Functional Aspects of a Second Mechanism of Candidacidal Activity by Human Neutrophils

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ABSTRACT We tested the ability of human neutrophils to kill five *Candida* species and the yeast *Torulopsis glabrata*. *C. parapsilosis* and *C. pseudotropicalis* were found to be killed readily by normal and myeloperoxidase-deficient neutrophils and were selected to probe the myeloperoxidase-independent fungicidal mechanisms of the neutrophil.

These organisms were killed with relatively normal (*C. parapsilosis*) or moderately reduced (*C. pseudotropicalis*) effectiveness by neutrophils from two boys with X-linked chronic granulomatous disease. Azide (2 mM) and sulfadiazine (4 mM) exerted a relatively small inhibitory effect on the ability of normal neutrophils to kill *C. parapsilosis*. These compounds did not, however, inhibit the killing of *C. parapsilosis* by myeloperoxidase-deficient neutrophils, although they blocked their iodination of ingested *Candida* cells. Anaerobic incubation conditions inhibited the ability of normal neutrophils to kill *C. parapsilosis* slightly but did not impair this function in myeloperoxidase-deficient cells.

All of the *Candida* species tested had catalase activity, yet their sensitivity to H_2O_2 in cell-free systems varied considerably. Our *C. parapsilosis* strain was extraordinarily resistant to H_2O_2 (LD_{75}: 0.14 M), as compared with *C. pseudotropicalis* or with our reference strain of *C. albicans* (LD_{75}: 2.3×10^{-3} M and 3.4×10^{-3} M, respectively).

These data establish the existence in human neutrophils of a second mechanism that exerts microbicidal activity against certain *Candida* species; the mechanism is unrelated to myeloperoxidase, iodination, or to the direct effects of H_2O_2 generated by the endogenous metabolic processes of the neutrophil. As yet unidentified, this mechanism appears to remain operative in the neutrophils of subjects with hereditary myeloperoxidase deficiency or chronic granulomatous disease.

An abstract was presented to the national meeting of the American Federation for Clinical Research, Atlantic City, N. J., April 1972.

Received for publication 22 February 1972 and in revised form 14 June 1972.

INTRODUCTION

Despite progress in our understanding of the phagocytic process, the actual mechanisms whereby human neutrophils kill microorganisms are incompletely understood. Myeloperoxidase (MPO)[1] participates in some of these bactericidal and fungicidal processes, and the role of this enzyme in the microbicidal activity of intact normal neutrophils may be considerable for certain organisms (1–3).

MPO-deficient individuals may remain in good health, without excessive or unusual infections, for prolonged periods of time; this fact is difficult to reconcile with the presumed importance of MPO to the economy of the neutrophil. Recently, Klebanoff and Pincus have attempted to explain this paradox by suggesting that peroxidase-deficient neutrophils can exert microbicidal activity by a compensatory increase in the nonenzymatic effects of endogenously generated H_2O_2 (4).

We have previously shown that human neutrophils with deficiencies in MPO have an impaired ability to kill certain bacteria and the fungus, *Candida albicans* (2, 3). We now report the ability of human neutrophils to kill several other species of yeast-like fungi and present evidence for the existence of a fungicidal mechanism that is independent of MPO and that is not a consequence of the direct action of H_2O_2. Such mechanisms offer an alternative explanation for the microbicidal activity of MPO-deficient neutrophils.

METHODS

Fungi. The organisms used in these studies included three strains of *C. albicans:* strain 820 (5), strain B-311, and strain Mo (a recent isolate from a patient with systemic

[1] *Abbreviations used in this paper:* CFU, colony-forming unit; MPO, myeloperoxidase.

candidiasis); three strains of *C. tropicalis*, including one recently recovered from a patient with *Candida* pyelonephritis; two strains each of *C. parapsilosis* and *C. krusei;* one strain of *C. pseudotropicalis;* and four strains of *Torulopsis glabrata.*[2]

The fungi were grown in Sabouraud's 2% dextrose broth[3] except for *C. pseudotropicalis*, which was cultured exclusively in tryptose phosphate broth,[4] and *C. parapsilosis*, which was grown either in Sabouraud's or tryptose phosphate broth. The cultures were incubated at 33°C for 2–3 days (*C. pseudotropicalis* and *C. parapsilosis*), 5–7 days (*C. albicans*), or 3–7 days (all others). Under these growth conditions, the fungi manifested the following characteristics: (*a*) stationary growth phase with high viability; (*b*) few autolysed or mycelial-phase cells;[5] (*c*) good agreement between observed colony counts and those predicted by enumeration of colony-forming units (CFU) with an hemocytometer; (*d*) a lag phase of at least 90 min before the colony count fell (*C. albicans*) or rose significantly (all others) after the introduction of fungi to medium; and (*e*) a mean of 1.3–2.0 cells per CFU.

Fungi were washed twice with sterile distilled water containing 0.01% gelatin (gel-water), counted in an hemocytometer, and suspended at the desired concentration in gel-water or phosphate-buffered saline.

Leukocytes. Mixed peripheral blood leukocytes (containing 55–75% neutrophils and 2–7% monocytes) were prepared as previously described (5). Purified neutrophil preparations (85–95% neutrophils, less than 0.2% monocytes) were prepared by a minor modification of Böyum's technique (6); a mixture of 24 vol of 9% Ficoll[6] and 10 vol of 32.6% sodium diatrizoate[7] replaced his Isopaque-Ficoll formulation.

Leukocytes were obtained from normal subjects, a subject (C. J. B.) with hereditary myeloperoxidase deficiency (2), and two boys (M. B. and D. D.), aged 8 and 9 yr, who have classic X-linked chronic granulomatous disease. At the time of these studies, C. J. B. continued to be free of all significant infections, M. B. had evidence of resolving, suppurative cervical adenitis (*Neisseria* sp.), and D. D. had recovered some 2 months earlier from severe bilateral pneumonitis, thought to have been caused by *Aspergillus fumigatus.*

Aerobic assays. Aerobic assays were conducted in 12×75-mm polystyrene tubes[8] that contained, in a final volume of 1.6 ml, 0.4 ml of normal group AB serum, leukocytes sufficient to provide 4×10^6 neutrophils, Hanks' balanced salt solution[9] and fungi. After the other components had been incubated together for 15 min at 37°C, fungi were added at ratios that approximated one CFU per two neutrophils in studies with mixed leukocyte populations and one CFU per five neutrophils in studies with purified neutrophil preparations. After addition of fungi, the tubes were thoroughly mixed and triplicate samples withdrawn for dilution, plating, and colony counting. The tubes were then incubated at 37°C while rotating end-over-end at 30 rpm. Additional triplicate samples were secured for colony counting after 45 min (in some experiments), 90 min, and 180 min of incubation. In experiments wherein the colony-count results were to be compared with the specific-staining method of evaluating candidacidal activity, samples were also removed after 150 min of incubation and slides were prepared, stained, and interpreted as previously described (7). Sabouraud's 2% dextrose agar[10] was used in the colony-count experiments; the plates were incubated for 48–96 hr at 33°C to permit maximal colony development before being counted.

Anaerobic assays. Anaerobic assays were conducted in 15-ml polycarbonate plastic tubes with tightly fitting sleeve-type rubber stoppers. The final volume in these tubes was 4.0 ml and contained leukocytes, fungi, and serum in the same relative proportions as were present in the aerobic experiments with purified neutrophils. The tubes were capped, and those to be rendered anaerobic were flushed for 30 min with a stream of highly purified nitrogen[11] that was passed through an alkaline solution of pyrogallol (8) and then through distilled water before entering the tubes via appropriately sized and placed needles. The fungi to be added were similarly capped, flushed with nitrogen, and inoculated into the assay tubes through the stopper with appropriate precautions to exclude contamination by room air. At intervals (45, 90, and 180 min) samples were withdrawn from the incubation mixtures by syringe, and these were diluted and prepared for colony counting as described above. Slides were prepared from the samples taken at 45 min and examined to evaluate the extent of phagocytosis.

At the conclusion of the experiment, the residual contents of representative tubes were found to have an oxygen tension less than 1% that of air-saturated medium similarly tested in a Gilson Model KM oxygraph with a Clark electrode.[12] To further confirm the adequacy of this technique of achieving anaerobiosis, leukocytes treated in an analogous manner were shown to have no stimulation of hexose-monophosphate shunt activity after ingestion of heat-killed *Candida* cells.

Quantitative iodination. The ability of normal or MPO-deficient neutrophils to iodinate ingested microorganisms was tested by a modification of the method of Pincus and Klebanoff (9). The standard reaction mixture consisted of 2.5×10^6 neutrophils (prepared by the Hypaque-Ficoll method described above), 10% normal group AB serum, 10 nmoles of NaI (0.2 μCi of ^{125}I), approximately 700 nmoles of glucose (including that added in the serum component), and calcium-free Krebs-Ringer phosphate buffer, in a final volume of 0.5 ml. To this were added the particles: 5×10^6

[2] I am indebted to Dr. C. Halde for providing and identifying many of the *Candida* isolates, to Dr. H. C. Hasenclever for providing *C. albicans* B-311, to Dr. S. J. Klebanoff for providing a strain of *C. tropicalis* used in his earlier experiments, and to Dr. T. Eickhoff for providing three strains of *T. glabrata.*

[3] Difco Laboratories, Detroit, Mich., or BBL, Division of BioQuest, Cockeysville, Md.

[4] Difco Laboratories, Detroit, Mich.

[5] Certain lots of Sabouraud's broth available from commercial suppliers proved unsatisfactory for these tests. In them, fungi attained a relatively low final concentration in stationary phase, and numerous (20–30%) nonviable and autolysed cells were present. Such preparations would have provided unacceptably high background values for the specific staining assay; therefore, lots of broth were screened for their ability to produce maximally viable fungal cultures before they were used in these studies.

[6] Pharmacia, Uppsala, Sweden.

[7] Hypaque, Winthrop Laboratories, New York.

[8] Falcon Plastics, Division of BioQuest, Los Angeles, Calif.

[9] Grand Island Biological Co., Berkeley, Calif.

[10] Difco Laboratories, Detroit, Mich.

[11] Hi-pure grade, Liquid Carbonic Division, General Dynamics Corp., New York.

[12] Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio.

heat-killed *C. albicans*, or, in some experments, 3×10^6 viable *C. parapsilosis*. Tubes were incubated for 60 min and processed as described (9).

Effect of hydrogen peroxide. The susceptibility of various *Candida* species to hydrogen peroxide was measured by minor modifications of a previously described technique (10). Fungi were washed twice with, and then suspended in, distilled water. They were exposed to various concentrations of H_2O_2 in tubes containing, in a final volume of 0.5 ml, approximately 1×10^6 CFU of fungi, citrate-phosphate buffer (50 μmoles), and water. The reactions were started by adding H_2O_2 (or water), and the tubes were incubated for 45–60 min at 37°C. Colony counts were then obtained by the usual method. During the incubations with H_2O_2, the evolution of bubbles was noted with all species tested, indicating that all contained catalase.

RESULTS

Measurements of fungicidal activity

The first series of experiments was performed with mixed peripheral blood leukocytes. Although such preparations also contained monocytes and eosinophils, well over 90% of the added organisms were ingested by neutrophils. Accordingly, these studies were taken to indicate neutrophil function. The assay conditions insured that the added fungi were rapidly ingested, and few yeasts remained in extracellular locations after 15 min of incubation.

The susceptibility of various *Candida* species to normal and MPO-deficient neutrophils is shown in Figs. 1 and 2. In both figures, killing by neutrophils is reflected by the percentage fall from initial colony counts. Fig. 2 shows, in addition, the percentage of *Candida* cells within neutrophils that have acquired the Giemsa-staining characteristics of ghosts after an incubation period of 2.5 hr.

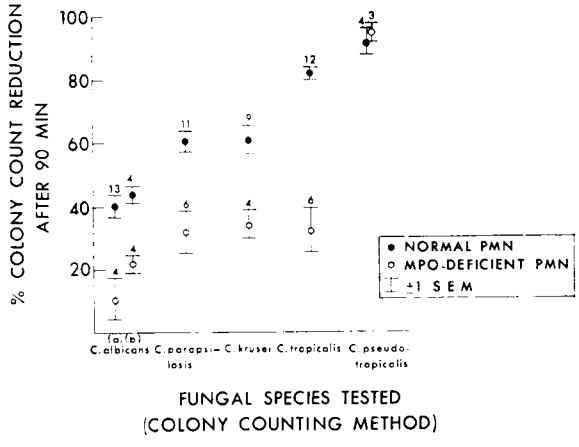

FIGURE 1 Ability of normal and MPO-deficient neutrophils to kill *Candida* species. Results with *C. albicans* strain 820 are indicated by (*a*); *C. albicans* strains Mo and B-311 have been combined (*b*). Results with two strains each of *C. parapsilosis* and *C. krusei* and with three strains of *C. tropicalis* were concordant within each species, and have been combined. The ordinate shows the mean percentage reduction (from original colony count levels) 90 min after fungi were added to mixed peripheral blood leukocytes. The numbers of experiments with different normal subjects and of experiments (each on a different day) with C. J. B.'s MPO-deficient leukocytes are shown above the bars denoting 1 SEM.

The results with both methods, colony counting and specific staining, are reasonably concordant. Under these test conditions, the various *Candida* species differed in their sensitivity to normal neutrophils, ranging from the somewhat resistant *C. albicans* to the highly susceptible *C. pseudotropicalis*. The *Candida* species showed an even greater range of susceptibility to MPO-deficient neutrophils; the ability of these leukocytes to kill *C. albicans* was decidedly impaired whereas their ability to kill *C. parapsilosis* or *C. pseudotropicalis* was relatively or completely intact.

In another experiment, the ability of normal and MPO-deficient neutrophils to kill four strains of *T. glabrata* was compared (Fig. 3). Although killing of this organism by the peroxidase-deficient cells proceeded at a subnormal rate, it reached approximately normal levels at the end of 3 hr of incubation. One of these *T. glabrata* isolates (strain D) was also studied in an additional series of experiments with normal and MPO-deficient mixed leukocytes, and this same pattern of delayed, but ultimately normal, killing by the MPO-deficient cells was seen consistently.

Characterization of the candidacidal mechanism

In the experiments summarized in Figs. 1 and 2, mean percentage reductions in colony counts (±SEM) with *C. parapsilosis*, strain 12-10, were 42% (±4.4%) for MPO-deficient leukocytes and 61.8% (±4.9%) for normal leukocytes after 90 min; after 180 min, MPO-deficient leukocytes had a 72% (±3.8%) reduction and normal leukocytes had a 79% (±4.2%) reduction. *C. parapsilosis*, strain 12-10 and *C. pseudotropicalis* appeared to be suitable species for exploring the nature of the MPO-independent candidacidal mechanisms of the human neutrophil. Accordingly, experiments were conducted with these organisms to elucidate the following points: (*a*) the effects of azide and sulfadiazine on the candidacidal function and the iodinating capacity of normal and MPO-deficient neutrophils; (*b*) the candidacidal activity of neutrophils from children with chronic granulomatous disease; (*c*) the effects of anaerobiosis on neutrophil candidacidal activity; and (*d*) the comparative sensitivity of *C. parapsilosis*, *C. pseudotropicalis*, and *C. albicans* to the direct toxic effects of H_2O_2.

Effects of azide and sulfadiazine. Azide and sulfadiazine are known to inhibit the function of MPO-mediated bactericidal and candidacidal activity (*C. albicans*)

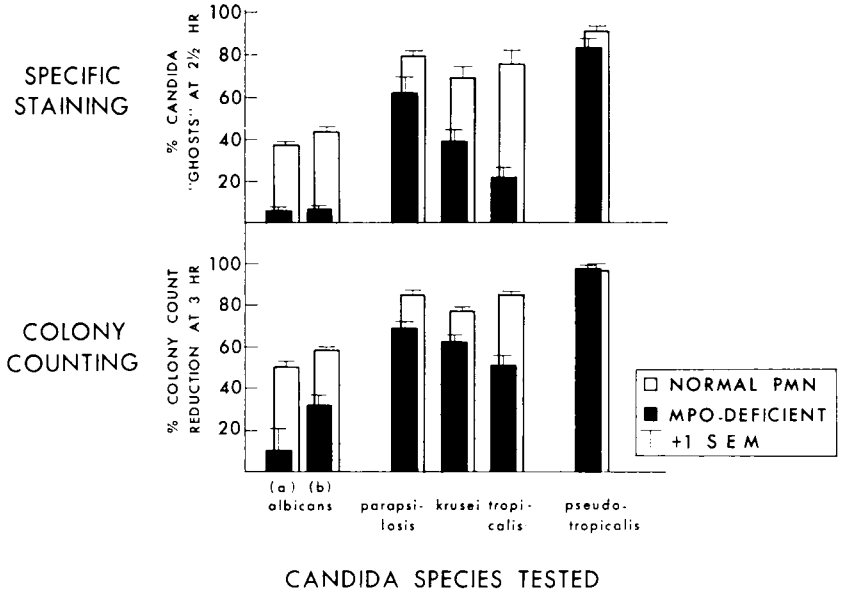

FIGURE 2 Ability of normal and MPO-deficient neutrophils to kill *Candida* species. Top, percentage of *Candida* cells within neutrophils that were killed and digested after 2.5 hr of incubation, as determined by the specific staining procedure; bottom, percentage fall in colony count 3 hr after addition of fungi to mixed peripheral blood leukocytes. Other experimental conditions are as described in Fig. 1.

in the normal human neutrophil (1, 11). The effect of these two agents on the ability of normal and MPO-deficient neutrophils to kill *C. parapsilosis* is shown in Fig. 4. Both sulfadiazine (4 mM) and azide (2 mM) caused a consistent, but relatively small, decrease in the ability of normal neutrophils to kill this organism after 90 min (not shown) and 180 min. Sulfadiazine was slightly more effective than was azide in this regard. A combination of sulfadiazine and azide had inhibitory effects that were about as effective as azide alone; no potentiation of inhibition was observed. In contrast, azide, sulfadiazine, or a combination of both caused a slight enhancement of the candidacidal activity of MPO-deficient neutrophils. This effect was

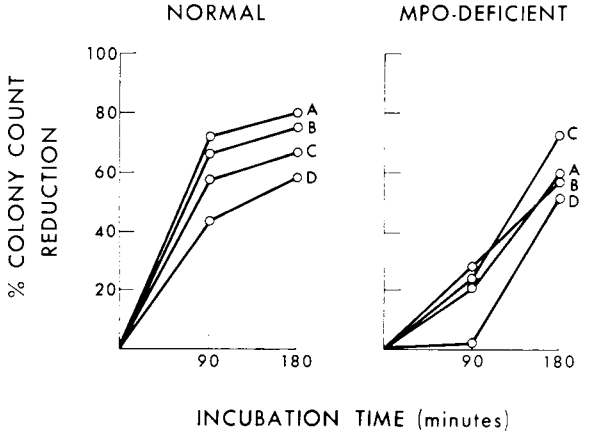

FIGURE 3 Ability of normal and MPO-deficient neutrophils to kill *Torulopsis glabrata*. Four strains of *T. glabrata* (A, B, C, D) were added to mixed peripheral blood leukocytes as described in the text. Colony counts in cell-free control tubes averaged 106.7 and 118.7% of initial values at 90 and 180 min, respectively.

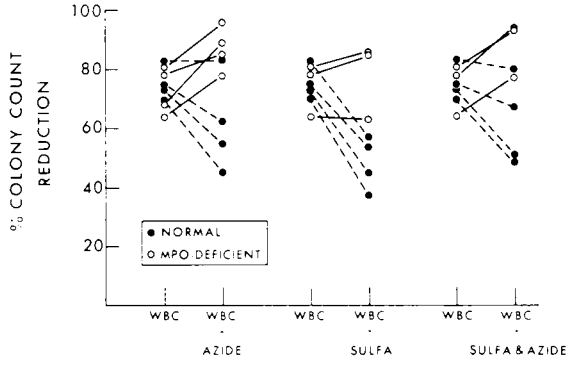

FIGURE 4 Effect of azide (2 mM) and sulfadiazine (4 mM) on the ability of normal and MPO-deficient leukocytes to kill *C. parapsilosis*, strain 12-10. Assays were performed with mixed peripheral blood leukocytes incubated with fungi for 3 hr. After 3 hr, colony counts in cell-free controls were (mean ±SEM) 118.3±22.5% (balanced salt solution), 92.0 ±5.6% (sodium azide), 98.0±7.4% (sulfadiazine), 94.8 ±14.3% (sulfadiazine + azide) of the initial values.

somewhat more prominent after 90 min of incubation (not shown).

Quantitative iodination. Iodination of ingested microorganisms has been proposed as a microbicidal mechanism in the human neutrophil (12). When we measured the ability of MPO-deficient neutrophils to iodinate ingested *C. albicans* cells, we were surprised to observe that a substantial degree of iodination occurred, attaining a mean of 41.2% of normal activity in five separate paired experiments. Three of these experiments, summarized in Table I, show that viable *C. parapsilosis* cells were iodinated by normal and (less well) by MPO-deficient neutrophils. Azide, sulfadiazine, and aminotriazole caused essentially complete inhibition of the iodinating capacity of MPO-deficient leukocytes.

Chronic granulomatous disease. Neutrophils from children with X-linked chronic granulomatous disease of childhood do not kill ingested *C. albicans* in vitro (5, 7). In contrast, their ability to deal with *C. parapsilosis* and *C. pseudotropicalis* is shown in Fig. 5. It is apparent that the neutrophils of the two boys had an appreciable ability to kill *C. pseudotropicalis* and could kill *C. parapsilosis* with normal or nearly normal efficacy. As such affected neutrophils almost certainly develop subnormal intracellular concentrations of H_2O_2, these results suggested that human neutrophils might contain a mechanism other than MPO or endogenously produced H_2O_2 that could kill these *Candida* species.

The results with MPO-deficient neutrophils in Fig. 5 show a higher absolute ability to kill *C. parapsilosis*

TABLE I

Iodination of Candida by Normal and Myeloperoxidase-Deficient Neutrophils

Particle‡	Agent§	Iodination* by MPO-deficient neutrophils	Iodination* by Normal neutrophils
None	None	0.92	0.85
C. albicans	None	2.02	4.06
	Azide	0.10	0.20
	Sulfadiazine	0.13	0.57
	Aminotriazole	0.08	0.26
C. parapsilosis	None	1.21	2.23
	Azide	0.06	0.18
	Sulfadiazine	0.13	0.47
	Aminotriazole	0.07	0.22

* Iodination expressed as nmoles of iodide converted to acid-precipitable form per 1×10^7 neutrophils in 1 hr. Each result is the mean of three paired experiments with a different normal subject and with C. J. B. Within a given experiment, each value is the mean of triplicate samples. (Less than 0.10 nmoles of iodide was "fixed" to *C. albicans* or *C. parapsilosis* in cell-free controls.)

‡ All tubes contained neutrophils and other components as described in the text.

§ Added at the following concentrations: azide and aminotriazole, 2 mM; sulfadiazine, 4 mM.

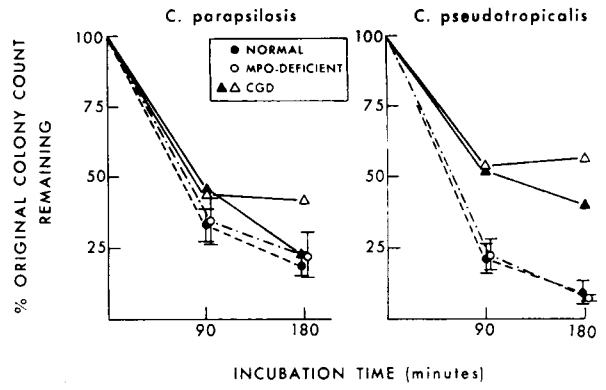

FIGURE 5 Ability of normal, MPO-deficient, and chronic granulomatous disease neutrophils to kill *Candida* species. The ordinate shows the percentage of originally added colonies that remained viable after incubation for specified periods with purified neutrophil populations from seven normal subjects (●), MPO-deficient neutrophils from C. J. B. (four experiments ○), and neutrophils from M. B. and D. D., two children with chronic granulomatous disease (CGD) (△, ▲). In two of these experiments, candidacidal activity was also measured at 45 min with normal and MPO-deficient neutrophils. The mean percentage of *C. parapsilosis* remaining viable at 45, 90, and 180 min in these two experiments was 34.6, 21.9, and 15.4% (normal) and 29.8, 26.2, and 13% (MPO-deficient). In the experiments with M. B. and D. D., 48.5 and 35.4% of *C. parapsilosis* remained viable after 45 min. Cell-free control tubes contained (mean ±SEM) 105.4±5.8% (*C. pseudotropicalis*) and 99.2±5.3% (*C. parapsilosis*) of the original colony counts after 90 min of incubation, and 144.8±14.9% (*C. pseudotropicalis*) and 120.2±7.7% (*C. parapsilosis*) after 3 hr.

after 90 min than is shown in Fig. 1. Although technical considerations may suffice to explain these differences, we cannot exclude alterations in characteristics of the organism or in performance of the MPO-deficient leukocytes during the interval separating the two sets of experiments. Technical differences can be summarized as follows: experiments summarized in Fig. 5 were done 6–8 months later; purified neutrophil populations were used in place of mixed leukocytes; and only one strain of *C. parapsilosis*, strain 12-10, was used after incubation for 2 or 3 days in tryptose phosphate broth, rather than combining the results with two strains that had been incubated for 5 days in Sabouraud's dextrose broth.

Anaerobic conditions. We compared the effects of anaerobic conditions on the ability of normal and MPO-deficient neutrophils to kill *C. parapsilosis*. We reasoned that if either normal or peroxidase-deficient leukocytes were exerting candidacidal activity by virtue of the system operative in the leukocytes of children with chronic granulomatous disease, they might continue to function well under anaerobic conditions. On the other hand, if candidacidal activity depended on the capacity

of either cell type to produce normal or elevated concentrations of H_2O_2, anaerobic conditions should greatly inhibit killing. Anaerobiosis caused a relatively slight decrease in the function of normal leukocytes, similar to that observed in the earlier experiments with azide, but it did not inhibit the function of MPO-deficient leukocytes (Fig. 6).

Sensitivity of the Candida species to hydrogen peroxide. The likelihood that normal or MPO-deficient neutrophils employed H_2O_2 itself to kill these organisms was examined indirectly by studies wherein we determined the susceptibilities of *C. parapsilosis, C. pseudotropicalis,* and the reference strain of *C. albicans* (UC 820) to H_2O_2 (Fig. 7).

DISCUSSION

Previous studies from this laboratory have indicated that the ability of human neutrophils to kill *C. albicans* derives from the operation of a microbicidal system that includes myeloperoxidase and hydrogen peroxide (2, 5, 10). This system, originally described by Klebanoff, is inhibited by azide (1) and sulfadiazine (11), and its operation in intact neutrophils is attended by iodination of the ingested microorganisms (1). It has been suggested that iodination may serve as an actual intraleukocytic microbicidal event mediated by this system (12), but this hypothesis has been seriously challenged (13).

Although Pincus and Klebanoff reported that MPO-deficient leukocytes iodinated ingested *Lactobacillus acidophilus* only about 7.6% (heat-killed organisms) or 16.8% (viable organisms) as effectively as normal leukocytes (9), we found a much smaller abnormality in their ability to iodinate heat-killed *C. albicans.* This iodination, which reached approximately one-half of normal levels, was blocked by aminotriazole, suggesting that it may have been mediated by catalase. Klebanoff has

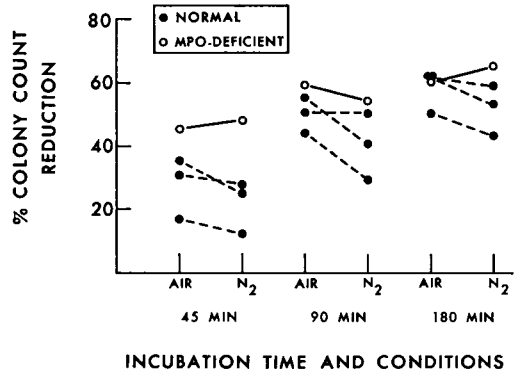

FIGURE 6 Effect of anaerobic conditions on ability of normal and MPO-deficient neutrophils to kill *C. parapsilosis* 12-10. Purified neutrophil populations of three normal subjects (●) and of the MPO-deficient subject C. J. B. (○) were used.

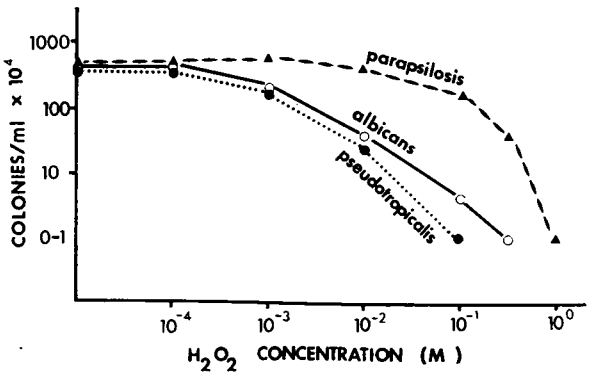

FIGURE 7 Relative susceptibility of *Candida* species to hydrogen peroxide. Absolute values of colony counts after exposure of the *Candida* species to H_2O_2 at pH 5.0 are shown under conditions described in the text. Similar results were obtained at pH 6.0 and 7.0. ▲, *C. parapsilosis;* ○, *C. albicans;* ●, *C. pseudotropicalis.*

shown that mixtures of catalase, H_2O_2, and iodide can catalyze the iodination of microorganisms in vitro and can exert microbicidal activity (14). However, the observation that azide and sulfadiazine blocked the ability of MPO-deficient neutrophils to iodinate ingested *Candida* species, but did not interfere with their ability to kill *C. parapsilosis,* indicates that iodination is not the mechanism of this candidacidal activity.

Was the ability of normal or MPO-deficient neutrophils to kill *C. parapsilosis* a consequence of the direct effects of endogenously generated H_2O_2? This possibility is suggested by recent studies of Klebanoff and Pincus, who reported that the leukocytes of our MPO-deficient patient (C. J. B.) developed a greater than normal increase in activity of the hexose-monophosphate shunt, and in oxidation of formate, after exposure to polystyrene microspheres (4). Although such studies indicate alterations in the activity of pathways that use H_2O_2, they do not necessarily indicate changes in the rate of generation or accumulation of H_2O_2 within MPO-deficient neutrophils; however, we evaluated the possible contribution of H_2O_2 to the ability of MPO-deficient neutrophils to kill certain *Candida* species.

Neutrophils from children with X-linked chronic granulomatous disease have a genetically determined abnormal metabolic response to phagocytosis that includes a markedly reduced ability to generate H_2O_2 (15). That such neutrophils manifest a normal or relatively intact ability to kill *C. parapsilosis* and *C. pseudotropicalis* indicates that these species are susceptible to some microbicidal system that does not depend on the production of supranormal (or even normal) concentrations of H_2O_2. Anaerobic incubation did not decrease the candidacidal activity of normal neutrophils substantially, suggesting that the same system operates in normal neutrophils. If

MPO-deficient neutrophils compensated for their primary abnormality by a secondary increase in the direct effects of H_2O_2, their ability to kill *C. parapsilosis* should have been even more sensitive to anaerobiosis than that of normal neutrophils. In fact, these MPO-deficient neutrophils were less sensitive, and the effect of anaerobic conditions resembled that of azide. Finally, if the hypothesis advanced by Pincus and Klebanoff is correct and serves as the basis of MPO-independent candidacidal activity in the human neutrophil, then the ability of MPO-deficient leukocytes to kill *Candida* species should be in direct proportion to the susceptibility of these fungi to H_2O_2. However, MPO-deficient neutrophils killed *C. parapsilosis* normally, but had little effect on a strain of *C. albicans* that was 50-fold more sensitive to H_2O_2 in a cell-free system.

We conclude, therefore, that a distinct microbicidal system in the human neutrophil exists that is independent of MPO, iodination, or the direct effects of endogenously generated H_2O_2. Narrowly interpreted, these data can provide an explanation for the ability of MPO-deficient neutrophils and those from patients with chronic granulomatous disease to kill certain species of *Candida*. More broadly interpreted, these studies should serve to redirect our attention to the importance of microbicidal systems in the human neutrophil that are not primarily dependent on the remarkable oxidative metabolic events that occur after phagocytosis.

ACKNOWLEDGMENTS

This study was supported by grants CA-11067 and AI-10547 from the U. S. Public Health Service.

REFERENCES

1. Klebanoff, S. J. 1970. Myeloperoxidase: contribution to the microbicidal activity of intact leukocytes. *Science (Wash. D. C.)*. **169**: 1095.
2. Lehrer, R. I., and M. J. Cline. 1969. Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to *Candida* infection. *J. Clin. Invest.* **48**: 1478.
3. Lehrer, R. I., J. Hanifin, and M. J. Cline. 1969. Defective bactericidal activity in myeloperoxidase-deficient human neutrophils. *Nature (Lond)*. **223**: 78.
4. Klebanoff, S. J., and S. H. Pincus. 1971. Hydrogen peroxide utilization in myeloperoxidase-deficient leukocytes: a possible microbicidal control mechanism. *J. Clin. Invest.* **50**: 2226.
5. Lehrer, R. I., and M. J. Cline. 1969. Interaction of *Candida albicans* with human leukocytes and serum. *J. Bacteriol.* **98**: 996.
6. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand. J. Clin. Lab. Invest. Suppl.* **97**: 77.
7. Lehrer, R. I. 1970. Measurement of candidacidal activity of specific leukocyte types in mixed cell populations. I. Normal, myeloperoxidase-deficient, and chronic granulomatous disease neutrophils. *Infect. Immun.* **2**: 42.
8. Stauffer, J. F. 1957. Anaerobic measurements. *In* Manometric Techniques. By W. W. Umbreit, R. H. Burris, and J. F. Stauffer, Burgess Publishing Co., Minneapolis, Minn. 3rd edition. 69.
9. Pincus, S. H., and S. J. Klebanoff. 1971. Quantitative leukocyte iodination. *N. Engl. J. Med.* **284**: 744.
10. Lehrer, R. I. 1969. Antifungal effects of peroxidase systems. *J. Bacteriol.* **99**: 361.
11. Lehrer, R. I. 1971. Inhibition by sulfonamides of the candidacidal activity of human neutrophils. *J. Clin. Invest.* **50**: 2498.
12. Klebanoff, S. J. 1967. Iodination of bacteria: a bactericidal mechanism. *J. Exp. Med.* **126**: 1063.
13. McCall, C. E., L. R. DeChatelet, M. R. Cooper, and P. Ashburn. 1971. The effects of ascorbic acid on bactericidal mechanisms of neutrophils. *J. Infect. Dis.* **124**: 194.
14. Klebanoff, S. J. 1970. Myeloperoxidase-mediated antimicrobial systems and their role in leukocyte function. *In* Biochemistry of the Phagocytic Process: Localization and the Role of Myeloperoxidase and the Mechanism of the Halogenation Reaction. J. Schultz, editor. North Holland Publishing Co., Amsterdam. 89.
15. Holmes, B., A. R. Page, and R. A. Good. 1967. Studies of the metabolic activity of leukocytes from patients with a genetic abnormality of phagocytic function. *J. Clin. Invest.* **46**: 1422.