

Leukocyte Myeloperoxidase Deficiency and Disseminated Candidiasis: the Role of Myeloperoxidase in Resistance to *Candida* Infection

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ABSTRACT The neutrophils and monocytes of a patient with disseminated candidiasis were found to lack detectable levels of the lysosomal enzyme myeloperoxidase (MPO), although they had normal levels of other granule-associated enzymes. Leukocytes from one of the patient's sisters also lacked detectable MPO; leukocytes from his four sons contained approximately one-third of mean normal peroxidase levels. Neither the patient nor his affected relatives had experienced frequent or unusual bacterial infections.

The phagocytic activity of the patient's MPO-deficient neutrophils was intact, and the cells displayed normal morphologic and metabolic responses to phagocytosis. In contrast to normal leukocytes which killed $30.5 \pm 7.3\%$ of ingested *Candida albicans* in 1 hr, however, the patient's neutrophils killed virtually none. His leukocytes also failed to kill the strain of *C. albicans* recovered from his lesions, as well as other *Candida* species. These MPO-deficient neutrophils killed *Serratia marcescens* and *Staphylococcus aureus* 502A at an abnormally slow rate, requiring 3–4 hr to achieve the bactericidal effect attained by normal leukocytes after 45 min. No other abnormalities in his cellular or humoral immune responses were demonstrated.

These findings suggest that hereditary MPO deficiency is transmitted as an autosomal recessive characteristic, that the homozygous state conveys enhanced susceptibility to disseminated candidiasis, and that MPO is necessary for candidacidal activity in human neutrophils.

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Although lending support to the suggested bactericidal role of MPO in leukocytes, the data indicate that alternative bactericidal mechanisms, effective in the absence of MPO, are functionally dominant in the human neutrophil.

INTRODUCTION

Normal neutrophils contain the lysosomal enzyme myeloperoxidase (MPO) in amounts that may approximate 5% of their dry weight (2–4). First detected in pus over a century ago (5), MPO has since been purified and extensively studied (6, 7); however, its metabolic functions are incompletely defined. The enzyme may protect neutrophils from the deleterious effects of hydrogen peroxide (8), may participate in the oxidative catabolism of uric acid (9), and may inactivate certain bacterial toxins (10). Also, by participating in the oxidation of reduced triphosphopyridine nucleotide, it has been suggested as a regulator of the hexose monophosphate shunt stimulation that follows particle ingestion by neutrophils (11, 12). Finally, substantial evidence suggests that MPO participates in the antibacterial activities of the neutrophil (13–16).

This report describes a patient with disseminated candidiasis whose neutrophils and monocytes lacked MPO, although his eosinophils and their precursors had peroxidase activity. This pattern of peroxidase deficiency has been reported previously in only three subjects, otherwise normal, two of whom were brother and sister (17, 18). Morphologic, biochemical, and functional studies on the leukocytes of the patient and available family members confirmed the genetic nature of the defect, clarified its mode of transmission, and allowed appraisal

of some of the suggested biochemical and functional contributions of MPO to the intracellular economy of the neutrophil.

METHODS

Case report

C. J. B., a 49 yr old white man, was hospitalized at the University of California Medical Center during a 4 wk period in January–February 1968. In December 1964, he had been admitted to a hospital elsewhere because of hemoptysis and an infiltrate in the middle lobe of the right lung. The lobe was excised and histologically showed acute and chronic inflammatory changes without significant numbers of bacteria or fungi; no cultures were made. Diabetes mellitus was detected, and treatment with oral hypoglycemic agents was begun. In October 1967, he was admitted to a hospital because of increasing fatigue, a gradual loss of 50 lb of weight, and recent shoulder pain. A nontender subcutaneous mass on his right scapula was biopsied and proved to be an abscess overlying an osteomyelitic lesion. The biopsy specimens grew *Candida albicans* in pure culture. In January 1968, he was referred to the Medical Center with a diagnosis of suspected systemic candidiasis.

A review of the family history indicated that none of the patient's relatives have had frequent or unusual infections. His mother, a diabetic, had died of a brain neoplasm at 62 yr of age. His father, two sisters, and four sons are in good health. There is no known consanguinity.

At the age of 6 yr, the patient had an episode of acute polyarthritis, which lasted about a week. Diagnosed as acute rheumatic fever and treated with bed rest, it resolved spontaneously and did not recur. At the age of 12, he lacerated a finger while playing football; a subungual infection and lymphangitis developed, which responded to local measures. Aside from these illnesses, the usual childhood exanthemata, and occasional upper respiratory infections, the patient had been well and did not suffer from frequent or unusual infections until the events of 1964 and 1967.

At the time of admission, the patient appeared chronically ill. Temperature, blood pressure, and pulse were normal. He had mild oral thrush, but his skin and nails appeared normal. The biopsy incision had dehiscd, and the tip of the right scapula was visible beneath a large ulcer. On cardiac examination a soft basal systolic murmur was audible. The physical examination was otherwise unremarkable. X-ray films of the chest showed evidence of prior lobectomy and atelectasis and bronchiectasis of the upper lobe of the right lung. Pulmonary function studies indicated moderate restrictive lung disease. No abnormalities were found by bronchoscopic examination, lymphangiography, or percutaneous biopsy of liver and bone marrow.

The total leukocyte count was 16,500/mm³, with 64% neutrophils, 19% lymphocytes, 12% monocytes, and 5% eosinophils. Results of the following determinations were within normal limits: hemoglobin, hematocrit, serum creatinine, creatinine clearance, blood urea nitrogen, serum electrolytes, uric acid, bilirubin, cephalin flocculation, prothrombin time, and erythrocyte triiodothyronine uptake. Fasting blood glucose levels ranged from 160 to 220 mg/100 ml. Sulfobromophthalein retention was 7% at 45 min. Serum alkaline phosphatase was 7.6 Bodansky units; the acid phosphatase level was normal. The serum total protein was 8.1 g/100 ml, with albumin 3.9 g, α_1 -globulin 0.32 g, α_2 -globulin 1.1 g, β -globulin 1.3 g, and γ -globulin 1.5 g/100 ml.

Serum immunoelectrophoresis showed a slight increase in IgG, IgM, and IgA levels. Chromosomal studies of peripheral blood and bone marrow leukocytes (19) showed a normal complement of 46 chromosomes; no abnormal chromosomes or aneuploid cells were seen. *C. albicans* was identified in cultures of material from oral swabs and specimens of stool, urine, and sputum. Cultures of blood, bronchial washings, and biopsy specimens of liver and bone marrow showed no growth.

A week after admission, a painful mass developed in the extensor muscles of the patient's left forearm. The mass was incised and exuded yellow pus which grew *C. albicans* in pure culture. Intravenous administration of amphotericin B, 270 mg, over a 3 wk period resulted in complete healing of the forearm abscess and partial healing of the scapular ulcer.

The patient was discharged in February 1968 and seen subsequently on an outpatient basis. He received 30 mg of amphotericin B once a week for 5 months and then once every other week for 3 months. In all, he received 1200 mg of amphotericin without untoward effects. He has resumed work, felt well, and regained 32 lb of weight; the diabetes has remained under good control with oral hypoglycemic agents. The scapular ulcer has healed completely, he no longer has thrush, and periodic urine cultures have been negative for *C. albicans*. He is still being observed closely for evidence of recurring infection.

Special studies

Skin tests and contact sensitization. Intradermal skin tests were done with standard antigens. Dermatophytin-O (Hollister-Stier Laboratories Inc., Spokane, Wash.) was used for *C. albicans* testing. Hypersensitivity to 2,4-dinitrochlorobenzene was tested 1 month after initial sensitization by the method of Epstein (20).

Lymphocyte transformation. The response of lymphocytes *in vitro* to tetanus toxoid and Dermatophytin-O was assessed by a previously described technique (21). Undiluted Dermatophytin-O, dialyzed overnight against saline to remove the phenol preservative, was added to the cultures in final dilutions of 1:5 to 1:150. After 5 days of incubation, thymidine-¹⁴C was added; the cultures were then incubated for an additional 24 hr. The cells were harvested and cell transformation and thymidine incorporation were determined.

Electron microscopy. Peripheral blood leukocytes were separated by dextran sedimentation and centrifugation (22) fixed overnight in 3% distilled glutaraldehyde buffered to pH 7.4 with sodium cacodylate, postfixed in 1% osmium tetroxide (23), dehydrated in ethanol, and embedded in Araldite (Ciba Products Co., Fair Lawn, N. J.) Ultrathin sections were stained with uranyl acetate and lead citrate. Electron micrographs were made with a Siemens Elmiskop IA (Siemens Co., Karlsruhe, Germany).

Histochemical staining. Peripheral blood and bone marrow preparations were stained for alkaline phosphatase by the method of Climie, Heinrichs, and Foster (24) and for acid phosphatase by the method of Gomori, as described by Pearse (25). Staining with Sudan black B and periodic acid-Schiff (PAS) was done by standard techniques (25). Staining for peroxidase was done by the method of Goodpasture, as modified by Beacom (26).

Biochemical enzyme determinations. Leukocytes were separated from heparinized venous blood by dextran sedimentation, and the red cells were removed by differential hypotonic lysis (27). The cells were washed in phosphate-

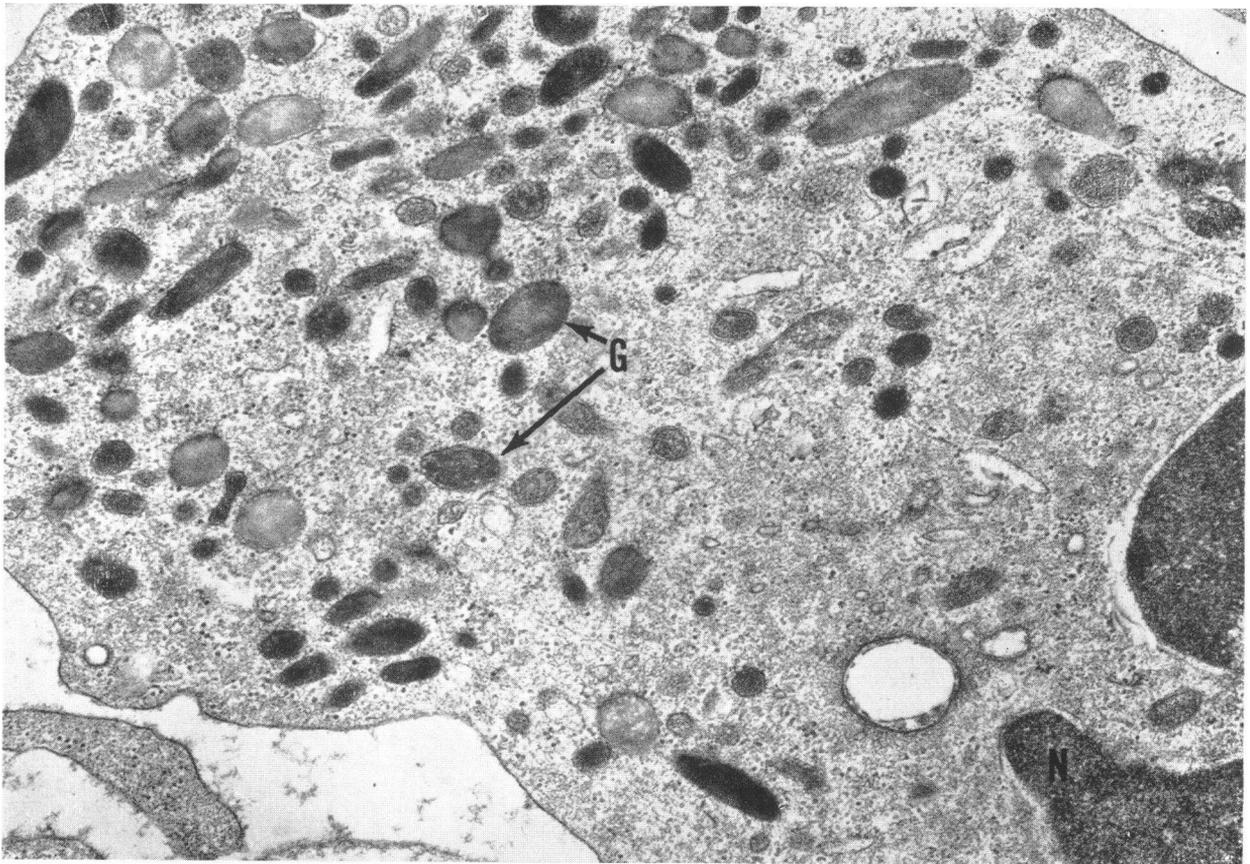


FIGURE 1 Patient's polymorphonuclear leukocyte. The granules (*G*) appear normal in number and configuration. *N*, nucleus. $\times 30,400$.

buffered saline and homogenized for 60 sec in 0.01 M acetate buffer, pH 3.8. The homogenate was centrifuged at 20,000 *g* for 20 min. The supernatant fraction was assayed for lysozyme, cathepsin, and ribonuclease by the method of Cohn and Hirsch (28) and for MPO by a modification of the method of Klebanoff (29), with *o*-anisidine as substrate. D-amino acid oxidase (DAAO) activity was measured by a previously described technique (30). Protein concentrations were determined by the method of Lowry, Rosebrough, Farr, and Randall (31).

Metabolic studies. Leukocytes were separated by dextran sedimentation and freed of red cells as described above. The cells were suspended in Eagle's minimal essential medium containing 20% AB serum at a concentration of 2×10^7 /ml. Production of $^{14}\text{CO}_2$ from glucose-1- ^{14}C and the incorporation of uridine- ^3H into ribonucleic acid (RNA) of resting and phagocytic leukocytes was determined as described previously (32), utilizing polystyrene particles (1.3 μ in diameter, 1 mg/ml). Leukocyte oxygen consumption before and after the addition of the test particles was measured at 37°C in a Gilson model KM oxygraph with a Clark electrode (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) calibrated against the air-saturated suspending medium. Studies were terminated 30 min after the addition of the test particles unless otherwise noted.

Leukocyte mobilization. Leukocyte migration was studied by the procedure of Rebeck and Crowley (33).

Candida serology. Serum from the patient and from 10 normal control subjects was tested for the presence of agglutinins to *C. albicans* B311 as described by Preisler, Hasenclever, Levitan, and Henderson (34).

Phagocytosis and microbicidal activity. Strains of *C. albicans*, *C. stellatoidea*, *C. tropicalis*, *C. krusei*, *Geotrichum candidum*, *Saccharomyces cerevisiae*, and a *Rhodotorula* species were maintained on Sabouraud's 2% dextrose agar slants. Fungi to be used in phagocytic or candidacidal assays were inoculated into Sabouraud's 2% dextrose broth, cultured for 3-7 days at 33°C, washed with Hanks' balanced salt solution (BSS), and counted with a hemocytometer. Leukocytes obtained by sedimenting heparinized venous blood with dextran (22) were washed twice with BSS containing 20% fetal calf serum and 5 USP units of heparin per ml and then counted in a hemocytometer. For assessment of phagocytosis, equal numbers of neutrophils and fungi, 2.5×10^6 , were suspended in 1 ml of BSS containing 25% normal AB serum and incubated in rotating (30 rpm) polyethylene tubes at 37°C. Samples of the incubation mixtures were taken at intervals, prepared on glass slides in a Shandon cytocentrifuge (Shandon Scientific Company, Ltd., Willesden, London), fixed with methanol, and stained with Wright's or Giemsa's stain. Leukocyte candidacidal activity was assayed by a differential staining technique employing 2×10^{-4} M methylene blue to discriminate between viable and nonviable *Candida* (35).

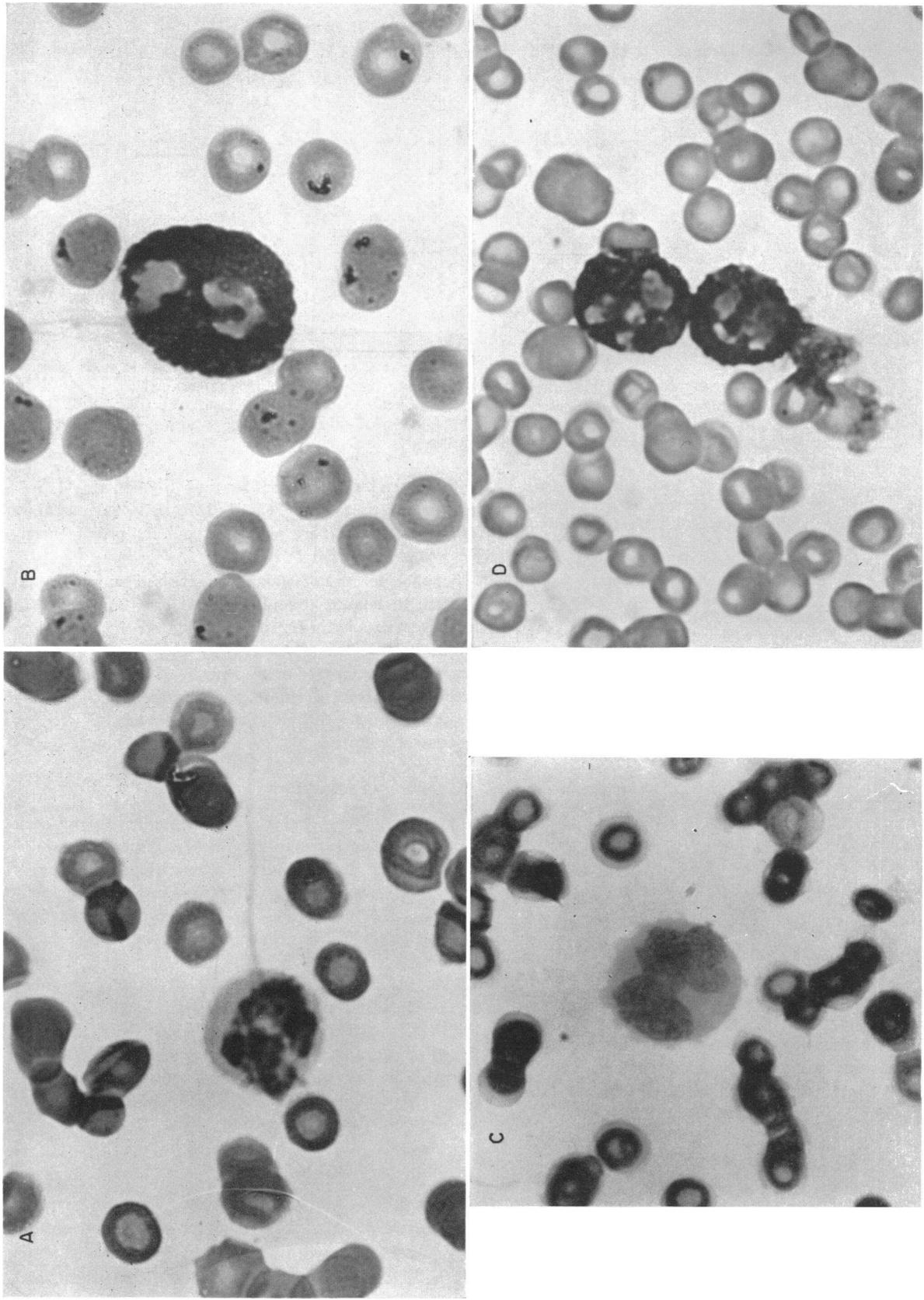


FIGURE 2 Histochemically stained preparation of patient's peripheral blood leukocytes. Neutrophil (A) and monocyte (C) are completely lacking in peroxidase activity; eosinophil (B) shows normal peroxidase activity. Similarly stained preparation of normal granulocytes (D) is shown for comparison (modified Goodpasture's stain). $\times 830$.

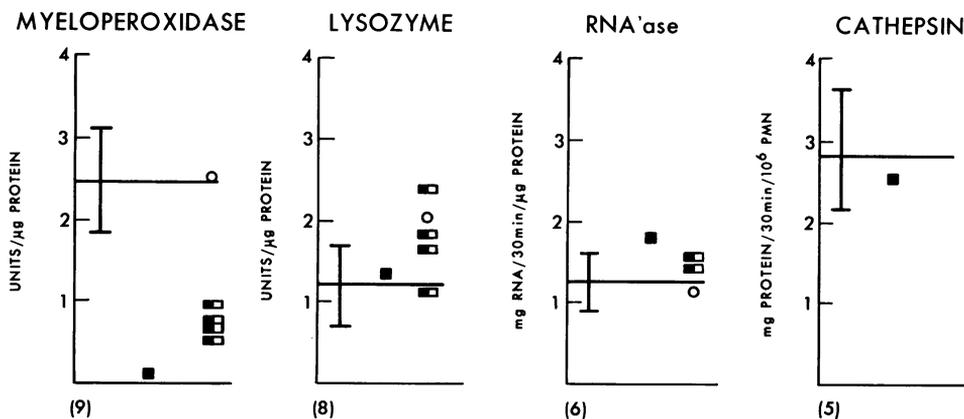


FIGURE 3 Biochemically determined enzyme activity of homogenates of leukocytes from normal subjects, myeloperoxidase-deficient patient C. J. B. (closed square), his wife (open circle), and sons (half-opened and half-closed rectangles). Number of normal subjects studied is shown in parentheses. PMN, polymorphonuclear neutrophilic leukocytes.

Bactericidal activity was measured¹ by a slight modification of the method of Hirsch and Strauss (36). *Staphylococcus aureus* 502A and a chromogenic strain of *Serratia marcescens* were grown for 18 hr at 37°C in nutrient broth,² washed twice with bicarbonate-free BSS containing 0.1% gelatin (BSS-gel), and diluted to an appropriate optical density in BSS-gel. The final assay mixture contained 8% fresh-frozen normal AB serum, 5×10^6 neutrophils, and from 0.5 to 1.0×10^8 colony-forming bacterial units in 1 ml of BSS. The mixtures, in small polyethylene tubes, were incubated at 37°C with rotation (30 rpm). At intervals, six to eight replicate samples were taken, diluted with distilled water containing 0.01% human serum albumin, and plated on Trypticase soy agar (Baltimore Biological Laboratories, Baltimore, Md.). Neither test organism was killed by BSS-serum controls.

In additional studies, cultures of *C. albicans* or *Escherichia coli* ATCC-1175 were washed twice with BSS-gel, incubated with the patient's leukocytes for 30 min at 37°C, and prepared for electron microscopy as described.

RESULTS

Immunologic studies

The patient could manifest delayed hypersensitivity, as indicated by positive reactions to skin tests with mumps antigen and Dermatophytin-O. Skin tests for histoplasmin, coccidioidin, and tuberculosis (PPD) were negative. Dinitrochlorobenzene challenge 1 month after the initial sensitization elicited a positive reaction. The patient's serum contained agglutinins to *C. albicans* B311 at a titer of 1:64, in contrast to 10 normal control sera whose agglutinin titers were 1:16 or less. 2 wk after immunization with typhoid-paratyphoid vaccine, the pa-

tient's serum antibody titer against *Salmonella* H, initially positive at 1:40, was 1:160, and titers against *Salmonella* O and paratyphoid A and B, initially negative, were positive at 1:160.

Lymphocyte transformation increased normally in response to tetanus toxoid and to Dermatophytin-O in vitro. Dermatophytin-O, in a dilution of 1:15, resulted in morphologic transformation of 11% of the patient's lymphocytes, compared with a mean of less than 4% in unstimulated control cultures; thymidine incorporation was 365 dpm/ 3×10^6 cells, compared with 47 dpm in the control cultures.

Morphologic studies

Structure. No morphologic abnormalities were seen in the patient's neutrophils by light microscopic examination of Romanowsky-stained preparations or by direct phase microscopy. By electron microscopy, nuclear configuration and cytoplasmic morphology appeared normal, and granules were normal in number, size, shape, and density (Fig. 1).

Histochemistry. Alkaline phosphatase, acid phosphatase, PAS, and Sudan black B stains of the patient's peripheral blood were indistinguishable from similarly stained preparations of normal cells. No peroxidase was demonstrable in his neutrophils and monocytes, although eosinophils and their bone marrow precursors had abundant peroxidase activity (Fig. 2).

Biochemical studies

The results of biochemical enzyme determinations on homogenates of leukocytes from the patient and normal subjects are compared in Fig. 3. As shown, the patient's cells had normal levels of lysozyme, ribonuclease, and

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²Bacto Tryptic Soy Broth, Difco Laboratories, Inc., Detroit, Mich.



FIGURE 4 Patient's polymorphonuclear leukocyte incubated 30 min with *Escherichia coli*. Multiple bacteria (B) are contained within a large phagocytic vacuole adjacent to the nucleus (N). Several granules are seen within a smaller phagocytic vacuole (arrow) containing a single bacterium. Cytoplasmic granules are decreased in number as compared with his nonphagocytic neutrophils. $\times 17,100$.

cathepsin. The DAAO activity of his leukocytes was 2.73×10^{-5} μ moles of O_2 per sec per mg protein (normal, $0.7-5.3 \times 10^{-5}$ μ moles). The peroxidase activity of homogenates of his leukocytes, however, was less than 5% of the mean normal level, a finding compatible with the 3-6% eosinophil contamination of the leukocyte preparations.

The metabolic response to phagocytosis of polystyrene particles was determined in studies on leukocytes from the patient and from eight normal subjects. The patient's cells responded to particle ingestion by increasing their rate of oxygen consumption to 227% of their basal rate (normal, $243 \pm 33\%$ of basal oxygen consumption). After phagocytosis, oxidation of glucose-1- ^{14}C increased to 850% of control levels (normal, $1540 \pm 390\%$ of control levels), and incorporation of uridine- 3H increased to 232% of control levels (normal $184 \pm 21\%$ of control levels).

Functional studies

Migration of the patient's leukocytes into a "Rebuck skin window" occurred normally.

The patient's leukocytes phagocytized all *Candida* species tested, as well as *G. candidum*, *S. cerevisiae*, and *Rhodotorula*, as rapidly and as completely as normal control cells, ingesting all added organisms within 15 min. Phagocytosis was associated with normal vacuole formation as assessed by examination of stained smears and by direct phase microscopy. The occurrence of vacuole formation and degranulation after phagocytosis of *E. coli* (Figs. 4 and 5) and *C. albicans* (Fig. 6) was confirmed by electron microscopy.

The results of candidacidal assays on leukocytes from the patient and normal control subjects are compared in Table I. With the assay system employed, normal neutrophils kill $30.5 \pm 7.3\%$ of ingested *C. albicans* in 1 hr; extending the incubation period to 3 hr does not result in increased killing (1, 35). In 11 determinations with

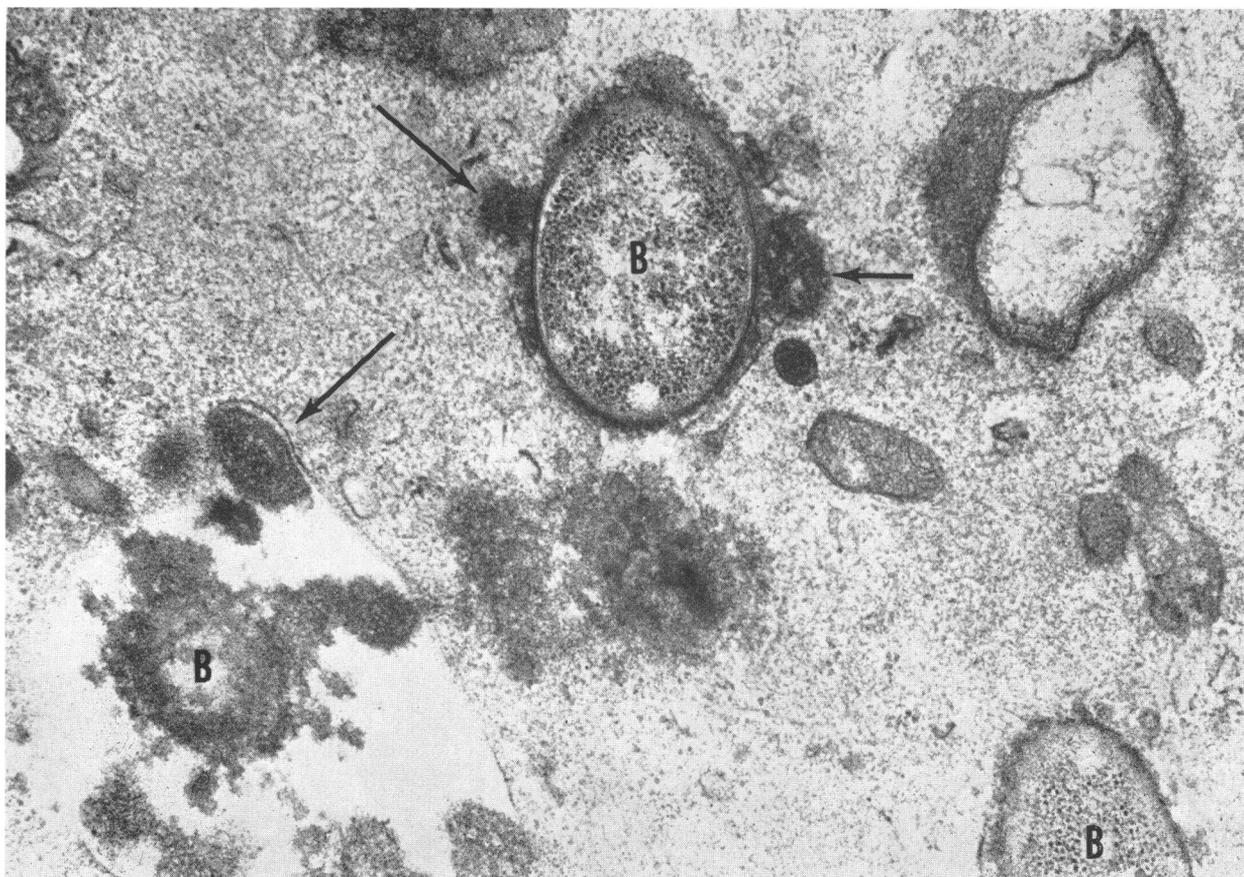


FIGURE 5 Higher magnification of phagocytized bacteria (*B*) within patient's polymorphonuclear leukocyte. The arrows indicate lysosomal granules adjacent to and within the phagocytic vacuole. $\times 42,500$.

autologous serum or serum from each of four normal AB donors, the patient's neutrophils killed only $0.1 \pm 0.2\%$ of ingested *C. albicans* at 1 hr, and little or no additional killing was observed after 3 hr.³ Similar results were obtained in assays with the four other strains of *Candida* tested, including the strain of *C. albicans* recovered from the patient's abscess (Table I). Normal cells assayed in the patient's serum were able to phagocytize and kill *Candida* normally.

The bactericidal activity of leukocytes from the patient and normal control subjects is compared in Table II. For studies made between April and June 1968, blood was obtained just before the administration of his weekly dose of amphotericin B. The studies of March 1969

³ *C. albicans*, when incubated in BSS containing serum, forms pseudo-germ tubes after 90–120 min, making it virtually impossible to do precise microscopy and differential counting. This problem can be circumvented by using *C. tropicalis*, which remains in yeast phase during the incubation. In a study with *C. tropicalis*, the patient's cells killed 1.3% of the ingested organisms after 3 hr.

were performed 4 months after the completion of amphotericin B therapy. All the assays gave comparable results. The patient's MPO-deficient leukocytes killed *S. marcescens* and *S. aureus* 502A at an abnormally slow rate, requiring 3–4 hr to accomplish the extent of killing attained by normal leukocytes after 45 min. Phagocytosis of both organisms, as judged by examination of stained smears of incubation mixtures with varying bacteria: neutrophil ratios, occurred in a comparable manner in MPO-deficient and normal human leukocytes. Differential centrifugation studies revealed that over 90% of the organisms added to the patient's leukocytes were cell associated within 15 min. The intracellular location of a major fraction of the cell-associated *Serratia* was confirmed by the ability of the organism to resist an antibiotic exposure (50 μ g of streptomycin and 50 U of penicillin G per ml for 10 min) which reduced the viable count of extracellular organisms over 100-fold.⁴

⁴ Lehrer, R. I., J. Hanifin, and M. J. Cline. Unpublished observations.

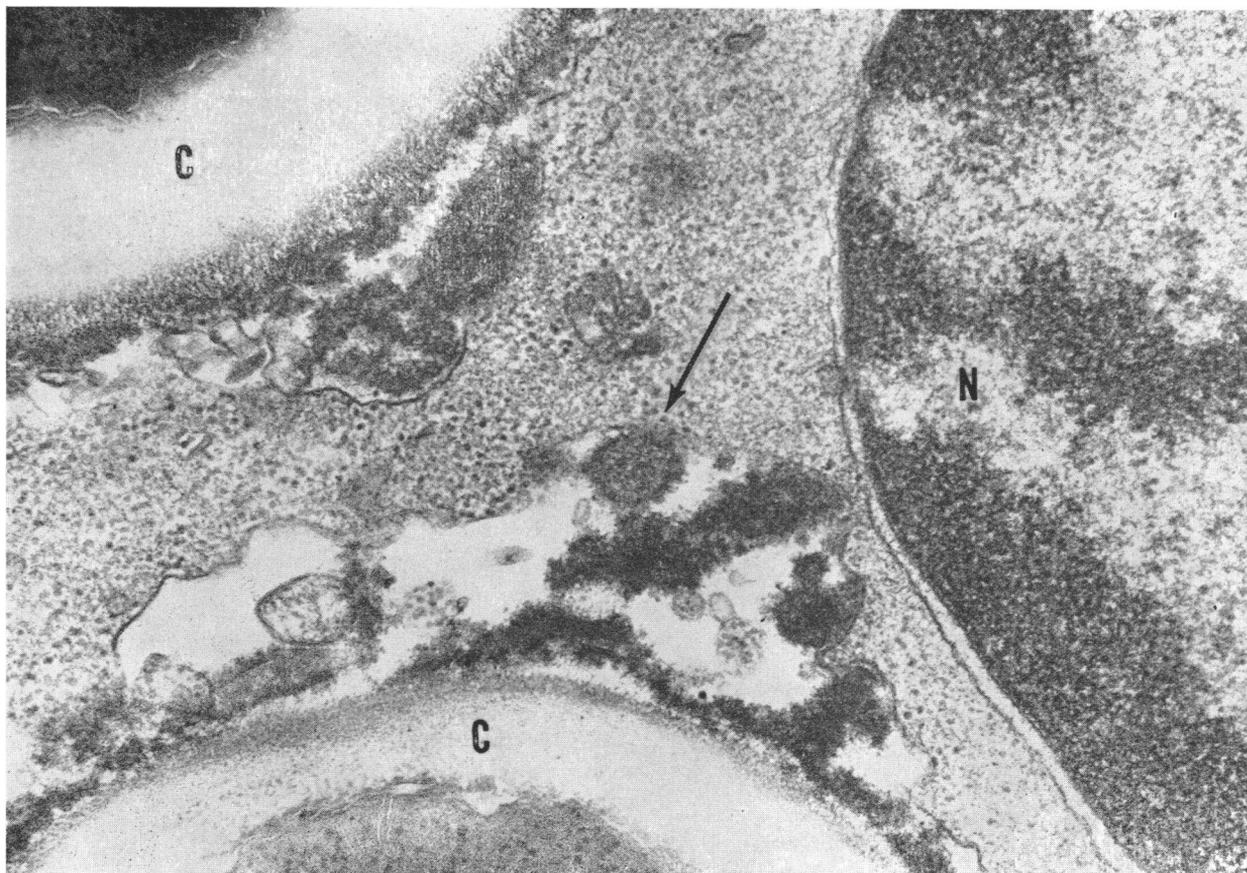


FIGURE 6 Patient's polymorphonuclear leukocyte 30 min after incubation with *Candida albicans*. The thick-walled *Candida* (C) are within phagocytic vacuoles containing electron-opaque material derived from the leukocyte. The arrow indicates a lysosomal granule entering the vacuole. N, nucleus. $\times 51,300$.

Family studies

Leukocytes from three of the patient's four sons were assayed with the test strain of *C. albicans* and showed normal candidacidal activity. The peroxidase activity of homogenates of leukocytes from the four sons ranged from 22 to 38% of the mean normal level. By histochemical staining, the neutrophils of all four showed a uniform distribution of peroxidase activity. Leukocyte lysozyme and ribonuclease levels were normal in all family members tested (Fig. 3).

The peroxidase activity of leukocytes from the patient's two sisters and his father was determined by histochemical staining only. Stained smears of the peripheral blood of one sister were indistinguishable from those of the patient; peroxidase activity was not present in her neutrophils and monocytes but was present in her eosinophils. Peroxidase was histochemically demonstrable in the neutrophils and monocytes of the father and the other sister. Confirmation that they, like the

four sons, are heterozygous must await biochemical measurements of peroxidase activity.

TABLE I
Candidacidal Activity of Myeloperoxidase (MPO)-Deficient Leukocytes from Patient C. J. B. and Normal Leukocytes

Organism	Per cent of ingested <i>Candida</i> killed in 1 hr	
	MPO-deficient leukocytes	Normal leukocytes
<i>Candida albicans</i>	0.1 \pm 0.2 (11)*	30.5 \pm 7.3 (60)*
<i>C. albicans</i> , recovered from patient's lesions	0	43.9
<i>C. stellatoidea</i>	0	25.5
<i>C. tropicalis</i>	0	25.8
<i>C. krusei</i>	2.0	48.3

* Results are given as the mean \pm 1 SD of the mean; numbers in parentheses refer to number of studies on patient's leukocytes or number of different normal subjects studied.

TABLE II
Bactericidal Activity of Normal and Myeloperoxidase-Deficient Leukocytes (Patient C. J. B.)*

Leukocyte donor	Dates of studies	Bacteria tested	Per cent reduction in colony count at			
			45 min	90 min	180 min	240 min
Normal subjects	April-June 1968	<i>Serratia marcescens</i>	96.7 ±1.6 (5)	99.3 (2)	NT	NT
C. J. B.		<i>S. marcescens</i>	41.7 ±10.3 (5)	76.1 ±7.6 (3)	96.9 ±1.2 (3)	NT
Normal subjects	March 1969	<i>S. marcescens</i>	98.9 (1)	99.5 (1)	99.9 (1)	99.8 (1)
C. J. B.		<i>S. marcescens</i>	56.6 (1)	75.3 (1)	93.4 (1)	94.7 (1)
Normal subjects	April-June 1968	<i>Staphylococcus aureus</i> 502A	91.8 ±2.2 (4)	93.0 (1)	NT	NT
C. J. B.		<i>S. aureus</i> 502A	36.0 ±9.1 (5)	43.6 ±9.7 (3)	79.7 (2)	91.8 (2)

* These results are expressed as the per cent reduction of initial colony counts at the specified time. The figure in parentheses indicates either the number of normal subjects or the number of experiments with C. J. B.'s leukocytes. When three or more experiments were performed, data are expressed as mean ±SEM. When two experiments were performed, the arithmetic mean is shown. NT signifies not tested.

DISCUSSION

In this report, we describe a patient whose normally phagocytic neutrophils could kill neither the strain of *C. albicans* recovered from his lesions nor several other *Candida* species. These functionally defective neutrophils lacked the lysosomal enzyme MPO.

The presence of peroxidase activity in the patient's eosinophils, despite its complete absence in his neutrophils and monocytes, suggests that eosinophil peroxidase (EPO) and MPO are under separate genetic control in man. This inference is strengthened by the fact that, unlike neutrophils, the eosinophils of several mammalian species lack peroxidase (4, 37). Other observations suggest that EPO and MPO differ in chemical structure (38, 39). In recent immunologic and spectrophotometric investigations,⁵ we found normal EPO and MPO to be immunologically distinct and demonstrated that our patient's neutrophils also lack MPO detectable by these analytic techniques. The family studies reported here, as well as histochemical observation in family members of the three subjects with hereditary MPO deficiency (17, 18), support an autosomal recessive mode of transmission for this condition.

Systemic candidiasis is representative of those opportunistic mycoses that may occur as a complication of major underlying disorders such as leukemia (40) or lymphoma (41, 42). Often the affected patients have been leukopenic or have received antibiotic, immunosuppressive, or cytotoxic therapy (40, 43, 44). Our patient had taken no drugs other than tolbutamide, prescribed for asymptomatic adult-onset diabetes mellitus. Despite intensive physical, laboratory, and radiologic examinations performed during his initial hospitalization

and the ensuing 14 months of study and treatment, we have detected no other disorder. We found no abnormalities other than MPO deficiency either in our patient's neutrophils or in his cellular and humoral immune responses. Did the *Candida* infection result from a specific susceptibility engendered by lack of leukocyte MPO? This question cannot be answered definitively until all the factors involved in resistance to systemic candidiasis are known. That the simultaneous occurrence of systemic candidiasis, defective neutrophil candidacidal activity, and hereditary MPO deficiency was on the basis of chance alone, however, seems unlikely.

MPO has been demonstrated to exert bactericidal (14, 15) and candidacidal (45) activity in the presence of hydrogen peroxide and suitable halides. Conditions inhibitory to the function of MPO have been shown to induce bactericidal and candidacidal defects in normal neutrophils (1, 16). On the basis of these observations, we believe that the deficient candidacidal activity of our patient's neutrophils was attributable to their deficiency of MPO.

The ability of our patient to escape significant bacterial infection for over four decades, the continued well being of his sister and the reported good health of the three previously described subjects with MPO deficiency (17, 18) seem incompatible with suggestions that MPO is the major mediator of neutrophil bactericidal function in man. Our patient's MPO-deficient cells killed *S. aureus* 502A and *S. marcescens* at distinctly subnormal rates; however, killing of the ingested bacteria eventually approached completion.⁶ These observations indicate the presence of alternative bactericidal mechanisms effective in the absence of MPO and suggest that

⁵ Salmon, S., J. Schultz, M. J. Cline, and R. I. Lehrer. Unpublished observations.

⁶ Lehrer, R. I., J. Hanifin, and M. J. Cline. Defective bactericidal activity in myeloperoxidase-deficient human neutrophils. *Nature (London)*. In press.

MPO may serve an ancillary role in intracellular bactericidal events in the human neutrophil. Additional studies of the bactericidal activity of our patient's neutrophils will be presented elsewhere.⁶

Relatively little is known of the fungicidal armaments of mammalian leukocytes. Zeya and Spitznagel (46) have isolated a family of cationic proteins with bactericidal and candidacidal activity from guinea pig and rabbit neutrophils. Our data suggest that MPO may be the major determinant of candidacidal activity in the human neutrophil. Occasional reports have associated leukocyte peroxidase deficiencies with other disorders (47-50). With time it should be possible to evaluate the relative significance of hereditary MPO deficiency as a predisposing cause of systemic candidiasis or other infections in man.

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REFERENCES

1. Lehrer, R. I., and M. J. Cline. 1968. Absent myeloperoxidase and leukocyte candidacidal activity in a patient with systemic candidiasis. *Clin. Res.* **16**: 331. (Abstr.)
2. Agner, K. 1941. Verdoperoxidase: a ferment isolated from leucocytes. *Acta Physiol. Scand.* **2** (Suppl. 8): 1.
3. Schultz, J., and K. Kaminker. 1962. Myeloperoxidase of the leucocyte of normal human blood. I. Content and localization. *Arch. Biochem. Biophys.* **96**: 465.
4. Schultz, J., R. Corlin, F. Oddi, K. Kaminker, and W. Jones. 1965. Myeloperoxidase of the leucocyte of normal human blood. III. Isolation of the peroxidase granule. *Arch. Biochem. Biophys.* **111**: 73.
5. Klebs, E. 1868. Die pyrogene Substanz. *Zentralbl. Med. Wiss.* **6**: 417.
6. Agner, K. 1958. Crystalline myeloperoxidase. *Acta Chem. Scand.* **12**: 89.
7. Schultz, J., and H. W. Shmukler. 1964. Myeloperoxidase of the leucocyte of normal human blood. II. Isolation, spectrophotometry, and amino acid analysis. *Biochemistry*. **3**: 1234.
8. Rechcigl, M., Jr., and W. H. Evans. 1963. Role of catalase and peroxidase in the metabolism of leucocytes. *Nature (London)*. **199**: 1001.
9. Howell, R. R., and J. E. Seegmiller. 1962. Uricolysis by human leucocytes. *Nature (London)*. **196**: 482.
10. Agner, K. 1950. Studies of peroxidative detoxification of purified diphtheria toxin. *J. Exp. Med.* **92**: 337.
11. Roberts, J., and J. H. Quastel. 1964. Oxidation of reduced triphosphopyridine nucleotide by guinea pig polymorphonuclear leukocytes. *Nature (London)*. **202**: 85.
12. Sbarra, A. J., and M. L. Karnovsky. 1959. The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. *J. Biol. Chem.* **234**: 1355.
13. Klebanoff, S. J. 1967. A peroxidase-mediated antimicrobial system in leukocytes. *J. Clin. Invest.* **46**: 1078. (Abstr.)
14. Klebanoff, S. J. 1967. Iodination of bacteria: a bactericidal mechanism. *J. Exp. Med.* **126**: 1063.
15. Klebanoff, S. J. 1968. Myeloperoxidase-halide-hydrogen peroxide antibacterial system. *J. Bacteriol.* **95**: 2131.
16. McRipley, R. J., and A. J. Sbarra. 1967. Role of the phagocyte in host-parasite interactions. XII. Hydrogen peroxide-myeloperoxidase bactericidal system in the phagocyte. *J. Bacteriol.* **94**: 1425.
17. Grignaschi, V. J., A. M. Sperperato, M. J. Etcheverry, and A. J. L. Macario. 1963. Un nuevo cuadro citoquímico: negatividad espontánea de las reacciones de peroxidasa, oxidasas y lípido en la progenie neutrofila y en los monocitos de dos hermanos. *Rev. Asoc. Med. Argent.* **77**: 218.
18. Undritz, E. 1968. Die Alius-Grignaschi-Anomalie: der erblichkonstitutionelle Peroxydasedefekt der Neutrophilen und Monozyten. *Blut.* **14**: 129.
19. Moorhead, P. S., P. C. Nowell, W. J. Mellman, D. M. Battips, and D. A. Hungerford. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res.* **20**: 613.
20. Epstein, W. L. 1958. Induction of allergic contact dermatitis in patients with the lymphoma-leukemia complex. *J. Invest. Dermatol.* **30**: 39.
21. Hartog, M., M. J. Cline, and G. M. Grodsky. 1967. The response of human leucocyte cultures to stimulation by tuberculin and phytohaemagglutinin measured by the uptake of radioactive thymidine. *Clin. Exp. Immunol.* **2**: 217.
22. Skoog, W. A., and W. S. Beck. 1956. Studies on the fibrinogen, dextran, and phytohemagglutinin methods of isolating leukocytes. *Blood J. Hematol.* **11**: 436.
23. Caulfield, J. B. 1957. Effects of varying the vehicle for O₂O₄ in tissue fixation. *J. Biophys. Biochem. Cytol.* **3**: 827.
24. Climie, A. R. W., W. L. Heinrichs, and I. J. Foster. 1962. Neutrophilic alkaline phosphatase test. A review with emphasis on findings in pregnancy. *Tech. Bull. Regist. Med. Technol.* **32**: 95.
25. Pearse, A. G. E. 1960. Histochemistry, Theoretical and Applied. J. & A. Churchill, Ltd., London. 2nd edition. 831, 850, 881.
26. Beacom, D. N. 1926. Modification of Goodpasture's technic for the peroxidase reaction in blood smears. *J. Lab. Clin. Med.* **11**: 1092.
27. Fallon, H. J., E. Frei III, J. D. Davidson, J. S. Trier, and D. Burk. 1962. Leukocyte preparations from human blood: evaluation of their morphologic and metabolic state. *J. Lab. Clin. Med.* **59**: 779.
28. Cohn, Z. A., and J. G. Hirsch. 1960. The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leukocytes. *J. Exp. Med.* **112**: 983.
29. Klebanoff, S. J. 1965. Inactivation of estrogen by rat uterine preparations. *Endocrinology*. **76**: 301.
30. Cline, M. J., and R. I. Lehrer. 1969. D-amino acid oxidase in leukocytes: a possible D-amino-acid linked antimicrobial system. *Proc. Nat. Acad. Sci. U. S.* In press.
31. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265.

32. Cline, M. J. 1966. Phagocytosis and synthesis of ribonucleic acid in human granulocytes. *Nature (London)*. **212**: 1431.
33. Rebeck, J. W., and J. H. Crowley. 1955. A method of studying leukocytic functions in vivo. *Ann. N. Y. Acad. Sci.* **59**: 757.
34. Preisler, H., H. F. Hasenclever, A. A. Levitan, and E. S. Henderson. 1969. Serologic diagnosis of disseminated candidiasis in patients with acute leukemia. *Ann. Intern. Med.* **70**: 19.
35. Lehrer, R. I., and M. J. Cline. 1969. Interaction of *Candida albicans* with human leukocytes and serum. *J. Bacteriol.* In press.
36. Hirsch, J. G., and B. Strauss. 1964. Studies on heat-labile opsonin in rabbit serum. *J. Immunol.* **92**: 145.
37. Undritz, E., E. M. Lang, and E. Van Oye. 1956. La réaction peroxydasique des éosinophiles comme moyen de taxonomie (classification) des mammifères. *Sang.* **27**: 513.
38. Rytömaa, T., and H. Teir. 1961. Relationship between tissue eosinophils and peroxidase activity. *Nature (London)*. **192**: 271.
39. Archer, G. T., G. Air, M. Jackas, and D. M. Morell. 1965. Studies on rat eosinophil peroxidase. *Biochim. Biophys. Acta.* **99**: 96.
40. Bodey, G. P. 1966. Fungal infections complicating acute leukemia. *J. Chronic Dis.* **19**: 667.
41. Casazza, A. R., C. P. Duvall, and P. P. Carbone. 1966. Infection in lymphoma. Histology, treatment, and duration in relation to incidence and survival. *J. Amer. Med. Ass.* **197**: 710.
42. Hutter, R. V. P., and H. S. Collins. 1962. The occurrence of opportunistic fungus infections in a cancer hospital. *Lab. Invest.* **11**: 1035.
43. Hurley, R. 1964. Acute disseminated (septicaemic) moniliasis in adults and children. *Postgrad. Med. J.* **40**: 644.
44. Louria, D. B., D. P. Stiff, and B. Bennett. 1962. Disseminated moniliasis in the adult. *Medicine.* **41**: 307.
45. Lehrer, R. I. 1969. Antifungal effects of peroxidase systems. *J. Bacteriol.* In press.
46. Zeya, H. I., and J. K. Spitznagel. 1966. Cationic proteins of polymorphonuclear leukocyte lysosomes. I. Resolution of antibacterial and enzymatic activities. *J. Bacteriol.* **91**: 750.
47. Sato, A., and S. Yoshimatsu. 1925. The peroxidase reaction in epidemic encephalitis. A new diagnostic and prognostic method. *Amer. J. Dis. Child.* **29**: 301.
48. Arakawa, T., Y. Wada, T. Hayashi, R. Kakizaki, N. Chida, R. Chiba, T. Konno, and H. Shioura. 1965. Uracil-uric refractory anemia with peroxidase negative neutrophils. *Tohoku J. Exp. Med.* **87**: 52.
49. Higashi, O., N. Katsuyama, and R. Satodate. 1965. A case with hematological abnormality characterized by the absence of peroxidase activity in blood polymorphonuclear leukocytes. *Tohoku J. Exp. Med.* **87**: 77.
50. Sato, A. 1965. Peroxidase-negative neutrophils in Arakawa-Higashi's syndrome, in encephalitis Economo, further in "healthy brethren." *Tohoku J. Exp. Med.* **87**: 94.