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Diminished Neutrophil Oxidative Metabolism After Phagocytosis of Virulent *Salmonella typhi*

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The interactions of human polymorphonuclear neutrophils (PMNs) with virulent and avirulent strains of *Salmonella typhi* were examined. Ingestion of the *S. typhi* strains by PMNs was evaluated with three techniques: visual examination of PMN monolayers (phagocytic index); uptake of radiolabeled *S. typhi* by PMNs; and removal of *S. typhi* from the supernatant of suspensions of PMNs and bacteria. All three techniques indicated equivalent phagocytosis of the strains. Postphagocytic PMN oxidative metabolism was quantitated with measurements of oxygen consumption, protein iodination, and chemiluminescence. We found that although PMNs ingested equal numbers of virulent and avirulent *S. typhi*, those PMNs ingesting the virulent organisms exhibited a significantly smaller increase in postphagocytic oxidative metabolism than PMNs ingesting avirulent *S. typhi*. Despite this muted oxidative burst the virulent bacteria were killed as well as the avirulent strains. Virulent *S. typhi* either fail to stimulate receptors that trigger PMN oxidative metabolism or inhibit PMN oxidative metabolism. Our data support the former hypothesis.

Although the act of ingesting a particle is usually sufficient to stimulate oxidative metabolism of polymorphonuclear neutrophils (PMNs) (12), recent studies suggest that these two events are separable (8, 14, 19). Miller et al. (17) reported that PMNs interacting with the virulent Quailes strain of *Salmonella typhi* failed to exhibit the usual enhanced postphagocytic oxygen consumption. Possible explanations for this phenomenon include (i) a decrease in the number of particles ingested, resulting in less triggering of oxidative burst; (ii) inhibition of oxidative metabolism by ingestion of the virulent *S. typhi*; or (iii) diminished stimulation of oxidative metabolism by ingestion of the virulent *S. typhi* because of failure to stimulate appropriate receptors. We carefully quantitated ingestion of organisms by PMNs and studied PMN postphagocytic oxidative metabolism. Because the relationship of this phenomenon to virulence of the organism is unclear, we examined the ability of PMNs to kill both virulent and avirulent *S. typhi* strains. (A portion of this work was published in abstract form [R. E. Kossack, J. Schadelin, R. Guerrant, P. Densen, and G. L. Mandell, Clin. Res. **26**:28A, 1978].)

MATERIALS AND METHODS

Bacteria. *S. typhi* strains (Quailes, Ty2V, and O-901) were kindly supplied by Richard B. Hornick of the University of Rochester School of Medicine and Dentistry, Rochester, N.Y. The Quailes and Ty2V strains contain high titers of both Vi and O antigens and are both known to produce clinical typhoid fever in humans (5, 11, 18). The O-901 strain is a nonmotile derivative of Ty2V which lacks both O and Vi antigens and is relatively nonvirulent for humans (5).

Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) (18-h) cultures were centrifuged at 800 × *g* for 15 min, and the resultant pellet was washed in 0.9% saline at 37°C and suspended in Hanks balanced salt solution (HBSS) (Microbiological Associates, Bethesda, Md.). The bacterial concentration was adjusted to 10^9 organisms per ml (an optical density of 1.10 at 580 nm), by using a Gilford 2450 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Bacterial counts by serial dilution and pour plates confirmed that this method yields equal numbers of all strains of *S. typhi*.

^{14}C-labeled bacteria were prepared by incubation of bacteria in Trypticase soy broth with 10 μCi of L-^{14}C-amino acids (New England Nuclear Corp., Boston, Mass.) for 18 h. These bacteria were then prepared as described above.

PMNs. Whole blood was obtained from healthy volunteers with no history of salmonella infection or recent (10 years) typhoid fever vaccination.

PMNs were obtained by sedimentation of whole blood in an equal volume of heparinized (10 U/ml) 3% Dextran-70 (Pharmacia Laboratories, Inc., Piscataway, N.J.) for 1 h at 22°C at a 60° angle. The leukocyte-rich supernatant was removed and centrifuged at 200 × *g* for 12 min at 22°C. For oxygen consumption experiments, the erythrocytes were lysed once using 3 ml of iced 0.22% saline plus heparin (10 U/ml). Isotonicity was restored after 45 s with 0.99 ml of 3% saline followed by 5 ml of HBSS. Centrifugation was re-

peated, and the pellet was washed twice in HBSS. The concentration of PMNs in the final suspension (usually 10^7 PMNs per ml) was determined by hemacytometer count.

Phagocytosis. (i) Phagocytic index. Samples of whole venous blood (0.5 nl) were placed on cleaned cover slips and incubated in a moist chamber at 37°C for 90 min. The resulting clot was washed off with 0.9% saline at 37°C, and a suspension of 10^7 bacteria in HBSS and 10% fresh autologous serum was added to the adherent leukocytes. These cover slips were then replaced in the moist chamber. Two cover slips were removed at 0, 5, 10, 20, and 30 min, rinsed with saline to remove cell-free bacteria, fixed with methanol, and stained with Giemsa stain. The resultant preparations were mounted on glass microscope slides and were examined with oil immersion microscopy. The preparations were coded so that the examiner was "blinded" as to the strain of bacteria used. Both the number of PMNs with associated bacteria and the total number of cell-associated bacteria per 100 PMNs were recorded.

(ii) Cell association of ^{14}C-labeled bacteria (4). PMNs, 2.5×10^6/ml, in HBSS and 10% fresh autologous serum were mixed with ^{14}C-labeled *S. typhi* at about 15 *Salmonella* colony-forming units per PMN in polypropylene tubes (12 by 75 mm; Falcon, Oxnard, Calif.) in a final volume of 3 ml. The tubes were tumbled at 12 rpm at 37°C, and at 0, 5, 10, and 20 min 0.2-ml samples were withdrawn, placed in 4 ml of HBSS (plus heparin, 10 U/ml), and centrifuged at 250 $\times$ *g* for 5 min. The supernatant was discarded, and the pellet was washed again as described above. The final pellet was suspended in 0.1 ml of Protosol (New England Nuclear Corp.). A 2.5-ml volume of Bray scintillation fluid was added, and the mixture was poured into a glass scintillation vial and counted in a liquid scintillation counter (LS250; Beckman Instrument Co., Inc., Fullerton, Calif.). The results are expressed as the percentage of total bacteria that were cell associated.

(iii) Removal of bacteria from supernatant fluid by PMNs. Phagocytosis was assayed by using the technique of differential centrifugation as described below.

Postphagocytic oxidative metabolism. (i) Oxygen consumption. PMNs (10^7) and *S. typhi* cells (1.25×10^8) in a final volume of 3 ml of HBSS with 10% serum were incubated in the chambers of a polarographic oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio). The incubation mixture contained 1 mM KCN, which abolished oxygen consumption of live *S. typhi* (unpublished data) without interfering with either PMN oxygen consumption or the attachment or ingestion of *S. typhi* by PMNs (12). The oxygen consumption of PMNs alone or with boiled *Staphylococcus aureus* 502A was also measured. Oxygen consumption was displayed on a linear recorder, and the rate during the period of maximal uptake was measured and expressed as microliters of O_2 per minute.

(ii) Myeloperoxidase-mediated iodination by granulocytes. The method of Klebanoff (13) as modified by Root et al. (20) was employed. PMNs (5×10^6) and 2.5×10^8 *S. typhi* units were incubated in 0.5 ml of Ca^{2+}-free Krebs-Ringer phosphate plus 0.10 μCi of $Na^{125}I$ (New England Nuclear Corp.) and nonradioactive NaI at a final total concentration of 0.08 mM. The samples were tumbled at 12 rpm for 30 min at 37°C, and the reaction was stopped by the addition of 0.1 ml of sodium thiosulfate. Iodinated protein was precipitated and washed three times with 1 ml of 10% trichloroacetic acid, centrifuging the protein suspension at 2,000 $\times$ *g* for 5 min after each trichloroacetic acid addition. The final button was taken up in 1 ml of 10% trichloroacetic acid and counted in a gamma counter (Beckman Biogamma II, Beckman Instruments).

(iii) Chemiluminescence. PMNs (10^6) and bacteria (5×10^7) in a total volume of 0.5 ml with 10% fresh autologous serum and 5×10^{-5} M luminol (Sigma Chemical Co, St. Louis, Mo.) were incubated in small glass vials. Luminescence was continuously measured in a Chemglow Photometer at 37°C (Aminco, Silver Spring, Md.). The data were fed into a linear recorder, and measurements were made until peak luminescence was achieved. Chemiluminescence was expressed as intensity units (J. Schadelin and G. L. Mandell, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., abstr. no. 209, 1977).

Bactericidal activity. *S. typhi* were added to PMNs in 2 ml of HBSS with 10% heat-inactivated autologous serum in polypropylene tubes (12 by 17 mm; Falcon). *S. typhi*/PMN ratios of 0.33:1 to 17:1 were used. The tubes were tumbled at 12 rpm at 37°C for 120 min. Samples (0.2 ml) were removed after 0, 15, 30, 60, and 120 min of incubation (3). Total counts of viable bacteria were performed after hypotonic lysis of the PMNs, serial dilution, and pour-plate preparation. Additional 0.2-ml samples were placed in 1.8 ml of 0.9% saline and centrifuged at 250 $\times$ *g* for 5 min. Serial dilutions and pour-plate counts were done on the supernatant and on the pellet after hypotonic lysis to determine numbers of viable cell-free (supernatant) and cell-associated (sediment) bacteria.

RESULTS

Phagocytosis. (i) Phagocytic index. Microscopic examination of PMN monolayers revealed consistently similar uptake of both virulent (Quailes) and avirulent (O-901) strains of *S. typhi* by PMNs. By 30 min, >88% of the PMN were found to have bacteria associated with them (88.5 $\pm$ 1.5% with Quailes, 88.5 $\pm$ 3.5% with O-901). In addition, the number of bacteria per PMN was similar for incubation with the two *S. typhi* strains. At 30 min those PMNs incubated with the Quailes strain contained 4.3 $\pm$ 0.6 bacteria per PMN, whereas those incubated with the O-901 strain contained 4.4 $\pm$ 0.4 bacteria per PMN. Preparations treated with 0.5% trypan blue revealed >95% dye exclusion by PMNs in all samples at all time periods, indicating PMN viability.

(ii) Cell association of radiolabeled bacteria. Studies with ^{14}C-labeled O-901 and Quailes strains of *S. typhi* likewise revealed sim-

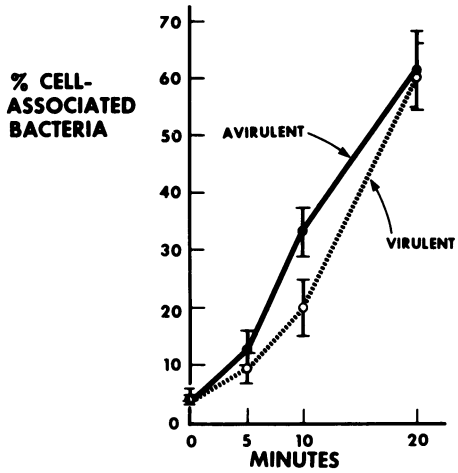

FIG. 1. *Cell association of S. typhi. PMNs were incubated with ^{14}C-labeled S. typhi. Cell-associated counts were determined at various time periods. Results are expressed as percentage of bacteria that are cell associated (mean ± standard error of the mean; n = 3).*

ilar uptake of stains by PMNs. Figure 1 shows uptake versus time for PMNs incubated with the virulent (Quailes) and avirulent (O-901) *S. typhi* strains. Uptake was similar at all time periods, except for a small, but significant, difference at 10 min.

(iii) Differential centrifugation. There was equivalent removal of both strains of *S. typhi* from the supernatant at 15, 30, and 60 min (Table 1). There was no change in numbers of bacteria in the supernatant over the time course of the experiment when PMNs were omitted.

Metabolic studies. (i) Oxygen consumption. Figure 2 shows cumulative O_2 consumption as a function of time for PMNs incubated with the virulent (Quailes) strain and the avirulent (O-901) strain of *S. typhi*. PMNs incubated with the avirulent strain showed a marked increase of oxygen consumption which was similar to that obtained when heat-killed staphylococci were ingested. Ingestion of the virulent strain stimulated a significantly smaller consumption of oxygen.

There was also a difference in the rate of O_2 consumption. Nonphagocytizing PMNs consumed 0.063 ± 0.012 μl of O_2 per 10^7 PMNs per min. After 10 min of incubation of PMNs with the avirulent (O-901) strain, O_2 consumption increased to a maximum of 0.432 ± 0.080 μl per 10^7 PMNs per min ($P = 0.002$ for non-phagocytizing PMNs versus PMNs plus the avirulent strain). In contrast, PMNs incubated with the virulent strain showed a muted response to the phagocytic stimulus, only reaching 0.153 ± 0.24 μl of O_2 per 10^7 PMNs per min ($P = 0.061$ for PMNs alone versus PMNs plus virulent *S. typhi*). Maximal O_2 uptake of PMNs ingesting the avirulent strain was significantly greater than for PMNs ingesting the virulent strain ($P = 0.01$). PMNs ingesting a different virulent strain (Ty2V) showed a similar muted consumption of oxygen (0.062 μl of O_2 per 10^7 PMNs per min).

(ii) Iodination of protein. The ability of PMNs incubated with virulent (Quailes) *S. typhi* to iodinate protein was only 60% of that of PMNs incubated with the avirulent strain (O-901) ($P = 0.0029$) (Fig. 3).

(iii) Chemiluminescence. The magnitude of the PMN chemiluminescent response reflects the generation of excited metabolites of oxygen

TABLE 1. *Percentage of bacteria removed from supernatant*[a]

Time (min)	% of bacteria		P (virulent versus avirulent)
	Virulent (Quailes)	Avirulent (O-901)	
15	60.6 ± 16.3	55.5 ± 23.6	0.346
30	74.3 ± 10.6	85.1 ± 7.7	0.108
60	98.9 ± 0.9	99.1 ± 0.8	0.470

[a] PMNs and *S. typhi* were tumbled together at a ratio of 15 bacteria per PMN. At the indicated time periods, the numbers of bacteria remaining in the supernatant were compared to numbers at 0 time. These data from four separate experiments are expressed as mean ± standard error of the mean.

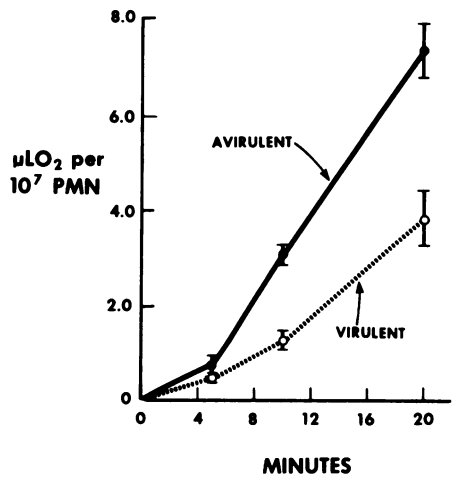

FIG. 2. *Oxygen consumption by PMNs. PMNs and S. typhi were incubated together in a polarographic oxygen monitor. KCN (1 mM) was employed to inhibit bacterial consumption of oxygen. Cumulative oxygen consumption is plotted against time. Ingestion of the virulent (Quailes) S. typhi strain resulted in significantly less O_2 consumption than did ingestion of the avirulent (O-901) S. typhi strain.*

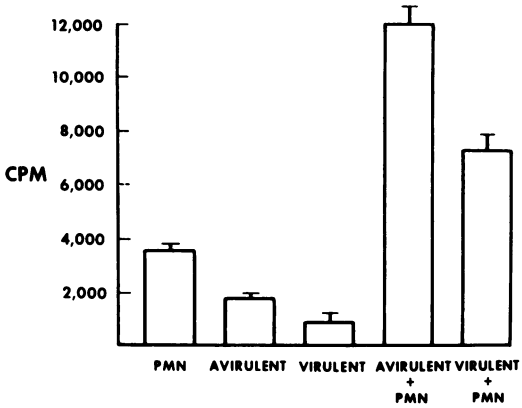

FIG. 3. *Iodination of protein by PMNs ingesting S. typhi. PMNs were incubated with S. typhi strains or heat-killed staphylococci in the presence of ^{125}I. The amount of protein iodination was quantitated over time. Ingestion of virulent (Quailes) S. typhi resulted in significantly less protein iodination than did ingestion of avirulent (O-901) S. typhi. Bars represent mean ± standard error.*

(J. Schadelin, R. Schadelin, and G. L. Mandell, Crit. Rev. Clin. Lab. Sci., in press). PMNs incubated with the avirulent (O-901) strain produced a quick rise in luminescence to a peak of 25.3 ± 3.1 luminescence units. PMNs incubated with the virulent (Quailes) strain responded more slowly and reached a peak value of only 14.8 ± 1.4 luminescence units ($n = 4$, $P = 0.005$). Results of a representative experiment are shown in Fig. 4. The luminescence response to ingestion of the avirulent strain was similar to that obtained with preopsonized zymosan particles (26.3 ± 3.3 luminescence units). When zymosan was added to suspensions of PMNs that were preincubated with virulent (Quailes) *S. typhi,* the resulting chemiluminescence peak (29.5 units) was as great as that observed when zymosan was added to suspensions of PMNs alone, indicating that the Quailes strain does not "poison" the PMNs. This indicates that the PMNs were not damaged or inhibited by interacting with the virulent *S. typhi.*

Bactericidal activity. PMNs killed both the virulent (Quailes) and the avirulent (O-901) *S. typhi* strain equally well at a ratio of one bacterium to one PMN (Table 2). When the number of organisms was increased to a multiplicity of 17 bacteria per PMN, 98.8% of the Quailes strain and 99.7% of the O-901 strain were killed by PMNs by 60 min.

DISCUSSION

Differences in the virulence of strains of *S. typhi* have been ascribed to differences in surface antigens. Felix and Pitt (5) showed that those strains possessing greatest quantities of Vi and O antigens were most virulent for mice. Hornick et al. (10) demonstrated that infection with Vi antigen-containing strains was more likely to produce clinical disease in humans than that with strains lacking Vi antigen.

Though these surface antigens appear to play a role in the pathogenesis of typhoid fever, the mechanisms have not been clearly defined. It has been suggested that these antigens may provide protection against phagocytosis (5) or enhance intracellular survival of virulent strains (11). Our studies demonstrate that despite the presence of both Vi and O antigens on the virulent (Quailes) strain the organism was efficiently ingested. We found that the virulent (Quailes) strain was ingested as well as the avirulent (O-901) strain.

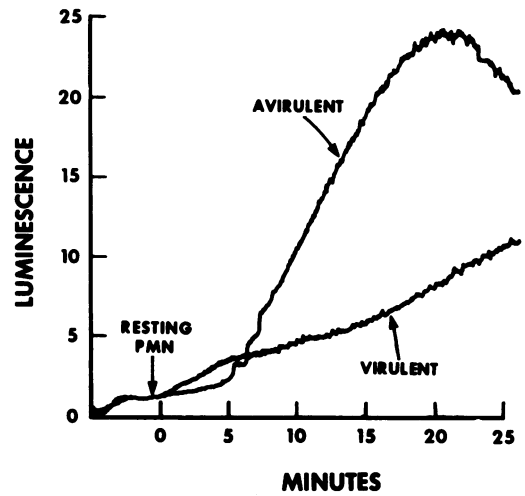

FIG. 4. *Chemiluminescence of PMNs ingesting S. typhi. PMNs were incubated with either virulent (Quailes) or avirulent (O-901) S. typhi in a photometer to measure light emission. PMNs with the virulent strain showed a muted chemiluminescent response.*

TABLE 2. *Percentage of bacteria killed*[a]

Time (min)	% of bacteria killed		P value
	Virulent (Quailes)	Avirulent (O-901)	
15	45.5 ± 23	37.9 ± 26	0.24
30	67.1 ± 12	76.5 ± 13	0.16
60	96.5 ± 4	99.2 ± 0.6	0.16

[a] PMNs and *S. typhi* were tumbled together at a ratio of 1 bacteria per PMN. At the indicated time periods, numbers of surviving bacteria were quantitated by lysis of PMNs, serial dilution, and pour-plate counts. Results from four separate experiments are expressed as mean (standard error of the mean) percent of bacterial inoculum killed.

It is clear that the nature of the ingested particle has a profound effect on PMN postphagocytic metabolic events (15). We observed that despite ingestion of equivalent numbers of bacteria, the oxygen consumption, protein iodination, and chemiluminescence were significantly muted in PMNs interacting with virulent (Quailes) *S. typhi*. Since these events have been closely linked with PMN bactericidal activity (13), we examined the ability of PMNs to kill virulent and avirulent *S. typhi* strains.

PMNs were able to kill virulent *S. typhi* and avirulent *S. typhi* equally well at multiplicities as high as 17 bacteria per PMN. Two possible explanations for this finding are: (i) PMN oxidative metabolism, stimulated by the virulent strain (albeit decreased), is sufficient for normal bactericidal activity, or (ii) PMNs kill *S. typhi* by nonoxidative means.

Substances associated with PMN granules can kill bacteria (6, 22, 23), and this killing does not appear to be dependent on oxidative activity. Cationic proteins with significant bactericidal activity towards gram-negative organisms have been identified in neutrophil granules (J. Weiss, O. Stendahl, and P. Elsbach, Clin. Res. **28**:515A, 1980). The activity of these substances may explain the ability of PMNs to kill virulent *S. typhi* despite muted oxidative metabolism. Friedberg and Shilo (6, 7) have shown that surface differences of various strains of *Salmonella typhimurium* can enhance differences in the ability of these organisms to resist killing by the oxygen-independent pathways.

The mechanism by which the virulent *S. typhi* strains dampen or fail to fully stimulate the oxidative activity may be related to interactions with PMN surface receptors. Stimuli that trigger the metabolic burst and those that induce ingestion may be separated (8, 14, 17, 19). It is conceivable that Vi or O antigen masks a surface moiety that normally triggers oxidative metabolism. A direct inhibitory effect is less likely because we found that PMNs that have interacted with virulent *S. typhi* are capable of a maximal metabolic burst upon further stimulation.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Butur, D.** 1977. L'action de le neurotoxine de *Salmonella typhimurium* S sur la NADH-oxidase des polynucleaires et des macrophages des cobayes. Rev. Roum. Biochim. **14**:7-12.
2. **Cagan, R. H., and M. L. Karnovsky.** 1964. Enzymatic basis of respiratory stimulation during phagocytosis. Nature (London) **204**:255-257.
3. **Dilworth, J. A., and G. L. Mandell.** 1977. Adults with chronic granulomatous disease of "childhood". Am. J. Med. **63**:233-243.
4. **Downey, R. J., and B. F. Diedrich.** 1968. A new method for assessing particle ingestion by phagocytic cells. Exp. Cell Res. **50**:483-489.
5. **Felix, A., and R. M. Pitt.** 1951. The pathogenic and immunogenic activities of *Salmonella typhi* in relation to its antigenic constituents. J. Hyg. **49**:92-110.
6. **Friedberg, D., and M. Shilo.** 1970. Interaction of gram-negative bacteria with the lysosomal fraction of polymorphonuclear leukocytes. I. Role of cell wall composition of *Salmonella typhimurium*. Infect. Immun. **1**: 305-310.
7. **Friedberg, D., and M. Shilo.** 1970. Role of cell wall structure of *Salmonella* in the interaction with phagocytes. Infect. Immun. **2**:279-285.
8. **Harvath, L., and B. R. Anderson.** 1979. Defective initiation of oxidative metabolism in polymorphonuclear leukocytes. N. Engl. J. Med. **300**:1130-1135.
9. **Harvath, L., and B. R. Anderson.** 1979. Defective initiation of oxidative metabolism in polymorphonuclear leukocytes. N. Engl. J. Med. **300**:1130-1135.
10. **Hornick, R. B., S. E. Greisman, T. E. Woodward, H. L. Dupont, A. T. Dawkins, and M. J. Snyder.** 1970. Typhoid fever: pathogenesis and immunologic control. N. Engl. J. Med. **283**:686-691, 739-746.
11. **Hornick, R. B., T. E. Woodward, F. R. McCrumb, M. J. Snyder, A. T. Dawkins, J. T. Bulkeley, F. De La Macorra, and F. A. Carozza.** 1966. Study of induced typhoid fever in man. I. Evaluation of vaccine effectiveness. Trans. Assoc. Am. Phys. **79**:361-367.
12. **Karnovsky, M. L.** 1962. Metabolic basis of phagocytic activity. Physiol. Rev. **42**:143-168.
13. **Klebanoff, S. J.** 1967. Iodination of bacteria: a bactericidal mechanism. J. Exp. Med. **126**:1063-1078.
14. **Malawista, S. E., and P. T. Bodel.** 1967. The dissociation by colchicine from phagocytosis from increased oxygen consumption in human leukocytes. J. Clin. Invest. **46**:786-796.
15. **Mandell, G. L.** 1971. Influence of type of ingested particle on human leukocyte metabolism. Proc. Soc. Exp. Biol. Med. **137**:1228-1230.
16. **Mandell, G. L., W. Rubin, and E. W. Hook.** 1970. The effect of an NADH oxidase inhibitor (hydrocortisone) on polymorphonuclear leukocyte bactericidal activity. J. Clin. Invest. **49**:1381-1388.
17. **Miller, R. M., J. Garbus, and R. B. Hornick.** 1972. Lack of enhanced oxygen consumption by polymorphonuclear leukocytes on phagocytosis of virulent *Salmonella typhi*. Science **175**:1010-1011.
18. **Olson, C. L., S. Gaines, and E. W. Hook.** 1961. Laboratory acquired typhoid fever. Infection with a laboratory strain of *Salmonella typhosa* isolated forty-one years earlier. Bull. Johns Hopkins Hosp. **109**:129-133.
19. **Roos, D., I. M. Goldstein, H. B. Kaplan, and G. Weissman.** 1976. Dissociation of phagocytosis, metabolic stimulation and lysosomal enzyme release in human leukocytes. Agents Actions **6**:256-259.
20. **Root, R. K., A. S. Rosenthal, and D. J. Balestra.** 1972. Abnormal bactericidal, metabolic and lysosomal functions of Chediak-Higashi Syndrome leukocytes. J. Clin. Invest. **51**:649-665.
21. **Root, R. K., and T. B. Stossel.** 1974. Myeloperoxidase-mediated iodination by granulocytes. Intracellular site of operation and some regulating factors. J. Clin. Invest. **53**:1207-1215.
22. **Zeya, H. I., and J. K. Spitznagel.** 1963. Antibacterial and enzymic basic proteins from leukocyte lysosomes: separation and identification. Science **142**:1085-1087.
23. **Zeya, H. I., and J. K. Spitznagel.** 1966. Cationic proteins of polymorphonuclear leukocyte lysosomes. I. Resolution of antibacterial and enzymatic activities. J. Bacteriol. **91**:750-754.