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Comparative Sensitivity of *Histoplasma capsulatum* Conidiospores and Blastospores to Oxidative Antifungal Systems†

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The comparative sensitivity of blastospores and conidiospores of *Histoplasma capsulatum* to hydrogen peroxide, to hydrogen peroxide and halides, and to a combination of hydrogen peroxide and halide with the enzyme myeloperoxidase was studied. Blastospores of different strains of *H. capsulatum* varied in their sensitivity to hydrogen peroxide. This variation correlated with the amount of catalase in cell-free extracts from the strains. Blastospores and conidiospores of a single isolate were about equally susceptible to hydrogen peroxide, but this sensitivity could obviously vary with the catalase content of the two types of spores. Halides augmented the antifungal activity of hydrogen peroxide for both types of spores. Iodide was far more efficient in this regard than was chloride. A crude granule lysate from guinea pig polymorphonuclear leukocytes was quite inhibitory to blastospore but not to conidiospore germination. A study of the myeloperoxidase activity of such preparations against blastospores was thus precluded. A sample of a very highly purified human myeloperoxidase functioned in the presence of hydrogen peroxide and either iodide or chloride to prevent germination of both blastospores and conidiospores. The preparation had no toxicity for spores apart from its interaction with hydrogen peroxide and halides.

The blastospores of *Histoplasma capsulatum* are killed by polymorphonuclear neutrophils (PMNs) from humans (9), guinea pigs (10), and mice (11). The most extensively examined of the antifungal properties of PMNs is the oxidative system involving H_2O_2, a halide, and the enzyme myeloperoxidase (11, 16). Previously I have reported on the sensitivity of blastospores of *H. capsulatum* to the myeloperoxidase system (10-12). The interaction of zoopathogenic fungi and phagocytic cells and cellular mechanisms mediating antifungal effects has been reviewed recently (13). In this paper I will describe results from experiments on the comparative sensitivity of conidiospores and blastospores to that oxidative system.

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MATERIALS AND METHODS

Fungi. Strain numbers 501, 505, and 507 of *H. capsulatum* from the Fungus Collection, University of California Los Angeles, were used in this study. Stock cultures of the yeast cells of *H. capsulatum* were maintained on glucose-cysteine blood agar (6) in a refrigerator and were subcultured every month. The mycelial phase of the fungus was kept on potato-dextrose agar in a freezer. Seed stocks of the mycelial phase were maintained on potato-dextrose agar by monthly transfers incubated at room temperature.

Preparation of spores. Blastospores were harvested by centrifugation from cultures of Salvin liquid medium (21) incubated for 48 to 72 h at 37°C on a gyratory shaker (New Brunswick Scientific Co., New Brunswick, N. J.) operating at 150 rpm. Harvested cells were washed in 0.02 M citrate phosphate buffer (pH 6.5) and suspended to the desired concentration in the buffer.

Preparing cultures of *H. capsulatum* which produce conidiospores abundantly presented some problems. A nutritionally lean medium is generally acknowledged to foster sporulation of fungi (2). A medium such as yeast extract agar (3) serves this purpose rather well with *H. capsulatum* (unpublished observation). Smith (22) studied the nutritional factors important in the sporulation of *H. capsulatum* and developed from the studies a defined medium upon which spores were abundantly produced. Moreover, Smith defined medium supported a wispy sort of mycelial growth which facilitated harvesting spores (see below). Accordingly, Smith defined medium contained in Roux bottles (125 ml of medium per bottle) was used to grow the fungus from which conidiospores were harvested. Spores prepared in this fashion from cultures of *H. capsulatum* strain 501 were mixtures of

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both macro- and microconidiospores. The microconidiospores were more abundant, usually constituting more than 90% of the spores harvested. The commercially available product (Meridian Diagnostics, Inc., Cincinnati, Ohio) of the same recipe was less suitable than the medium concocted freshly in the laboratory.

Conidiospores were harvested as follows: seed cultures grown on potato-dextrose agar were immersed in a salt solution of low ionic strength, and portions of the mycelial mat were placed in a tissue grinder. The material was triturated briefly, and the resulting suspension was used to inoculate the surface of Smith defined medium contained in Roux bottles. Cultures thus prepared were incubated at 30°C. After 1 month of incubation, the surface growth was wetted with about 10 ml of 0.02 M citrate phosphate buffer (pH 6.5). The hyphal growth was loosened with a pipette, and the whole of it was transferred to a tissue grinder. The material was triturated and passed through four layers of gauze, after which the harvested spores were washed three times in 0.02 M citrate phosphate buffer, and the suspension was standardized to contain approximately 1×10^6 spores per ml. Spore harvests did not always measure up to expectations, and smaller numbers of spores were sometimes used in the experiments outlined below.

Media and buffers. Glucose-cysteine blood agar was prepared according to a standard recipe (6). Potato-dextrose agar was made in this laboratory as described in a recent publication (14). The formula for Smith defined medium has been published (22). The medium used for plate count and slide cell cultures was 2% glucose–1% proteose peptone no. 3–2% agar (GPA). Yeast nitrogen base was prepared from a commercial source (Difco Laboratories, Detroit, Mich.).

The salt solution of low ionic strength contained 0.01 M KCl, 0.0024 M $CaCl_2$, and 0.0025 M $MgCl_2$. The citrate phosphate and phosphate buffers were prepared according to standard directions (7). Generally, buffers were used at 0.02 M or 0.05 M because of the well-described sensitivity of blastospores of *H. capsulatum* to more concentrated solutions (10).

Assays. Construction of the reaction mixtures used to assess the toxicity of hydrogen peroxide, hydrogen peroxide and halides, and hydrogen peroxide, halides, and myeloperoxidase will be explained with the experimental results. After incubation in the reaction mixtures, blastospores or conidiospores were serially diluted in 0.02 M phosphate buffer (pH 7.0) and plated on the surface of GPA plates, which were incubated at 30°C. Mycelial colonies were counted after 5 to 7 days of incubation. Spore germination was assessed by counting germlings which arose from specimens placed on the surface of agar blocks in slide cell cultures incubated for 48 h at 30°C. The slide cell cultures were prepared by the method pictured in a standard laboratory manual (15). In keeping with the observations of Goos (8), the micro- and macroconidiospores germinated equally well under these culture conditions, and even though the small spores predominated, both spore sizes were scored.

Cell-free extracts were prepared from blastospores grown on Salvin medium at 37°C. The cells were harvested by centrifugation, washed in 0.02 M phosphate buffer (pH 7.0), and concentrated. Cells were broken in a Braun cell homogenizer (model MSK; Bronwill Scientific Inc., San Francisco, Calif.). The catalase content of the cell-free extract was determined spectrophotometrically by the modified method of Beers and Sizer (1) described by Price et al. (19).

The peroxidase activities of the myeloperoxidase preparation from guinea pig granules, of the specimen of purified human myeloperoxidase, and of cell-free extracts of blastospores of *H. capsulatum* were measured by the O-dianisidine method described in the *Worthington Enzyme Manual* (5). Protein determinations were by the method of Lowry et al. with bovine serum albumin as standard (17).

Harvest of guinea pig PMN. Guinea pigs of both sexes, which weighed 300 to 600 g, were used. Leukocytes were induced by intraperitoneal injection of 15 ml of 12% sodium casinate (Difco) in distilled water. After 16 to 18 h, the animals were killed by chloroform anesthesia, and the exudate was aseptically harvested by lavage of the peritoneal cavity three times with 20 ml of Hanks balanced salt solution (HBSS). Suspensions of harvested cells were centrifuged ($500 \times g$ at 5°C), washed once in HBSS, and counted in a hemacytometer. The guinea pig leukocytes are properly called heterophils, and the abbreviation PMN is used throughout to mean those cells.

Preparation of granule lysates from guinea pig PMN. Harvested and washed cells were counted, and HBSS suspensions were adjusted to contain 2×10^8 to 3×10^8 cells. The suspensions were centrifuged ($480 \times g$ at 5°C), and the packed cells were suspended in 10 ml of 0.34 M sucrose solution. The cells were mixed and homogenized for 5 min in a motor-driven Teflon homogenizer (A. H. Thomas Co., Philadelphia, Pa.). The volume of the mixture was increased to 15 ml with 0.34 M sucrose. The disrupted cell suspension was centrifuged at $480 \times g$ for 10 min, and the opalescent supernatant fluid was collected. The pellet was suspended in 15 ml of cold 0.34 M sucrose and centrifuged again. The procedure was repeated three times or until the supernatant fluids were clear. The opalescent supernatant fluids were collected and centrifuged at $27,000 \times g$ for 25 min. The supernatant was discarded, and the pellet which contained the granules was frozen until used. The thawed pellets were mixed with 1 to 1.5 ml of 0.01 M citric acid at 4°C for 60 min. A one-tenth volume of 0.21 M Na_2HPO_4 was added to the extracts, which were then centrifuged at $27,000 \times g$ for 10 min in the cold. The lysates were kept in the freezer until used. The addition of Na_2HPO_4 raised the pH to about 5.0. Further additions of phosphate buffer were used to adjust the concentration, and the final pH was that of the buffer controls. The frozen material was thawed and suspended in 5 ml of the diluent appropriate to the system being studied.

Chemicals. Hydrogen peroxide was from Mallinckrodt (Paris, KT), and the inhibitor of catalase 3-amino-1,2,4-triazole was from Aldrich Chemical Co., Milwaukee, Wis.

RESULTS

Assessment of killing. Blastospores of *H. capsulatum* germinate on GPA incubated at 30°C and thereby form mycelial colonies which

can be counted (Fig. 1). The data recorded in Table 1 display the sensitivity of blastospores of *H. capsulatum* to hydrogen peroxide as revealed by routine colony counts and by direct assessment of the percentage of blastospores that form germ tubes in slide cell cultures. The semiquantitative data generated by the two methods lead to virtually the same conclusions. Conidiospore germination can also be used to assess viability of such spores after exposure to oxidative antifungal systems. Direct assessment of the percentage of spores that germinate after exposure to noxious substances obviates certain sources of inaccuracy, e.g., clumping, which can affect the reliability of plate counts.

Augmentation of peroxide toxicity by halides. It is well known that halides augment the fungicidal activity of hydrogen peroxide (9). Thus, 10^{-4} M hydrogen peroxide is nontoxic to *Histoplasma* blastospores, but addition of 10^{-3} M of iodide, which is itself nontoxic, creates a lethal combination (10–12). The data in Table 2 confirm that conidiospores of the fungus are also sensitive to halide augmentation of hydrogen peroxide toxicity. It is to be noted that 0.1 M chloride did not augment hydrogen peroxide toxicity. Higher concentrations of chloride reduced germination in blastospores of *H. capsulatum* (Table 3), so I did not continue work with chloride augmentation of hydrogen peroxide killing. Also recorded in Table 3 is the fact that the blastospores of this strain of *Histoplasma* are more resistant to hydrogen peroxide than were those of the strain used to obtain the data recorded in previous work (10–12; Table 1). The yeast cell phase of growth was only recently derived from strain 501 after it was deemed important to work with blastospores and co-

TABLE 1. *Fungicidal activity of hydrogen peroxide against blastospores of H. capsulatum (505)*[a]

Hydrogen peroxide concn (M)	Measurement of activity			
	Colony-forming units/ml[b]	% Reduction	Germ tube formation (%)[c]	% Reduction
0	2.7×10^6	—	90	—
0.1	$<1 \times 10^4$	>99	0	>99
0.01	$<1 \times 10^4$	>99	0	>99
0.001	4.2×10^5	93	72	20
0.0001	2.6×10^6	0	93	0

[a] Strain designation.

[b] The reaction mixtures contained, in a final volume of 2.0 ml, 5×10^6 blastospores per ml and H_2O_2 at the final concentration shown in the table. The diluent was distilled water. The mixtures were incubated at 37°C for 3 h on a rotator. Samples were plated on GPA incubated at 30°C, and the colony-forming units were recorded after 5 to 7 days.

[c] The reaction conditions were similar to those recorded in footnote *b*, except that there were about 1×10^7 blastospores per ml and that the diluent was 0.025 M citrate phosphate buffer (pH 6.5). One-loopful samples were placed on agar blocks of GPA in slide cell cultures incubated at 30°C for 48 h.

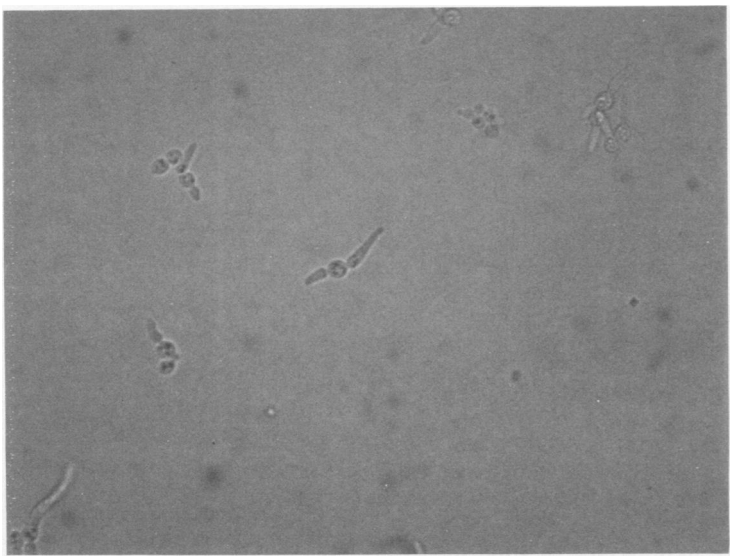

FIG. 1. *Germinating blastospores of H. capsulatum. Yeast cells from a culture grown on glucose-cysteine blood agar for 48 h at 37°C were harvested and washed in 0.02 M phosphate buffer (pH 7.0). One loopful of the cell suspension was placed on the surface of a block of glucose-peptone agar in slide cell culture. The cultures were incubated at 30°C, and the photograph shown was taken at 24 h.*

TABLE 2. *Augmentation by halides of the fungicidal effect of hydrogen peroxide on conidiospores of H. capsulatum (501)*[a]

Hydrogen peroxide concn (M)	% Germination[b] with:			
	Iodide (M)			Chloride (M)
	0	0.001	0.0001	0.1
0	90	ND[c]	ND	ND
0.001	82	0	41	81
0.0001	84	31	76	86

[a] Strain designation.

[b] The reaction mixtures contained, in a final volume of 2.0 ml, 4×10^5 conidiospores per ml and the halides and H_2O_2 at the final concentration shown in the table. The diluent was 0.025 M citrate phosphate buffer (pH 6.5). Mixtures were incubated at 37°C for 3 h on a rotator, and 1-loopful samples were placed on agar blocks of GPA in slide cell cultures incubated at 30°C for 48 h.

[c] Certain combinations known to be nontoxic on the basis of preliminary experiments were not done (ND).

TABLE 3. *Augmentation by halides of the fungicidal effect of hydrogen peroxide on blastospores of H. capsulatum (501)*[a]

Hydrogen peroxide concn (M)	% Germination[b] with:					
	Iodide (M)[c]			Chloride (M)[c]		
	0	0.01	0.001	1.0	0.15	0.1
0	90	90	77	0	45	81
0.1	0	ND	ND	ND	ND	ND
0.01	86	0	0	0	0	0
0.001	75	0	0	0	35	70
0.0001	85	ND	71	ND	ND	ND

[a] Strain designation.

[b] Certain combinations judged to be obviously toxic or somewhat irrelevant on the basis of preliminary work were not tested (ND).

[c] The reaction mixtures contained, in a final volume of 2.0 ml, about 1×10^7 blastospores per ml and the halides and H_2O_2 at the final concentration shown in the table. The diluent was 0.025 M citrate phosphate buffer (pH 6.5). Mixtures were incubated at 37°C for 3 h on a rotator, and 1-loopful samples were placed on agar blocks of GPA in slide cell cultures incubated at 30°C for 48 h.

nidiospores of the same strain. The disparate sensitivity of the two strains led to comparison of them in the rest of the study. Strain 505 does not form adequate numbers of conidiospores to allow full comparisons of both spore types of both strains.

Comparative sensitivity of blastospores and conidiospores of *H. capsulatum* to hydrogen peroxide. A rearrangement of data presented in previous tables is shown in Table 4. Here it is obvious that the sensitivity of two different strains of *Histoplasma* to hydrogen peroxide is different and that if one compared conidiospores of one strain to blastospores of another, different conclusions would emerge. Different levels of catalase might account for the differences in sensitivity of blastospores of the two strains. The data in Table 5 show that concentrations of hydrogen peroxide which are relatively nontoxic (10^{-3} M for strain 505 and 10^{-2} M for strain 501) become lethal in the presence of 40 mM of the catalase inhibitor 3-amino-1,2,4-triazole (AT). It should be noted that the inhibitory effect of AT on catalase is antagonized by sulfhydral compounds (20). The yeast cells used were grown in Salvin medium rich in cysteine. The cells thus had to be starved before use in the experiments recorded in Table 5, footnote *a*. In fact, AT inhibition of blastospore catalase could not be convincingly displayed with unstarved cells.

There were consistent differences in levels of catalase in cell-free extracts from the blastospores of three strains of *H. capsulatum* (Table

TABLE 4. *Fungicidal effect of hydrogen peroxide on blastospores and conidiospores of H. capsulatum*

Hydrogen peroxide concn (M)	% Germination[a]		
	Blastospores[b]		Conidiospores[b]
	505	501	501
0	90	80	90
0.1	0	1	0
0.01	0	86	48
0.001	72	75	93
0.0001	93	85	88

[a] Reaction conditions as recorded in footnotes to Tables 2 and 3.

[b] Numbers refer to strain used.

TABLE 5. *Effect of AT on the sensitivity of H. capsulatum to hydrogen peroxide*

Ingredients[a]	% Decrease in germination over controls	
	501	505
Buffer (Control)	—	—
Buffer + AT	2	5
Buffer + H_2O_2	16	13
Buffer + H_2O_2 + AT	85	100

[a] Reaction mixtures contained 2×10^6 to 7×10^6 yeast cells in 0.025 M citrate phosphate buffer (pH 6.5) to a final volume of 2.0 ml and the following supplements as indicated in the table: H_2O_2, 10^{-3} M (505) and 10^{-2} M (501); AT, 4×10^{-2} M. The yeast cells were starved for 5 days in yeast nitrogen base in shake cultures at 37°C before being washed and standardized. Blastospore germination was determined as described in Table 3, footnote *c*.

6). These differences corresponded to the relative sensitivity of the strains to hydrogen peroxide toxicity. The levels of peroxidase in these strains were too low to be detected by the o-dianisidine method.

Sensitivity of blastospores and conidiospores to myeloperoxidase. The preliminary data shown in Table 7 suggest that crude granule lysates from guinea pigs are quite toxic to blastospores of *H. capsulatum*, so much so that some purification will be required before further work on their myeloperoxidase (MPO) content can be pursued. The toxic component is one or more of the highly cationic proteins which such preparations also contain and which will be the subject of a later publication in this series. The conidiospores seem not to be sensitive to these cationic proteins, but the very low sensitivity to combinations of ingredients intended to show MPO activity makes the results inconclusive as to oxidative antifungal mechanisms.

A sample of highly purified human MPO was made available to us from the laboratory of R. I. Lehrer (Department of Medicine, UCLA). This material was used in a final series of experiments, the results of which are recorded in Table 8. The human MPO was devoid of intrinsic toxicity at the highest level used (Table 8). The enzyme produced a potent microbicide in the presence of hydrogen peroxide and both iodide and chloride. Both blastospores and conidiospores were sensitive, but chloride was not efficient against conidiospores at the lowest level of MPO tested (1 U).

DISCUSSION

It is apparent from the data presented that blastospores of different strains of *H. capsulatum* vary in their sensitivity to hydrogen peroxide. This variation was correlated with the content of catalase of the strains. Although blastospores and conidiospores of a single isolate were about equally susceptible to hydrogen peroxide, it is obvious that such susceptibility could vary in keeping with variations in catalase content of the two types of spores among other isolates. Hydrogen peroxide, halides, and MPO combine to form a potent antifungal system active against both conidiospores and blastospores of *H. capsulatum*. However, efforts to use granule lysates from guinea pig PMNs as a source of MPO activity revealed a component which was highly

TABLE 6. *Comparison of hydrogen peroxide toxicity and catalase content of three isolates of H. capsulatum*

Strain no.	Hydrogen peroxide toxicity[a] (M)	Catalase level (U/mg of protein)[b]
501	0.1	199
505	0.01	36
507	>0.1	307

[a] Recorded as molarity of hydrogen peroxide in 0.025 M citrate phosphate buffer (pH 6.5), which caused more than 25% reduction in blastospore germination over controls after 3 h at 37°C.

[b] Average value for enzyme activity of cell-free extracts tested in two separate experiments. One unit decomposes 1 μmol of H_2O_2 per min (19).

TABLE 7. *Comparative antifungal effect of granule lysate from guinea pig PMN on conidiospores and blastospores of H. capsulatum (501)*[a]

Ingredients[b]	% Germination[c]	
	Conidiospores	Blastospores
Diluent (pH 6.5)	90	90
H_2O_2, Cl^-	90	96
H_2O_2, I^-	91	91
GL	86	0
H_2O_2, I^-, GL	50	0
H_2O_2, Cl^-, GL	98	0

[a] Strain designation.

[b] Reaction mixtures contained 5×10^6 blastospores or conidiospores per 1 ml in 0.025 M citrate phosphate buffer (pH 6.5) to a final volume of 2.0 ml and the following ingredients (final concentration) as shown in the table: H_2O_2, 10^{-4} M; KI, 10^{-3} M; KCI, 10^{-1} M; 100 λ of a granule lysate (GL) prepared from 2×10^8 PMNs and with 249 U of o-dianisidine per 100 λ.

[c] Determined by the methods recorded in footnotes to Tables 2 and 3.

TABLE 8. *Effect of human MPO, hydrogen peroxide, and halides on conidiospores and blastospores of H. capsulatum (501)*[a]

Ingredients	% Germination[b,c]	
	Blastospores	Conidiospores
Diluent	97	91
H_2O_2	86	80
H_2O_2, KI	95	79
H_2O_2, KCl	85	81
MPO (100 U)	97	86
H_2O_2, KI, MPO (100 U)	0	0
H_2O_2, KI, MPO (10 U)	16	0
H_2O_2, KI, MPO (1 U)	16	0
H_2O_2, KCl, MPO (100 U)	0	0
H_2O_2, KCl, MPO (10 U)	31	0
H_2O_2, KCl, MPO (1 U)	29	84

[a] Strain designation.

[b] Reaction mixtures contained 2×10^7 blastospores or 1×10^7 conidiospores/ml in 0.025 M citrate phosphate buffer (pH 6.5) to a final volume of 2 ml and the following ingredients (final concentration) as shown in the table: H_2O_2, 10^{-4} M; KI, 10^{-3} M; KCl, 10^{-1} M; MPO, 100, 10, and 1 U by the o-dianisidine assay.

[c] Determined by the methods recorded in the footnotes to Tables 2 and 3.

toxic, in the absence of hydrogen peroxide and halides, to blastospores but not to conidiospores of the fungus.

In a study on the fate of *H. capsulatum* in guinea pig PMNs published earlier (10) eosin y dye exclusion was used to measure viability of the yeast cells after exposure to various oxidative antifungal systems. The dye exclusion method does not work well with spores other than blastospores (unpublished observations) and, accordingly, a method of viability assessment was sought which could be applied equally well to conidiospores and blastospores. Since both blastospores and conidiospores initiate mycelial colonies when incubated at 25 to 30°C, colony-forming units was one obvious possibility. Clumping of the spores either inherent in the strain used or engendered by the fungicidal environment caused irregular uncertainties which were difficult to control. Blastospores of *H. capsulatum* germinate at 30°C. Thus, percent germination in slide cell cultures could be used as one measure of spore viability after exposure to various mixtures of potentially fungicidal ingredients. Clumping was less of a consideration inasmuch as the behavior of each individual spore could be assessed. The data in Table 1 show that very similar conclusions can be derived from percent spore germination and colony-forming unit assessments. The obvious additional advantage of the spore germination technique lies in the fact that it is so much less laborious and time consuming than plate counting that a far larger number of experiments can be quickly done.

The data in Table 3 reaffirm the rather well-known fact that halides augment the fungicidal activity of H_2O_2 for blastospores of *H. capsulatum* (10). Moreover, iodide is very efficient at augmentation, whereas chloride is itself toxic for these spores. The data in Table 2 show that iodide augments the toxicity of hydrogen peroxide for conidiospores. However in this case, although chloride again didn't function in augmentation, it was not toxic for the spores even at a concentration of 0.1 M.

During these studies, it was discovered that three different isolates of *H. capsulatum* varied in the level of sensitivity of their blastospores to the inhibitory effects of hydrogen peroxide (Table 6). The differences in sensitivity were correlated with higher levels of catalase in the more resistant strains. Mandell (18) reported that the catalase but not the superoxide dismutase content of strains of *Staphylococcus aureus* was correlated with the intracellular survival of the strains in PMNs and with their virulence. Thus, he judged that catalase might be a virulence factor of staphylococci. In contrast, there was no correlation between the killing rate of H_2O_2 and catalase or superoxide dismutase levels in a strain of *Escherichia coli* (4). In this case there was a suggestion that the ability to repair the single-stranded DNA breaks induced by H_2O_2 was crucial to resistance to H_2O_2 toxicity (4). We will need to examine more isolates of *H. capsulatum* to be certain that catalase content alone and not some additional factors accounts for relative H_2O_2 sensitivity. The observation that AT increases sensitivity to H_2O_2 (Table 5) is additional evidence that levels of catalase may play some role in the toxicity of H_2O_2 for *H. capsulatum*.

In a previous study it was noted that granule lysates prepared from guinea pig PMNs could be used as a source of MPO activity against blastospores of *H. capsulatum* (10). These preparations were obtained from guinea pig PMN granules by repeated freezing and thawing. We now know that this procedure is not as efficient as the citric acid extraction which we currently use (see above). However, in these preparations we have also found highly cationic proteins by polyacrylamide gel electrophoresis which are very toxic to the blastospores of *H. capsulatum*. It is plausible that these substances account for the toxicity of the granule preparations. We are purifying these proteins to study them further. Moreover, we are setting about to purify guinea pig MPO away from the toxic proteins. In the meantime, we looked at a highly purified human MPO. It had no intrinsic toxicity for blastospores or conidiospores of *H. capsulatum*, but it was very efficient in the presence of H_2O_2 and a halide in generating a potent germicide which prevented both conidiospore and blastospore germination.

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