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Role of the Phagocyte in Host-Parasite Interactions

XI. Relationship Between Stimulated Oxidative Metabolism and Hydrogen Peroxide Formation, and Intracellular Killing

R. J. McRIPLEY AND A. J. SBARRA

Department of Pathology and Medical Research, St. Margaret's Hospital and Department of Obstetrics and Gynecology, Tufts University Medical School, Boston, Massachusetts 02125

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The increased respiratory and hexose monophosphate activities noted in phagocytizing cells results in the formation of hydrogen peroxide. This is brought about by the oxidation of reduced nicotinamide adenine dinucleotide phosphate by its oxidase. Evidence is presented which indicates that this H_2O_2 is involved in the intracellular killing of bacteria. When molecular oxygen was excluded from phagocytizing leukocytes by anaerobiosis, thus inhibiting H_2O_2 formation, reduced intracellular killing was observed. In some cases the impairment of leukocytic bactericidal activity by anaerobiosis could be partially reversed by the addition of H_2O_2. Exogenous catalase also could reduce intracellular killing. In addition, when leukocytic isolates were dialyzed so as to reduce endogenous H_2O_2, the bactericidal activity of the leukocytes was significantly decreased under both aerobic and anaerobic conditions. These results occurred with both guinea pig and human leukocytes and with several test microorganisms.

It is well known that a number of changes occur in the metabolic activities of polymorphonuclear neutrophils (PMN) during phagocytosis. For example, it has been shown that particle uptake causes significant increases in oxygen consumption, glycolysis, glycogenolysis, flow of glucose through the hexose monophosphate pathway (HMP), lactic acid production, and formate oxidation (2, 9, 11, 18). While it has been fairly well established that glycolysis and glycolytic-associated activities are stimulated in order to provide energy for the phagocytic act (4, 18), the significance of the elevated oxygen consumption and the direct oxidation of glucose through the HMP has not been clearly established. We have recently presented evidence which indicates that the increased oxidative metabolic activities were stimulated by particles only after they had gained entry into the PMN and could thus be considered as postphagocytic events (21). Further, it was suggested that these oxidative activities could lead to the formation of hydrogen peroxide, an agent which could be involved with the bactericidal activity of the PMN. Earlier, Iyer, Islam, and Quastel (9) proposed a pathway for the formation of hydrogen peroxide in the PMN. According to these investigators nicotinamide adenine dinucleotide phosphate (NADP), which is apparently the rate-limiting factor in HMP activity, is regenerated from reduced NADP by $NADPH_2$ oxidase. The regeneration of NADP requires molecular oxygen and results in the formation of H_2O_2. The bactericidal activity of H_2O_2 has long been recognized.

Preliminary data indicating the involvement of H_2O_2 in the intracellular bactericidal activity of the PMN have recently been presented (21). The purpose of this paper is to offer additional evidence for the role of H_2O_2 in the intracellular killing of bacteria by the phagocyte.

MATERIALS AND METHODS

Bacteria. Test organisms, *Escherichia coli*, *Shigella sonnei*, *Pseudomonas aeruginosa*, and *Staphylococcus albus*, from the department stock collection, were grown overnight in Trypticase Soy Broth (BBL). They were washed three times with calcium free Krebs-Ringer phosphate medium (KRPM), *p*H 7.4. The organisms were then standardized to a known concentration by means of a Klett-Summerson colorimeter (540-mμ filter).

Guinea pig peritoneal exudate leukocytes. Guinea pigs of both sexes, which weighed 300 to 400 g, were used. A leukocytic exudate was induced in the peritoneal cavity of a guinea pig by the intraperitoneal injection of 15 ml of a 12% sterile solution of sodium caseinate (18). After 16 to 18 hr, the animal was sacrificed and the exudate aseptically harvested by aspiration. The peritoneal cavity was then washed twice with KRPM. The washings were pooled and the resulting leukocytic exudate was washed twice with KRPM and made up to the desired volume. The con-

centration of leukocytes in the suspension was determined, either by estimation of the amount of cellular phosphorus by measuring the absorbance at 600 mμ of a suitable dilution, or by direct count in a hemocytometer. The leukocytic suspension, consisting of more than 90% PMN, was kept in an ice-water bath until used.

Human peripheral blood leukocytes. Leukocytes were isolated from the venous blood of apparently healthy individuals by using a previously described method (12). Briefly, blood was drawn into a syringe containing 0.5 ml of 30% dextran and 300 units of heparin for each 10 ml of blood. The syringe was allowed to stand in a vertical position for 45 min at room temperature. The resulting buffy-coat leukocytes were removed and washed twice with KRPM. The leukocytic isolate was then brought to the desired volume with KRPM, and the total and differential white-cell counts were performed by using conventional methods. Isolates generally contained more than 70% PMN.

Dialysis of leukocyte suspensions. Leukocytic isolates from humans were prepared as described above; 2 or 3 ml of the leukocyte suspension (10^7 to 5×10^7 leukocytes/ml) in cellulose sacs was dialyzed against a 40-fold volume of KRPM for 30 min at 37 C, to reduce the endogenous H_2O_2 concentrations. After dialysis, the leukocytes were placed in an ice-water bath before use. A sample of the initial leukocyte suspension, not dialyzed, was incubated with the dialyzed cells, and these leukocytes served as controls.

Assessment of phagocytosis and intracellular killing. The phagocytic and bactericidal activities of human and guinea pig leukocytes were determined by a slight modification of a method employed in previous studies (12). Initial reaction mixtures were prepared, in two siliconized test tubes, consisting of a known concentration of the test organism, 20% fresh autologous serum in the case of experiments utilizing human leukocytes, and KRPM in a final volume of 1.0 ml. Serum was not required for optimal phagocytosis when guinea pig PMN were used. A known number of phagocytes (10^7 to 5×10^7 leukocytes/ml) in a volume of 1.0 ml was added to one tube, and 1.0 ml of KRPM was added to the other. The latter served as a bacteria-serum control. Bacteria-to-phagocyte ratios which gave maximal phagocytosis were utilized: 2:1 to 4:1 for *P. aeruginosa*, *S. sonnei*, and *E. coli*, and 8:1 to 10:1 for *S. albus*. From each of the initial reaction tubes 0.2-ml samples were removed and placed into two series of four to eight siliconized tubes, one series for the control suspension and the other for the experimental system. These tubes were then incubated in a shaking water bath at 37 C. At selected time intervals, one control and two experimental tubes were removed. To one experimental tube and to the control tube was added 0.8 ml of 5% saponin, which served to lyse phagocytes and release intracellular bacteria. The concentration of saponin employed was not toxic for the test bacteria. The saponin-containing tubes were allowed to stand for 15 min at room temperature, after which appropriate dilutions were made and plated on Trypticase Soy Agar (BBL) plates by using conventional methods. Viable counts from these tubes gave total viable bacteria at the particular time interval in the absence and presence of leukocytes, thus indicating the bactericidal activity of the phagocytes. To the remaining experimental tube was added 9.8 ml of KRPM, and the tube was centrifuged at $250 \times g$ for 5 min; 0.2 ml was then removed from the supernatant liquid and added to 0.8 ml of 5% saponin. After 15 min, this mixture was appropriately diluted and plated. This gave the total extracellular count from which the phagocytic activity could be determined. Phagocytosis was also monitored by phase microscopy.

Nitrogen and oxygen atmospheres. Anaerobic conditions were obtained by carrying out the phagocytic and bactericidal experiments under nitrogen. Oxygen-free nitrogen (Liquid Carbonic, Cambridge, Mass.) was passed initially through alkaline pyrogallol, to remove trace amounts of oxygen, and then through nitrogen-saturated distilled water. Rubber-stoppered control and experimental tubes containing 0.2-ml samples of the appropriate initial reaction mixture were connected in a series by plastic tubing and needles and attached to the nitrogen train. The nitrogen was passed at the rate of approximately 250 ml/min. The tubes were flushed with nitrogen for 15 min while standing in an ice-water bath. Phagocytosis was initiated by transferring the tubes to a shaking water bath at 37 C while nitrogen continued to be passed. At predetermined time intervals, a set of tubes was removed and processed as described above. An aerobic experiment utilizing the same leukocytic isolate and similar conditions, with the exception of anaerobiosis, was carried out concomitantly as a control. An oxygen atmosphere was obtained in essentially the same way as anaerobiosis. Oxygen, USP (National Cylinder Gas Division of Chemetron Corp., Wakefield, Mass.), was passed through distilled water and connected to the series of tubes as was the nitrogen. The oxygen was passed at an approximate rate of 500 ml/min. The tubes were flushed for 10 min in an ice-water bath and then the experiment was performed as described above.

Chemicals. Reagent grade hydrogen peroxide (Superoxol, 30% H_2O_2) was obtained from Merck and Co., Inc., Rahway, N.J. The concentration of H_2O_2 used in experiments was determined spectrophotometrically from the molecular extinction coefficient of H_2O_2 at 230 mμ (0.067 cm^{-1} $mmole^{-1}$). Beef liver catalase, 2 × crystallized, 42,700 units/mg, was purchased from Mann Research Laboratories, Inc., New York, N.Y. When used in experiments, appropriate concentrations of H_2O_2 or catalase were added to both control and experimental reaction mixtures and the procedure described above was followed.

RESULTS

Effect of anaerobiosis on phagocytosis and intracellular killing. According to the proposed pathway for the formation of H_2O_2 in the phagocyte, molecular oxygen is required. If H_2O_2 is involved in the intracellular killing of bacteria, then the bactericidal activity of the phagocyte should be reduced by excluding molecular oxygen. Micro-

scopic and extracellular viability studies indicated that anaerobiosis did not affect the phagocytic activity (engulfment) of human or guinea pig leukocytes. This finding agreed with earlier results (13, 18) and made it possible to compare the bactericidal activity of a leukocytic isolate under aerobic and anaerobic conditions. The effect of anaerobiosis on the intracellular killing of *P. aeruginosa* by guinea pig PMN is shown in Fig. 1. Under anaerobic conditions, the bactericidal activity of PMN was somewhat reduced after 30 min and was markedly impaired at 60 min, with almost a 1-log difference in killing between aerobic and anaerobic conditions. This dramatic effect at the 60-min interval occurred because the killing under anaerobic conditions was essentially completed by 30 min, whereas under aerobic conditions the bactericidal activity was almost exponential for the entire 60-min test period. The effect of anaerobiosis on the bacterial control was insignificant.

Anaerobiosis also impaired the intracellular killing of *P. aeruginosa* and *S. albus* by human phagocytes (Fig. 2 and 3). In both cases, killing under anaerobic conditions was greatly reduced by 30 min, with little or no additional killing occurring after this time period. In contrast to results obtained with guinea pig phagocytes, the killing of bacteria by human phagocytes under aerobic conditions was almost 90% complete by the 30-min test period. Bacteria-serum controls were not affected by the aerobic or anaerobic conditions. It should be noted that experiments designed to detect the extracellular killing of test organisms were always negative, and this confirms earlier results (19).

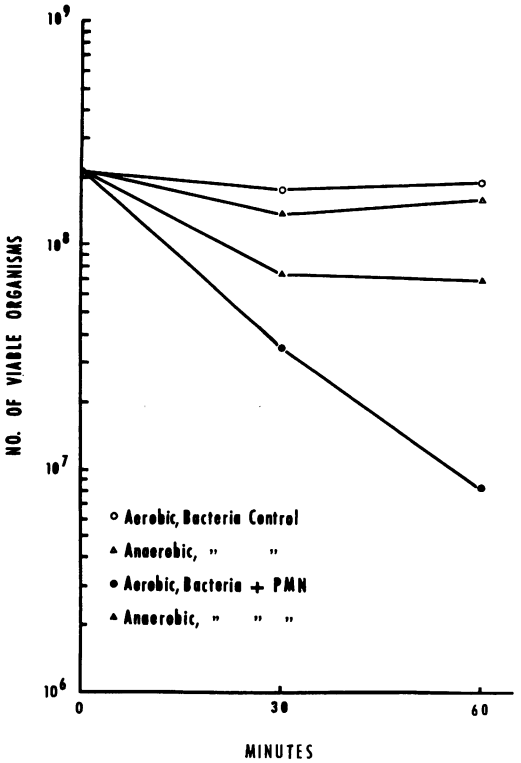

FIG. 1. *Effect of anaerobiosis on the intracellular killing of Pseudomonas aeruginosa by guinea pig leukocytes.*

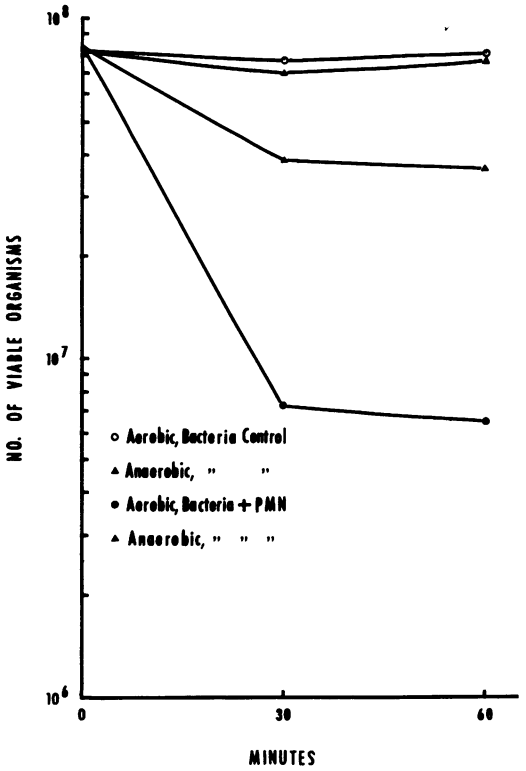

FIG. 2. *Effect of anaerobiosis on the intracellular killing of Pseudomonas aeruginosa by human leukocytes.*

Effect of molecular oxygen on phagocytosis and intracellular killing. Analogous to the anaerobic experiments with nitrogen, an atmosphere of 100% oxygen did not affect the ability of phagocytes to ingest particles. Also, the intracellular killing of the various test organisms by human and guinea pig phagocytes was unaltered. The same order of killing was demonstrated in the oxygen atmosphere as was exhibited under aerobiosis (Fig. 1–3). Preliminary experiments, however, have revealed that small but significant increases in the bactericidal activity of guinea pig PMN homogenates occur in the presence of

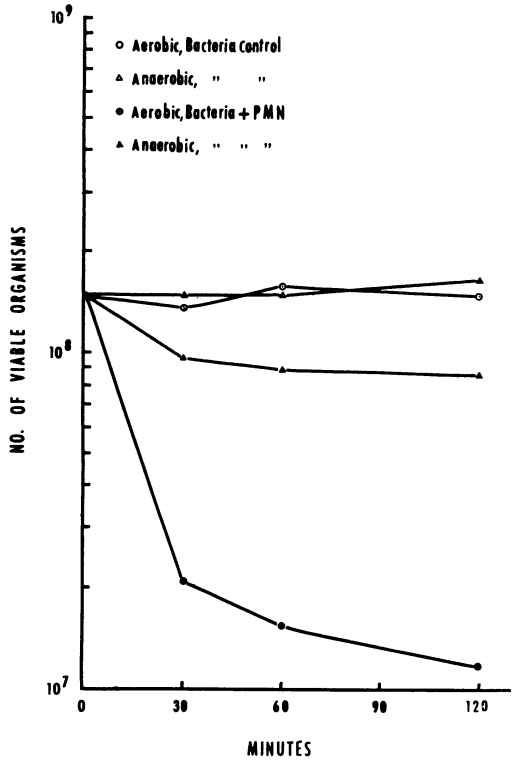

FIG. 3. *Effect of anaerobiosis on the intracellular killing of Staphylococcus albus by human leukocytes.*

oxygen. These experiments will be the subject of a subsequent publication.

Effect of added hydrogen peroxide on intracellular killing. Since the bactericidal activity of phagocytes was reduced under anaerobic conditions, and assuming this was due to impaired H_2O_2 formation, it seemed not unreasonable to postulate that exogenous H_2O_2 might restore the bactericidal activity of these cells. Thus, experiments in which various concentrations of H_2O_2 were added to the test system were carried out. The results of one series of experiments are shown in Table 1. The concentration of H_2O_2 utilized was not bactericidal to the concentration of test organisms employed. Moreover, the 2 mM H_2O_2 concentration did not affect the phagocytic and bactericidal activities of either the guinea pig or human leukocytes, since under aerobic conditions the activities were essentially the same in the absence and presence of the added H_2O_2. Under anaerobic conditions, H_2O_2 did stimulate intracellular killing of the test organisms by both leukocytic populations.

Effect of endogenous hydrogen peroxide levels on leukocytic bactericidal activity. In a further

TABLE 1. *Effect of added H_2O_2 on intracellular killing of Pseudomonas aeruginosa under anaerobic conditions*

Leukocyte	H_2O_2 (2 mM)	Per cent killed[a]	
		30 min	60 min
Guinea pig	−	51.2 ± 2.9	57.7 ± 1.9
	+	57.0 ± 3.4[b]	68.8 ± 2.1[c]
Human	−	66.7 ± 1.6	77.8 ± 0.4
	+	79.2 ± 3.0[c]	83.8 ± 2.3[b]

[a] Mean ± standard error of the mean for three or more experiments.
[b] Difference from control not significant.
[c] Difference from control significant at $P < 0.05$.

attempt to implicate H_2O_2 in the intracellular killing of bacteria, experiments were carried out in which the endogenous (intracellular) concentration of H_2O_2 in the PMN was reduced. Since catalase is an enzyme which decomposes hydrogen peroxide into water and oxygen, initial studies utilized catalase to reduce the H_2O_2 level in the PMN. Experiments were performed under an aerobic atmosphere to ensure optimal conditions for H_2O_2 formation and intracellular killing. The results (Table 2) indicate that catalase significantly decreased the bactericidal activity of the phagocytes at the 30-min test period, but by 60 min this activity was similar to that shown by the controls.

Preliminary results had indicated that endogenous H_2O_2 could be reduced in guinea pig PMN by dialysis against KRPM. Thus, guinea pig PMN were dialyzed and then utilized in our experimental system to assess the phagocytic and bactericidal activities of these cells. Microscopic studies revealed that dialysis did not impair the phagocytic activity of the PMN. However, the intracellular killing of *S. sonnei* was markedly reduced both under aerobic and anaerobic conditions (Fig. 4). This dramatic inhibition of killing was observed only at the 30-min test period and was even greater than that exhibited by the control PMN under anaerobic conditions. After 60 min of incubation, intracellular killing by dialyzed PMN under both aerobic and anaerobic conditions had returned to the level of killing exhibited by control PMN under anaerobic conditions. When *P. aeruginosa* was used as test organism, slightly different results were obtained (Fig. 5). The impaired bactericidal activity of dialyzed PMN under aerobic conditions was observed at both the 30-min and 60-min test periods and was less than that observed with

TABLE 2. *Effect of catalase on intracellular killing of Pseudomonas aeruginosa by human leukocytes under aerobic conditions*

Catalase (200 units)	Per cent killed[a]	
	30 min	60 min
−	91.4 ± 0.1	93.8 ± 0.8
+	80.7 ± 1.0[b]	93.6 ± 0.4

[a] Mean ± standard error of the mean for three or more experiments.

[b] Difference from control significant at $P < 0.001$.

control PMN under anaerobic conditions. In addition, the impaired activities of dialyzed and control PMN under anaerobic conditions were analogous at both test periods. Similar results were obtained when *P. aeruginosa* and human eukocytes were employed.

DISCUSSION

In spite of the many studies designed to elucidate the nature of intracellular killing in a phagocytic cell, the exact mechanism of action is yet unclear (7). Various intracellular antibacterial factors have been discovered and implicated: phagocytin (6), cationic proteins (23), lysozyme (5), lysosomes (3), and hydrogen peroxide (9). However, none of these has yet gained universal acceptance; as a result, no unified concept has been presented that has withstood the test of time. In addition, no satisfactory mechanism has been presented regarding the intracellular interaction between host cells and bacteria which will allow some microbes to be readily killed while others survive and in some cases even multiply. Recently, some insight into the mechanism of the intracellular killing of bacteria by phagocytes, based on hydrogen peroxide formation, has been brought into focus.

The metabolic pathway leading to hydrogen peroxide formation in the PMN, as originally proposed by Iyer et al. (9), is shown in Fig. 6. Evidence in support of this pathway and hydrogen peroxide formation in PMN is accumulating. The presence of hydrogen peroxide in PMN was originally postulated on the basis of the oxidation of formic acid (9). Recently, a technique for the direct estimation of hydrogen peroxide in PMN has been developed in this laboratory. A preliminary report of this procedure has been presented (B. Paul et al., Bacteriol. Proc., p. 92, 1967). By using this technique, it has been found that the H_2O_2 concentration of guinea pig PMN significantly increases during phagocytosis. This finding

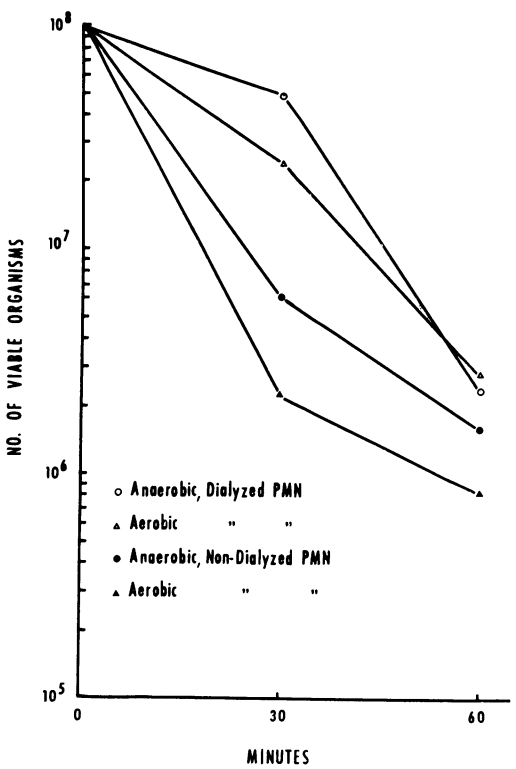

FIG. 4. *Effect of leukocytic dialysis on the intracellular killing of Shigella sonnei by guinea pig leukocytes under aerobic and anaerobic conditions.*

correlates well with the increased hexose-monophosphate shunt activity and oxygen consumption which accompanies phagocytosis (2, 4, 9, 18). In addition, other investigators (10, 17, 22) have provided evidence for increased $NADPH_2$ oxidase activity and concomitant stimulation of oxygen uptake during phagocytosis. Increased NADP-$NADPH_2$ ratios have been found in phagocytizing PMN, although the total NADP + $NADPH_2$ concentration remained constant (22).

Although there is good evidence for hydrogen peroxide formation in PMN, evidence implicating it in the bactericidal activity of the PMN is lacking. Several ingestigators, however, have postulated that it may be involved in the intracellular killing of bacteria (9). Data obtained in this study suggest that H_2O_2 is involved in the killing of ingested organisms. According to the proposed pathway of hydrogen peroxide formation, the exclusion of molecular oxygen from the PMN should result in the inhibition of H_2O_2 formation and consequently impaired intracellular bactericidal activity. This hypothesis was verified by the results obtained in the anaerobic killing experi-

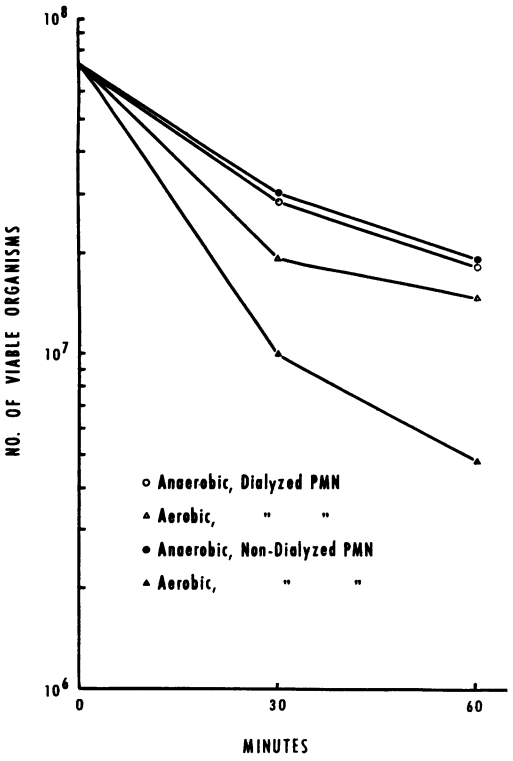

FIG. 5. *Effect of leukocytic dialysis on the intracellular killing of Pseudomonas aeruginosa by guinea pig leukocytes under aerobic and anaerobic conditions.*

ments. The bacterial killing that is observed under anaerobic conditions is apparently due to preformed H_2O_2 or other antimicrobial agents present in the PMN, or both. However, it should be pointed out that impaired intracellular killing under anaerobic conditions does not occur with all bacteria. This probably indicates, as might be expected, that certain bacteria are resistant to the hydrogen peroxide antibacterial system.

The fact that ingested bacteria were killed to the same extent in a 100% oxygen atmosphere as they were in air suggests that oxygen is not limiting in an air atmosphere. Apparently, the PMN produces the same amount of H_2O_2 under both conditions, and thus similar bactericidal activities are demonstrated. These results, when considered in light of the anaerobic experiments, give further support for the role of oxygen in H_2O_2 formation and intracellular killing in the PMN.

Experiments on the effect of added hydrogen peroxide on intracellular killing under anaerobic conditions yielded interesting results. Increased killing in the presence of exogenous H_2O_2 was demonstrated with both guinea pig and human

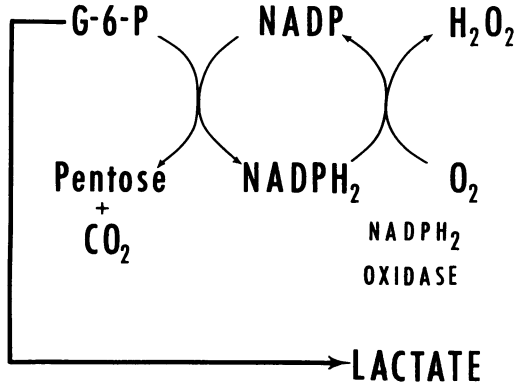

FIG. 6. *Postulated pathway for increased H_2O_2 formation in phagocytizing cells. G-6-P. is glucose-6-phosphate, NADP is nicotinamide adenine dinucleotide phosphate, and $NADPH_2$ is reduced nicotinamide adenine dinucleotide phosphate.*

leukocytes. This finding lends further support to the involvement of H_2O_2 in the intracellular killing of bacteria. The bactericidal activity of PMN under aerobic conditions was not enhanced by the added H_2O_2, probably because sufficient H_2O_2 was already available.

Added catalase is effective in impairing the intracellular killing of *P. aeruginosa* under aerobic conditions, presumably by decomposing hydrogen peroxide. However, the impaired killing is exhibited only at the 30-min test period. After 60 min, the extent of killing is the same in the absence as in the presence of added catalase. Thus, the PMN apparently is able to overcome the inhibitory effect of catalase. Preliminary results in our laboratory have suggested that an inhibitor of catalase is present in the PMN. The catalase appears to gain entry into the PMN during phagocytosis (14, 20). When PMN are preincubated with catalase and later tested for bactericidal activity, no impairment of killing is noted, indicating that under these conditions catalase did not gain entry into the cell. The catalase, once it has entered the PMN, apparently acts on the endogenous H_2O_2 before it is inactivated; thus, impaired killing is observed at the 30-min test interval. But, since the phagocytizing PMN is actively producing H_2O_2, killing in the normal range is demonstrated at the 60-min testing.

The involvement of hydrogen peroxide in intracellular killing is also indicated by the results of the dialysis experiments. It is possible to remove over 90% of the total cellular H_2O_2 by dialysis. When the bactericidal activities of dialyzed and nondialyzed control PMN are compared, a significant reduction in killing is observed with the dialyzed PMN under both aerobic and anaerobic

conditions. However, the extent of this reduction appears to be related to the test organism utilized. For example, when *P. aeruginosa* is the test organism, the impaired killing is observed throughout the experiment, whereas with *S. sonnei* reduced killing is shown only at the 30-min time interval and is not significantly different from the anerobic control at 60 min. The reason for the increased killing of *S. sonnei* with dialyzed PMN under anaerobic conditions between 30 and 60 min is presently unclear. It should be noted that *S. sonnei* and other enteric organisms do behave somewhat differently than do other test organisms under anaerobic conditions, and this may account for the observed results.

The findings in this study support the contention that hydrogen peroxide is involved in the intracellular killing of ingested bacteria by PMN. Because PMN are known to be rich in catalase (2), the relationship of the leukocytic catalase to the intracellular hydrogen peroxide must be resolved. Many possible relationships between the intracellular H_2O_2 and catalase may be postulated. For example, Rechcigal and Evans (16) have proposed that leukocytic catalase may prevent the accumulation of H_2O_2 levels which would be toxic to the cell. If this postulate is true, then it might be argued that the residual concentrations of H_2O_2 would be so low as not to exert a significant bactericidal effect on ingested bacteria. However, data in the following paper indicate that the bactericidal activity of low concentrations of H_2O_2 is markedly enhanced by myeloperoxidase found in leukocytic granules. Work is now in progress to elucidate the exact nature of the relationship between catalase, inhibitor, and H_2O_2 in PMN.

In addition to leukocytic catalase, many bacteria also exhibit catalase activity. Thus, future experiments utilizing catalase-producing and catalase-deficient organisms are planned in an attempt to determine systematically the relationship between catalase activity and resistance to intracellular killing.

It should be pointed out that the involvement of hydrogen peroxide in intracellular killing does not preclude the involvement of other antibacterial agents in the phagocyte. In fact, the involvement of an additional factor, myeloperoxidase, a "lysosomal" enzyme, will be developed in the following communication. However, the correlation between the observed phagocytic stimulated metabolic activities and intracellular killing implicate hydrogen peroxide as being involved with intracellular killing of at least some organisms. In addition, there appears to be clinical support for the proposed mechanism of H_2O_2 formation and its role in intracellular killing. Leukocytes from patients with chronic granulomatous disease are able to phagocytize bacteria readily, but they apparently are unable to kill the ingested organisms (8, 15). These leukocytes, while demonstrating normal glycolysis and lactic acid production, do not exhibit the stimulations of respiration and hexose monophosphate shunt activity that normally occur during phagocytosis (1). Thus, on the basis of our study these phagocytes are not producing H_2O_2, and this would explain the abnormal intracellular killing of bacteria. It should be mentioned that these patients are generally susceptible to bacterial infection (8, 15).

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LITERATURE CITED

1. BAEHNER, R. L., AND D. G. NATHAN. 1967. Leukocyte oxidase: defective activity in chronic granulomatous disease. Science **155**:835–836.
2. CLINE, M. J. 1965. Metabolism of the circulating leukocyte. Physiol. Rev. **45**:674–720.
3. COHN, Z. A., AND J. G. HIRSCH. 1960. The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leukocytes. J. Exptl. Med. **112**:983–1004.
4. COHN, Z. A., AND S. I. MORSE. 1960. Functional and metabolic properties of polymorphonuclear leukocytes. I. Observations on the requirements and consequences of particle ingestion. J. Exptl. Med. **111**:667–687.
5. FLEMING, A. 1922. On a remarkable bacteriolytic element found in tissue and secretions. Proc. Roy. Soc. (London) Ser. B **93**:306–317.
6. HIRSCH, J. G. 1956. Phagocytin: A bactericidal substance from polymorphonuclear leukocytes. J. Exptl. Med. **103**:589–611.
7. HIRSCH, J. G. 1965. Phagocytosis. Ann. Rev. Microbiol. **19**:339–350.
8. HOLMES, B., P. G. QUIE, D. B. WINDHORST, AND R. A. GOOD. 1966. Fatal granulomatous disease of childhood. Lancet **1**:1225–1228.
9. IYER, G. Y. N., M. F. ISLAM, AND J. H. QUASTEI. 1961. Biochemical aspects of phagocytosis. Nature **192**:535–541.
10. IYER, G. Y. N., AND J. M. QUASTEL. 1963. NADPH and NADH oxidation by guinea pig polymorphonuclear leukocytes. Can. J. Biochem. Physiol. **41**:427–434.
11. KARNOVSKY, M. L. 1962. Metabolic basis of phagocytic activity. Physiol. Rev. **42**:143–168.
12. MCRIPLEY, R. J., R. J. SELVARAJ, M. M. GLOVSKY, AND A. J. SBARRA. 1967. The role of the phagocyte in host-parasite interaction. V. Phagocytic

and bactericidal activities of leukocytes from patients with different neoplastic disorder. Cancer Res. **27**:674–685.

13. NUNGESTER, W. J. 1951. Mechanisms of man's resistance to infectious diseases. Bacteriol. Rev. **15**:105–129.
14. OXMAN, E., AND P. F. BONVENTRE. 1967. Facilitated uptake of streptomycin by Kupffer cells during phagocytosis. Nature **213**:294–295.
15. QUIE, P. G., J. G. WHITE, B. HOLMES, AND R. A. GOOD. 1966. Decreased bactericidal activity of polymorphonuclear leukocytes in children with chronic granulomatous disease. J. Clin. Invest. **45**:1058–1059.
16. RECHCIGAL, J. M., AND W. H. EVANS. 1963. Role of catalase and peroxidase in the metabolism of leukocytes. Nature **199**:1001–1002.
17. ROSSI, F., AND M. ZATTI. 1964. Changes in the metabolic pattern of polymorphonuclear leukocytes during phagocytosis. Brit. J. Exptl. Pathol. **45**:548–559.
18. 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**:1355–1362.
19. SBARRA, A. J., W. SHIRLEY, R. J. SELVARAJ, E. OUCHI, AND E. ROSENBAUM. 1964. The role of the phagocyte in host-parasite interactions. I. The phagocytic capabilities of leukocytes from lymphoproliferative disorders. Cancer Res. **24**: 1958–1968.
20. SBARRA, A. J., W. SHIRLEY, AND W. A. BARDAWIL. 1962. "Piggy-back phagocytosis." Nature **194**: 255–256.
21. SELVARAJ, R. J., AND A. J. SBARRA. 1966. Relationship of glycolytic and oxidative metabolism to particle entry and destruction in phagocytosing cells. Nature **211**:1271–1276.
22. SELVARAJ, R. J., AND A. J. SBARRA. 1967. The role of the phagocyte in host-parasite interactions. VII. Di- and tri-phosphopyridine nucleotide kinetics during phagocytosis. Biochim. Biophys. Acta **141**:243–249.
23. ZEYA, H. I., AND J. K. SPITZNAGEL. 1966. Cationic proteins of polymorphonuclear leukocyte lysosomes. II. Composition, properties, and mechanism of antibacterial action. J. Bacteriol. **91**: 755–762.