim, F. 1942. The effect of various substances on the oxygen uptake of *Blastomyces dermatitidis.* J. Bacteriol. 44: 533-539.

Cozad, G. C. 1958. A study of the whole yeast cell agglutination test in rabbits experimentally infected with *Histoplasma capsulatum*. J. Immunol. 81: 368-375.

Levine, S., and M. Novak. 1950. Studies on the metabolism of *Blastomyces dermatitidis* I. The effect of various substances on respiration. J. Bacteriol. 60: 333-339.

Pan, S. F., R. Yee, and H. M. Gezon. 1957. Studies on the metabolism of Shigella I. The occurrence of a tricarboxylic acid cycle in *Shigella flexneri*. J. Bacteriol. 73: 402-409.

Pine, L. 1954. Studies on the growth of *Histoplasma capsulatum* I. Growth of the yeast phase in liquid media. J. Bacteriol. 68: 671-679.

Pine, L. 1955. Studies on the growth of *Histoplasma capsulatum* II. Growth of the yeast phase on agar media. J. Bacteriol. 70: 375-381.

Pine, L. 1957. Studies on the growth of *Histoplasma capsulatum* III. Effect of thiamin and other vitamins on the growth of the yeast and mycelial phases of *Histoplasma capsulatum*. J. Bacteriol. 74: 239-245.

Salvin, S. B. 1949. Cysteine and related compounds in the growth of the yeast-like phase of *Histoplasma capsulatum*. J. Infectious Diseases 84: 275-283.

Salvin, S. B. 1950. Growth of the yeastlike phase of *Histoplasma capsulatum* in a fluid medium. J. Bacteriol. 59: 312-313.

Scherr, G. H. 1957. Studies on the dimorphism of *Histoplasma capsulatum* I. The roles of -SH groups and incubation temperature. Exptl. Cell Research 12: 92-107.

Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1949. Manometric techniques and tissue metabolism. Burgess Publishing Co., Minneapolis, Minn.