JCI The Journal of Clinical Investigation

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J Clin Invest. 1978;61(2):480-488. https://doi.org/10.1172/JCI108959.

Research Article

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Antimycin A, a specific mitochondrial respiratory chain inhibitor, depressed the oxygen consumption of monocytes by ≅ 70% but had no effect on neutrophil respiration. Therefore, the oxygen consumed by phagocytosing monocytes appeared to be metabolized in two distinct processes: ≅ 30% of the oxygen is converted to hydrogen peroxide, whereas the remaining 70% is metabolized via the mitochondrial respiratory chain. The release of superoxide and hydrogen peroxide was unaffected by antimycin in either cell type.

Phagocytosis of zymosan particles by monocytes was nearly abolished by antimycin, whereas no effect was noted with neutrophils. Thus, phagocytosis appears to be highly dependent on oxidative phosphorylation in monocytes but not in polymorphonuclear leukocytes. Moreover, in monocytes treated with antimycin, an addition of opsonized zymosan particles induced stimulation of the oxidative metabolism without occurrence of ingestion.

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Differences in Oxygen Metabolism of Phagocytosing Monocytes and Neutrophils

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ABSTRACT The oxidative metabolism of monocytes and polymorphonuclear leukocytes from human peripheral blood was studied in resting and phagocytosing cells. Monocytes, like neutrophils, showed an increase in oxygen consumption during phagocytosis with a concurrent release of superoxide anions and hydrogen peroxide. Both oxygen products are highly reactive agents with potential bactericidal activity. Neutrophils consumed two and a half times as much oxygen, generated about twice as much superoxide, and released five times as much hydrogen peroxide as monocytes did. Monocytes generated superoxide and hydrogen peroxide at equivalent rates.

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Received for publication 1 April 1977 and in revised form 29 September 1977.

INTRODUCTION

Peripheral blood monocytes and polymorphonuclear leukocytes (PMN)[1] are both able to ingest particulate materials. Neutrophils display a characteristic metabolic response to stimulation with phagocytosable particles: oxygen consumption is substantially increased (1) and the hexose monophosphate shunt is also activated (2). Oxygen is converted, in a cyanide-insensitive process, to highly reactive products, such as superoxide (3), hydrogen peroxide (4), and possibly hydroxyl radicals (5). Each of these agents may have bactericidal properties. Moreover, the action of hydrogen peroxide is considerably enhanced by myeloperoxidase together with a halide (6).

Comparison of the rate of phagocytosis and the subsequent metabolic reactions in monocytes and neutrophils has produced conflicting results. Phagocytosis of various particles appeared to be quantitatively similar in monocytes and PMN (7). Other reports (8, 9) indicate that monocytes ingest and kill bacteria at a significantly lower rate than neutrophils. Amounts of oxygen consumed and of formate oxidized were equivalent or even higher in monocytes than in PMN (8, 10). Recently, the generation of hydroxyl radicals (11), superoxide anions (10), and chemiluminescence (10, 12) by monocytes has been measured. With various stimulating agents, monocytes produced 20–70% of the chemiluminescence generated by neutrophils. Release of superoxide by monocytes amounted to ≅60% of the production by PMN (13).

In the present study we investigated the respiratory burst and the concurrent release of superoxide anion and hydrogen peroxide by monocytes and neutrophils after stimulation by serum-treated zymosan. It appeared that monocytes convert a smaller part of the

[1] *Abbreviations used in this paper*: DMSO, dimethyl sulfoxide; PMN, polymorphonuclear leukocytes.

consumed oxygen to superoxide and hydrogen peroxide than do PMN: the difference is attributable to a large increase in mitochondrial respiration by phagocytosing monocytes, but not by PMN.

METHODS

In each experiment in which the reactions of monocytes and PMN were compared, both types of cells were derived from the same donor. Suspensions of 1.5–2.5 × 10^6 cells/ml were prepared in Eagle's minimum essential medium, Grand Island Biological Co., Grand Island, N. Y. buffered with 25 mM Tris-HCl (pH 7.4 at 37°C), and supplemented with 5% (vol/vol) heat-inactivated fetal calf serum (Gibco Bio-Cult, Glasgow, Scotland). When indicated, these suspensions were incubated with metabolic inhibitors for ≅5 min at 37°C before addition of serum-treated zymosan.

Preparation of cells. Citrated venous blood from healthy individuals was used. Suspensions of mononuclear leukocytes and of PMN were prepared by centrifugation of the buffy coat on a Ficoll-Isopaque (Ficoll, Pharmacia, Uppsala, Sweden; Isopaque, Nyegaard, Oslo, Norway) layer as described by Böyum (14). A large cell separation procedure with the IBM 2991 blood-cell processor (IBM Corp., White Plains, N. Y.) was used (15, 16). Neutrophils were further purified from the heavy density layer according to the method described by Weening et al. (17). Monocytes were purified from the mononuclear fraction as follows: the mononuclear leukocytes were collected in a phosphate-buffered saline solution containing 0.38 g trisodium citrate-2-hydrate (E. Merck, Darmstadt, West Germany) per 100 ml and 3.5 mg human serum albumin/ml (Gepasteuriseerde Plasma Oplossing, GPO, Central Laboratory Netherlands Red Cross Blood Transfusion Service). The cells were spun down, washed once, and resuspended in Tris-buffered Eagle's minimum essential medium, supplemented with 20% (vol/vol) heat-inactivated fetal calf serum, to a final concentration of ≅10^7 cells/ml. This suspension was layered on glass Petri dishes and incubated at 37°C for 90 min. Nonadherent cells were then removed by rinsing thoroughly with medium. Adherent cells were scraped off with a piece of silicone rubber and suspended in the medium.

The preparations contained 82±11% (mean±SD, n = 52) monocytes as determined by electronic sizing with a Coulter counter, model ZF (Coulter Electronics Ltd., Dunstable, England) equipped with a pulse-height analyzer (Channelyzer, model C-1000 Coulter Electronics Ltd.) (18, 19). In the size distribution profiles so obtained, two populations of mononuclear leukocytes could be distinguished. The percentage of large cells was considered to indicate the fraction of monocytes, as these cells had been shown by Loos et al. (18) to display intracellular lysozyme activity, phagocytosis, and kidney-shaped nuclei. Other cells present in the preparations were granulocytes (≅3%) and lymphocytes (≅15%) (judged morphologically). All results were corrected for monocyte percentage in the preparations used. The nonadherent cells still contained 3–5% monocytes (electronic sizing). The purification method appeared neither to stimulate nor to damage the monocytes; moreover, no serious selection within the monocyte population was achieved (see Results).

Preparation of particles. Zymosan was incubated in fresh human serum (4 mg/ml) for 30 min at 37°C, washed twice with 154 mM NaCl, resuspended in 154 mM NaCl at a concentration of 10 mg/ml (i.e. ≅6–7 × 10^8 particles/ml), and stored at −80°C. This storage did not affect the structure or stimulating properties of the particles. The optimal (final) concentration of serum-treated zymosan for the metabolic studies was found to be 1 mg/ml (particle to cell ratio of ≅30). In the phagocytosis assay, the best results were obtained with a particle to cell ratio of ≅6 for monocytes and ≅15 for PMN.

Oxygen consumption. Oxygen consumption was measured polarographically with a Clark type electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio; model YSI 5331), as described by Weening et al. (17).

Hydrogen peroxide determination. The release of hydrogen peroxide into the incubation medium in the presence of sodium azide (2 mM) was measured fluorometrically as described by Homan-Müller et al. (20).

Superoxide anion production. The generation of superoxide anions was measured by the reduction of ferricytochrome c (150 μM) (Horse heart ferricytochrome c, grade III, Sigma Chemical Co., St. Louis, Mo.) (3, 21). The values found in the presence of 1.3 μM superoxide dismutase were subtracted from all other values.

Phagocytosis assay. For the determination of adherence plus ingestion (22), the cells were incubated with radioactive zymosan particles. These particles had been labeled with ^{125}I (lactoperoxidase method) and subsequently opsonized as described above. 0.3-ml samples of incubation mixture were added to 1 ml of ice-cold phosphate-buffered saline, containing sodium fluoride (10 mM) and human serum albumin (5 mg/ml). The mixture was then centrifuged on an ice-cold Ficoll-Isopaque layer of high density (sp gr 1.095 g/cm^3) (23) to separate the noningested particles from the cells.

Zymosan ingestion was measured as follows. A 500-μl sample of supernate was taken from the top of the gradient tube and counted separately (A). The rest of the supernate (800 μl), the interface (cells plus ^{125}I-zymosan), and the Ficoll-Isopaque solution was transferred to a clean test tube. With hot scissors the plastic gradient tube was then cut just above the pellet. The upper part was added to the test tube and the radioactivity in it was counted (B). The lower part of the gradient tube, containing the noningested zymosan particles, was put into another tube and also counted (C).

Calculation of percentage ingestion was made as follows. Soluble radioactivity, which remains in the supernate on the Ficoll-Isopaque, amounts to 13/5 × A. Therefore, the radioactivity in the cells is B + A − (13/5)A = B − (8/5)A. The uptake as percentage of total radioactivity added is (B − [8/5]A / B+C−[8/5]A) × 100. Although the particles used for this test were washed pellet particles after centrifugation over Ficoll-Isopaque with a density of 1.095 g/cm^3, we performed control incubations without cells to correct for noningested low density particles. From these control incubations samples were taken and the percentage radioactivity in the interface was subtracted from the values with cells at the corresponding incubation times. These controls varied from 20 to 40% of the cell values at time (t) = 0 to 5–10% at t = 45 min.

The uptake of zymosan by monocytes and neutrophils appeared to be nonlinear with regard to cell and particle concentration. Therefore, a fixed particle to cell ratio was used and the cell concentration was kept as close as possible to 4 × 10^6 phagocytes/ml.

Rosette formation. Adherence of serum-treated zymosan to cells was determined with rosette formation as described by Wong and Wilson (24).

Materials

Antimycin A (C. F. Boehringer, Mannheim, West Germany) and oligomycin (Upjohn Co. Fine Chemicals Div., Kala-

mazoo, Mich.) were dissolved in dimethyl sulfoxide (44 mM and 2 mg/ml, respectively). These stock solutions were diluted with medium and added to the cells in final concentrations indicated in the figure legends. Dimethyl sulfoxide had no demonstrable effect in the amounts used. Superoxide dismutase, isolated from bovine blood as described by McCord and Fridovich (25), was a gift from Dr. R. Wever, University of Amsterdam.

Statistical analysis. Statistical significance was assessed using Student's t test for paired observations.

RESULTS

First we checked whether the glass adherence and scraping procedure used to purify the monocytes, activated or damaged the cells. Half of a nonpurified mononuclear cell suspension was incubated for 90 min on plastic Petri dishes at 37°C, the other half was kept in siliconized glass tubes at 0°C. Afterwards, the monocytes were scraped from the dishes and resuspended in their own supernates. In this way, both suspensions had a similar composition of adherent and nonadherent cells. The resting level of oxygen consumption in both suspensions was not significantly different ($P > 0.1$, $n = 4$) and appeared to be similar to the level found in purified suspensions. Thus, no activation of monocyte metabolism by the plating procedure could be detected. After stimulation with opsonized zymosan, the oxygen consumption in the original and in the resuspended cell mixture was also not significantly different ($P > 0.1$, $n = 4$). Moreover, the purification procedure had no effect on cell viability: 98±1% (mean±SEM, $n = 8$) of the cells excluded trypan blue, either before or after adherence. Thus, no cell damage was apparent. Finally, we found that the respiration rate with zymosan on a "per monocyte" basis was the same in purified and in nonpurified suspensions. Thus, no serious selection within the monocyte population was introduced by the isolation procedure.

Monocyte metabolism. During phagocytosis of serum-opsonized zymosan, the oxygen consumption of purified monocytes increased 16-fold (Table I). In contrast, the respiration of nonadherent cells increased only two- to threefold by the addition of zymosan. The maximal rate of oxygen uptake by purified monocytes was reached within 5 min of the addition of the particles. Table I also shows the liberation of superoxide and hydrogen peroxide by these cells. During the first 10 min the total release of these products was ≅30% less than the maximal response. Although the differences in incubation condition do not allow a direct comparison between the amounts of oxygen products recovered,[2] the data in Table I suggest that monocytes produce superoxide and hydrogen peroxide in similar quantities.

Comparison of monocyte and neutrophil metabolism. Fig. 1 displays the oxygen consumption and hydrogen peroxide production in eight paired experiments of monocytes and neutrophils isolated from the same blood samples. The assays were performed under identical conditions in parallel incubations. Sodium azide (2 mM) was present in the incubation mixtures as an inhibitor of catalase and myeloperoxidase, two enzymes responsible for intracellular break down of hydrogen peroxide. This concentration of NaN_3 did not affect the oxygen consumption of monocytes significantly (Table II), but was essential for measuring H_2O_2 generation.

Fig. 1 clearly shows that the respiratory burst in phagocytosing PMN was much greater (on a per cell basis) than in monocytes from the same individual. The oxygen consumption of monocytes amounted to 39±6% of the oxygen uptake of PMN.[3] The maximal rate of hydrogen peroxide release by monocytes was 19±5% of that found with PMN. In neutrophils 89±3% of the oxygen consumed within the first 10 min was recovered as hydrogen peroxide; in monocytes this figure was only 29±5%.

In six other experiments, without NaN_3, the release

TABLE I
Oxidative Metabolism of Monocytes

Parameters	Additions	Maximal rate*	Number of experiments
Oxygen consumption	—	0.1±0.03	10
	Opsonized zymosan	1.6±0.2	10
Superoxide production	—	0	6
	Opsonized zymosan	0.8±0.4	6
Hydrogen peroxide production‡	—	0	11
	Opsonized zymosan	0.8±0.1	11

* Expressed in nmol/min per 10^6 monocytes (mean±SEM).
‡ In the presence of NaN_3 (2 mM).

[2] Cytochrome c, needed for the trapping of O_2^-, prevents measurement of oxygen consumption and H_2O_2 generation, by its reaction with O_2^- (27):

$$\text{cyt } c\ (Fe^{3+}) + O_2^- \rightarrow \text{cyt } c\ (Fe^{2+}) + O_2.$$

In this way oxygen is regenerated and no decrease in the oxygen concentration can be measured. Moreover, O_2^- is no longer able to react with protons to H_2O_2, and hydrogen peroxide production cannot be measured either.

[3] From each pair of observations the ratio was calculated between the values obtained with monocytes and with PMN, and the result was multiplied by 100%. The numbers given in this paragraph are the mean±SEM ($n = 8$) of these percentages.

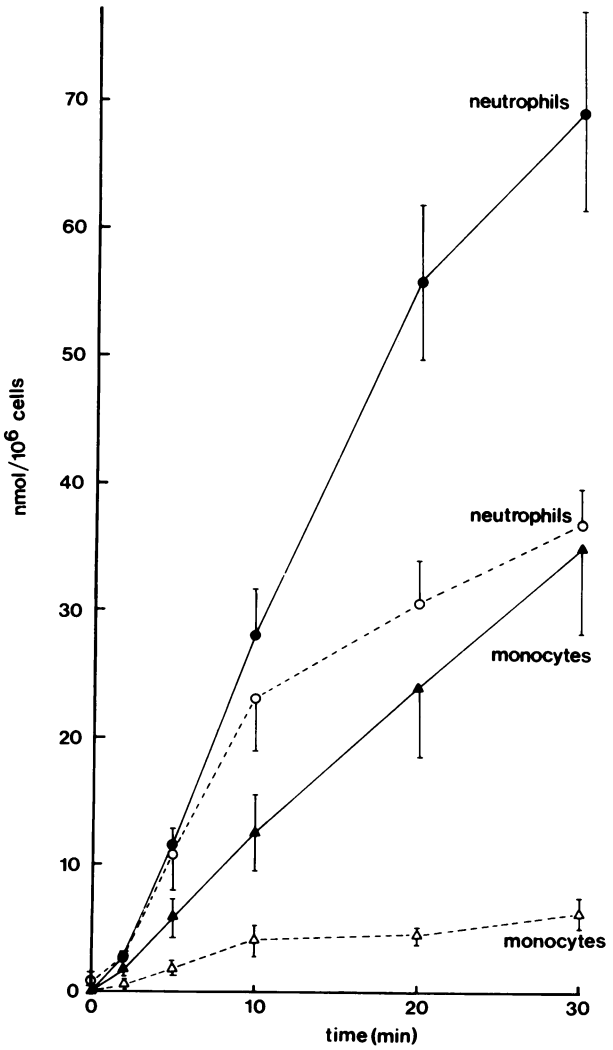

FIGURE 1 Oxygen consumption and hydrogen peroxide production of zymosan-stimulated monocytes and neutrophils from the same blood samples. Mean±SEM of eight experiments are shown. NaN_3 was present at a concentration of 2 mM. Addition of serum-treated zymosan (1 mg/ml) at t = 0 results in a burst of oxygen consumption and concurrent release of H_2O_2 in both cell types. The figure shows the O_2 consumption (nmol consumed/10^6 cells) by PMN (●) and monocytes (▲), and H_2O_2 production (nmol in supernates/10^6 cells) by PMN (○) and monocytes (△).

of superoxide by monocytes and neutrophils from the same donors was compared. Monocytes generated O_2^- at ≅60% of the rate of PMN.

Fig. 1 shows that the oxygen consumption of the cells continued at a virtually constant rate during the observation period, whereas hydrogen peroxide production, especially by monocytes, leveled off after ≅10 min. Control experiments were performed to detect the cause of this difference. Hydrogen peroxide, in a concentration similar to that found in the incubations (20 μM), was added to supernates or lysates (obtained by five time freeze-thawing or sonication) of monocytes stimulated for 0, 10, or 30 min with opsonized zymosan at 37°C. These mixtures were then incubated for another 30 min at 37°C, and the concentration of H_2O_2 was measured before and after this period. Because a recovery of 85–90% of the added H_2O_2 was found in all samples, an increased break down of H_2O_2 during the experiments of Fig. 1 is highly unlikely. Similar experiments with PMN had led to the same conclusion (20). Possibly, the amount of hydrogen peroxide released inside phagosomes increased with time, thus reducing its recovery from the surrounding medium.

TABLE II
Effect of Metabolic Inhibitors on Oxygen Consumption of Zymosan-Stimulated Monocytes

Inhibitor*	Inhibition‡	Number of experiments	Significance§
	%		
NaN_3 (2 mM)	1–19	3	$P > 0.05$
KCN (2 mM)	23–52	6	$P < 0.05$
Antimycin A (360 μM)	65–86	7	$P < 0.01$
Oligomycin (15 μg/ml)	38–79	3	$P < 0.025$

* Monocytes were incubated with the inhibitors for 5 min at 37°C before addition of serum-treated zymosan (1 mg/ml). The reaction was then followed for 30 min.
‡ The inhibition was calculated from the maximal rates of oxygen consumption obtained with and without the inhibitor (range).
§ Student's *t* test for paired observation.

Effects of metabolic inhibitors on monocyte respiration. In PMN, phagocytosis induces a KCN-insensitive burst of oxygen consumption (26) which is used mainly, if not entirely, for the generation of superoxide anions and hydrogen peroxide (27). The results, shown in Fig. 1, however, seem to indicate that only a minor part of the oxygen taken up by monocytes during phagocytosis is converted to these reactive products. Therefore, we decided to investigate the importance of the mitochondrial respiration in these cells. Table II shows the effects of four inhibitors of mitochondrial respiration on the oxygen consumption of phagocytosing monocytes. Sodium azide had no significant effect on oxygen consumption. KCN inhibited oxygen uptake by 23–52%. Both azide and cyanide are inhibitors of the respiratory chain in mitochondria; however, they also affect other heme group-containing enzymes involved in oxygen metabolism, e.g., myeloperoxidase and catalase. Therefore, we also used oligomycin, a selective blocker of the oxidative phosphorylation, and antimycin A, a specific inhibitor of the mitochondrial respiratory

chain at the level of cytochrome *b*. Both agents strongly inhibited the oxygen consumption by monocytes.

Because of its strong and specific effect, further experiments were carried out with antimycin. This agent had no effect on the assay of either oxygen or superoxide (checked with the xanthine plus xanthine oxidase method, [28]), nor did it affect the hydrogen peroxide assay (H_2O_2 standards in medium). Antimycin in concentrations up to 730 μM did not stimulate the oxygen consumption of resting monocytes or neutrophils. As shown in Fig. 2, antimycin inhibited the oxygen consumption of monocytes in a dose-dependent fashion, whereas no effect was found in PMN. In the presence of high concentrations of antimycin, monocytes still display ≅20–30% of the maximal oxygen consumption. Fig. 3 shows that in this situation the amount of hydrogen peroxide recovered from monocytes is roughly equivalent to the oxygen uptake. Figs. 4 and 5 illustrate that antimycin has no effect on the release of superoxide or hydrogen peroxide by either monocytes or PMN. No significant difference in the percentage of trypan blue excluding cells could be found before or after incubation with antimycin (360 μM) for 30 mih at 37°C ($P > 0.1, n = 7$). The effect of antimycin on monocyte respiration was not produced by the adherence and scraping procedure, because antimycin had essentially the same effect on total mononuclear leukocyte suspensions.

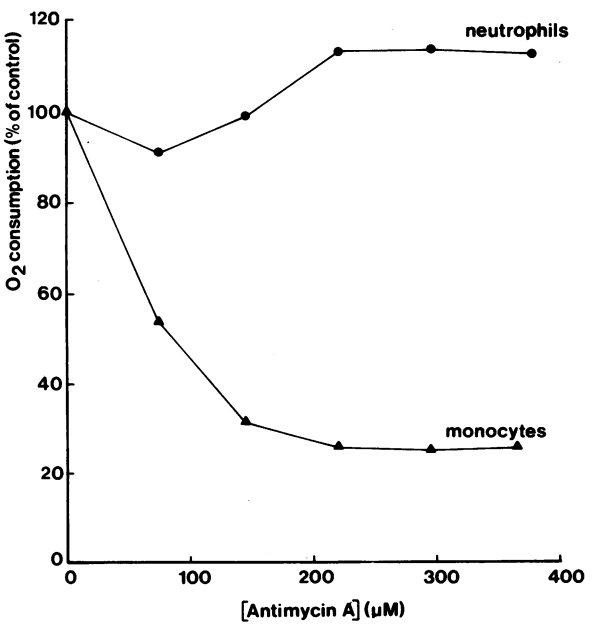

FIGURE 2 Effect of antimycin A on oxygen consumption of zymosan-stimulated monocytes (▲) and neutrophils (●) from the same blood sample. Values given are maximal rates of O_2 consumption, expressed as the percentage of the control value without antimycin (=100%). The reaction was followed for 30 min.

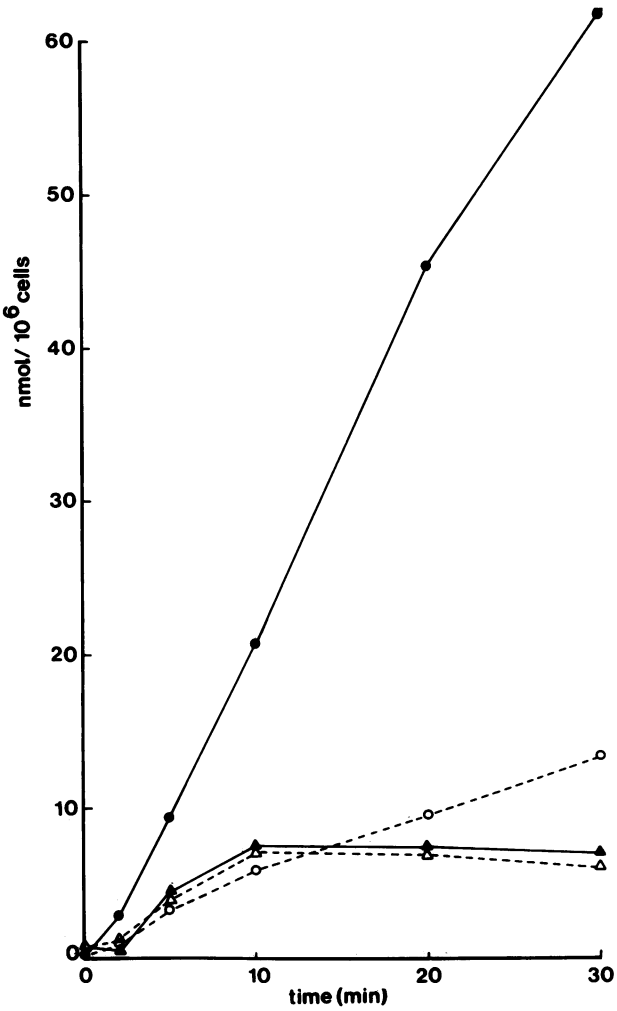

FIGURE 3 Oxygen consumption and hydrogen peroxide release by zymosan-stimulated monocytes in the presence of antimycin A (360 μM), compared to controls in the presence of DMSO only. NaN_3 was present at a concentration of 2 mM. Serum-treated zymosan (1 mg/ml) was added at t = 0. O_2 consumption (nmol consumed/10^6 monocytes) is shown with (○) and without (●) antimycin, and H_2O_2 production (nmol in supernates/10^6 monocytes) with (△) and without (▲) antimycin.

Phagocytosis. From the foregoing, it appears that phagocytosing monocytes display a strong burst of respiratory chain activity. Because phagocytosis is an energy-requiring process, we investigated the influence of antimycin on the ingestion of serum-treated zymosan by monocytes. Cells were incubated with ^{125}I-labeled zymosan (0.2 mg/ml) for 45 min, and ingestion (plus adherence) was measured as described under Methods. Fig. 6 illustrates our findings: monocytes ingested these particles at a fairly constant, slow rate for at least 45 min. In the presence of antimycin, ingestion was almost completely inhibited. Phagocytosis by neutrophils, on the other hand, proceeds at a faster rate (22), and was not

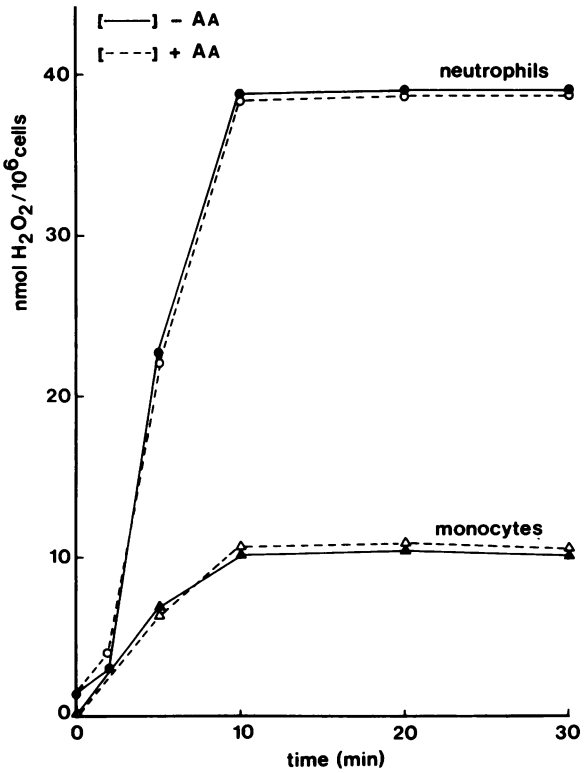

FIGURE 4 Effect of antimycin A (360 μM) on hydrogen peroxide release of zymosan-stimulated monocytes and neutrophils from the same blood sample. H_2O_2 (nmol/10^6 cells) was measured in the cell-free supernates of PMN, in the presence of antimycin (○) or DMSO only (●), and in the cell-free supernates of monocytes, also in the presence of antimycin A (△) or DMSO only (▲). NaN_3 was present at a concentration of 2 mM.

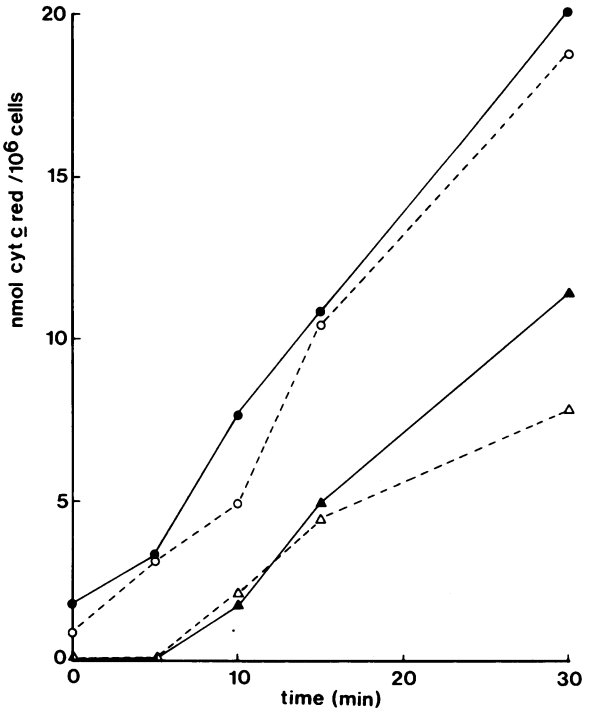

FIGURE 5 Effect of antimycin A (360 μM) on superoxide generation by zymosan-stimulated monocytes and neutrophils from the same blood sample, compared to controls in the presence of DMSO only. Superoxide generation is expressed as nmoles ferricytochrome *c* reduced per 10^6 cells in cell-free supernates. Values obtained in control experiments in the presence of superoxide dismutase (1.3 μM) were subtracted. The figure shows the reduction of cytochrome *c* by PMN with (○) and without (●) antimycin, and by monocytes with (△) and without (▲) antimycin.

influenced by antimycin at concentrations which had a significant effect on monocytes (Fig. 7). No higher concentration of antimycin than 150 μM could be tested, because these led to clumping of neutrophils after ≅15 min; this prevented accurate measurement of phagocytosis. Oligomycin (15 μg/ml) had a similar effect: it strongly inhibited uptake of ^{125}I-zymosan by monocytes, but not by PMN (data not shown).

The influence of antimycin on adherence of serum-opsonized zymosan to the cells was compared to the effect of dimethyl sulfoxide (DMSO) only. We found 27±2% (mean±SEM, n = 6) of rosette-forming cells in the presence of antimycin, vs. 26±2% (mean±SEM, n = 6) with DMSO only. The difference is not statistically significant ($P > 0.1$); thus, the inhibition of particle ingestion by antimycin did not appear to be due to decreased adherence.

DISCUSSION

At present it is unknown whether the myeloperoxidase-hydrogen peroxidase-halide system, which exists in PMN (6), plays an important role in the bactericidal reactions of monocytes. It has been shown by [^{14}C]-formate oxidation that human monocytes produce hydrogen peroxide (8, 10). However, this technique neither allows a reliable comparison between different cell types (because it depends on the intracellular catalase concentration) (4, 29), nor accurate stoichiometric oxygen recovery studies (because it detects only a fraction of the total H_2O_2 production) (4, 30–32). Sagone et al. (10) reported formate oxidation to be low and unpredictable in monocytes in comparison with neutrophils. Baehner and Johnston (8) found that formate oxidation by monocytes of children with cyclic and congenital neutropenia reached higher values than by control PMN. Using a more sensitive and specific fluorometric assay (20) we found that monocytes release ≅20% of the amount of hydrogen peroxide generated by neutrophils.

Several studies indicate that human monocytes also generate superoxide anions during phagocytosis (10, 13). Our results have confirmed this observation, albeit that monocytes generate less superoxide than neutrophils.

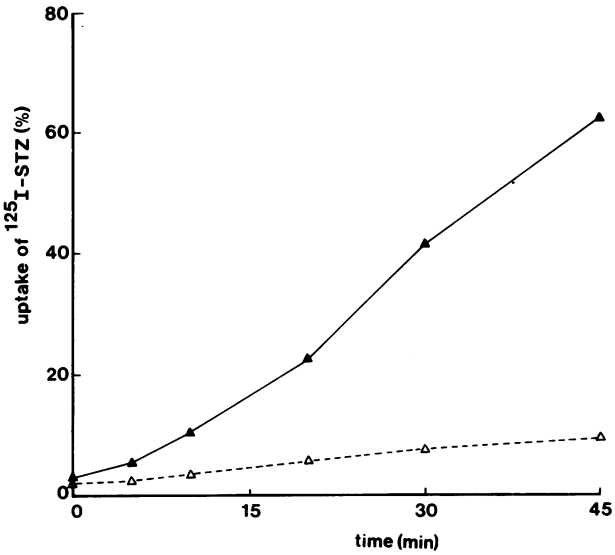

FIGURE 6 Phagocytosis of ^{125}I-zymosan by monocytes. Particle to cell ratio of ≅6. Cell-associated radioactivity (i.e. ingested plus adherent particles) is expressed as percentage of the total radioactivity per sample (see Methods). The figure shows the uptake of ^{125}I-zymosan in the presence of 360 μM antimycin (△) and in the presence of DMSO only (▲). The figure shows the results of one of five such experiments performed.

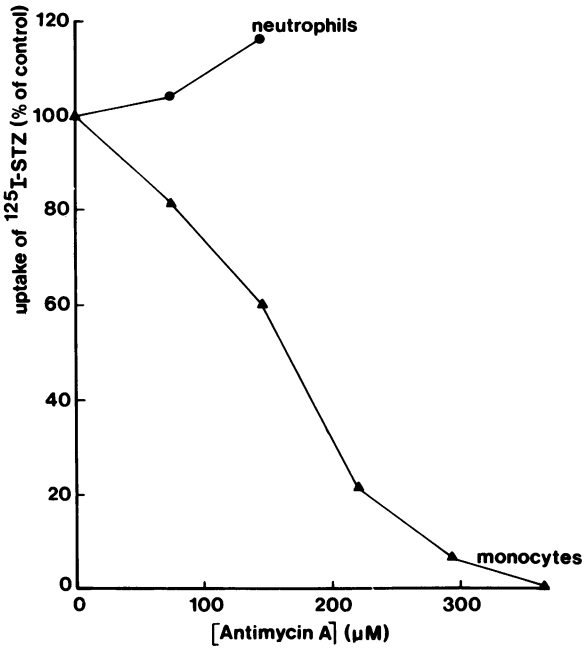

FIGURE 7 Effect of antimycin A on the uptake of ^{125}I-zymosan by monocytes (▲) and neutrophils (●) from the same blood sample. Values given represent total uptake of ^{125}I-zymosan in 30 min, expressed as the percentage of the values obtained without antimycin (=100%). Particle to cell ratio: for monocytes, ≅6; for PMN, ≅15. At concentrations of antimycin over 150 μM, clumping of neutrophils occurred; therefore, no values can be given for higher concentrations of antimycin.

These findings may contribute to our understanding of monocyte function. Monocytes kill bacteria less efficiently than PMN (8, 9, 33). The slower rate of phagocytosis by monocytes, confirmed with serum-treated zymosan, is often held responsible for this phenomenon. It may well be, however, that the lower rate of formation of bactericidal oxygen products by these cells also contributes to the difference in killing capacity. These findings would seem to lend support to the assumption that the role of monocytes in the killing of bacteria (and thus in the primary resistance to bacterial infections) is much less important than that of PMN. Possibly, the main function of monocytes is the "processing" and degradation of antigens in the afferent and efferent limb of the immune system, in which case there would be no need for a powerful intracellular bactericidal system.

The second question studied was the amount of the oxygen consumed which could be recovered as hydrogen peroxide. In phagocytosing PMN, this percentage amounted to ≅90%. In monocytes, we found a recovery of only ≅30% during the initial 10 min. This appeared to be due to a strong stimulation of mitochondrial respiration during phagocytosis of monocytes. Thus, a substantial amount of oxygen is used in the respiratory chain of these cells and not converted to reactive oxygen products.

It is noteworthy that blockade of the mitochondrial electron transport by antimycin leads to a complete recovery of oxygen as hydrogen peroxide in monocytes (Fig. 3). On the other hand, antimycin inhibits the ingestion of serum-treated zymosan almost completely (Fig. 6), without affecting the release of either superoxide (Fig. 5) or hydrogen peroxide (Fig. 4). Two conclusions may be drawn from these observations. First, it proves that actual ingestion of particles is not necessary to trigger monocytes for production of oxygen radicals. Second, the observation that a very large proportion of the highly instable superoxide anion is recovered in the incubation medium, points to the plasma membrane as the site of oxygen reduction, presumably first to superoxide and then, spontaneously, to hydrogen peroxide. These results are in accord with similar observations in PMN (27, 28, 34–39).

When several inhibitors of mitochondrial respiration were compared for their ability to reduce the respiratory burst in phagocytosing monocytes (Table II), cyanide and azide were clearly less effective than oligomycin and antimycin A. This was also demonstrated by the fact that antimycin inhibited the oxygen uptake of zymosan-stimulated monocytes as effectively in the presence as in the absence of azide (cf. Table II and Fig. 3). Both cyanide and azide inhibit

catalase, thus preventing dissociation of hydrogen peroxide into oxygen and water. The oxygen consumption observed is the result of the true oxygen uptake minus the oxygen formed in the reaction mediated by catalase. Thus, in PMN, azide and cyanide result in an apparent increase in cell respiration (17). In monocytes, this effect is counteracted by the inhibition of the mitochondrial respiration, which may explain the relatively small effect of azide and cyanide on the overall oxygen consumption. The difference in effect of cyanide and azide could be due to the influence of the pH on their inhibitory effect (40).

The finding that not only antimycin but also oligomycin inhibited the respiratory burst of monocytes proves that the respiratory chain in these cells is tightly coupled to ATP synthesis (phosphorylation). The observation that antimycin did not affect the formation of superoxide and hydrogen peroxide indicates that this process is not dependent on mitochondrial oxidation. This conclusion is in contrast to Miller's postulate for mouse peritoneal macrophages (41). Miller found a decrease in bactericidal activity of these cells in the presence of cyanide and antimycin, but not with oligomycin, and concluded that the respiratory chain provides at least part of the hydrogen peroxide needed for bacterial killing. Our results indicate, however, that bacterial killing may have been reduced as a result of the inhibition of bacteria uptake, although we would expect oligomycin to have the same effect. On the other hand, there may be a difference in killing mechanism between the two cell types.

The most striking metabolic difference between PMN and monocytes is the major role of oxidative phosphorylation in providing energy for phagocytosis in monocytes. In the presence of 150 μM antimycin, the ingestion of serum-opsonized zymosan by monocytes was depressed by ≅40%, but no effect was seen in PMN. Virtually complete inhibition of particle uptake by monocytes was noted at 360 μM antimycin. Cline and Lehrer (7) concluded that monocytes depend on glycolysis for phagocytosis: in their hands sodium fluoride and sodium monoiodoacetate inhibited particle ingestion, but cyanide and anaerobiosis did not. In human alveolar macrophages, however, phagocytosis was inhibited by low oxygen tension, cyanide, and inhibitors of glycolysis (42). We suggest that both peripheral blood monocytes and the alveolar macrophages presumably derived from them (43) depend on oxidative phosphorylation for their energy supply, whereas neutrophils and peritoneal macrophages do not (44).

It is difficult to ascribe a physiological function to our findings. Alveolar macrophages have been regarded as differentiated monocytes in that they rely more on oxidative phosphorylation in an environment of high oxygen tension (42), whereas peritoneal macrophages, living under less aerobic conditions, supposedly retain the monocyte property of dependence on glycolysis. Our results, on the other hand, show that monocytes are strongly dependent on oxidative phosphorylation. Thus, peritoneal macrophages may have "lost" their mitochondrial dependency during their differentiation from monocytes, possibly similar to the situation with PMN which mature from mitochondria-rich precursor cells in the bone marrow into peripheral blood cells with relatively few mitochondria (45). The dependence of monocytes on oxidative phosphorylation may indicate that these cells can only function in well-oxygenated areas, whereas PMN are able to do so in a more anaerobic environment, such as synovial fluid.

Thus, we conclude that phagocytosis activates two pathways of oxygen metabolism in monocytes. The major portion of the oxygen is used in the mitochondria, supplying the energy for particle ingestion. This process does not seem to be of critical importance in neutrophils. A minor portion of the oxygen consumed by monocytes is converted to superoxide and hydrogen peroxide. The latter reaction probably takes place in the plasma membrane and is insensitive to antimycin, in either monocytes or neutrophils.

ACKNOWLEDGMENT

We thank Dr. J. A. Loos and Dr. R. S. Weening for many fruitful discussions.

This study was supported in part by financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.), The Hague, The Netherlands (grant 91-5).

REFERENCES

1. Baldridge, C. W., and R. W. Gerard. 1933. The extra respiration of phagocytosis. *Am. J. Physiol.* **103:** 235–236.
2. Karnovsky, M. L. 1968. The metabolism of leukocytes. *Semin. Hematol.* **5:** 156–165.
3. Babior, B. M., R. S. Kipnes, and J. T. Curnutte. 1973. The production by leukocytes of superoxide, a potential bactericidal agent. *J. Clin. Invest.* **52:** 741–744.
4. Iyer, G. Y. N., D. M. F. Islam, and J. H. Quastel. 1961. Biochemical aspects of phagocytosis. *Nature (Lond.).* **192:** 535–541.
5. Johnston, R. B., Jr., B. B. Keele, M. P. Misra, J. E. Lehmeyer, L. S. Webb, R. L. Baehner, and K. V. Rajagopalan. 1975. The role of superoxide anion generation in phagocytic bactericidal activity. *J. Clin. Invest.* **55:** 1357–1372.
6. Klebanoff, S. J. 1975. Antimicrobial systems of the polymorphonuclear leukocytes. *In*: The Phagocytic Cell in Host Resistance. J. A. Bellanti and D. H. Dayton, editors. Raven Press, New York, 45–59.
7. Cline, M. J., and R. I. Lehrer. 1968. Phagocytosis by human monocytes. *Blood.* **32:** 423–435.
8. Baehner, R. L., and R. B. Johnston, Jr. 1972. Monocyte function in children with neutropenia and chronic infections. *Blood.* **40:** 31–41.
9. Steigbigel, R. T., L. H. Lambert, Jr., and J. S. Remington. 1974. Phagocytic and bactericidal properties of normal human monocytes. *J. Clin. Invest.* **53:** 131–142.

10. Sagone, A. L., Jr., G. W. King, and E. M. Metz. 1976. A comparison of the metabolic response to phagocytosis in human granulocytes and monocytes. *J. Clin. Invest.* **57:** 1352–1358.
11. Weiss, S. J. 1976. Hydroxyl radical (OH·) generation by human monocytes. *Fed. Proc.* **35:** 1396.
12. Nelson, R. D., E. L. Mills, R. L. Simmons, and P. G. Quie. 1976. Chemiluminescence response of phagocytizing human monocytes. *Infect. Immun.* **14:** 129–134.
13. Johnston, R. B., Jr., J. E. Lehmeyer, and L. A. Guthrie. 1976. Generation of superoxide and chemiluminescence by human monocytes during phagocytosis and on contact with surface-bound immunoglobulin G. *J. Exp. Med.* **143:** 1551–1556.
14. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* 21, suppl. **97:** 77–90.
15. Brutel de la Rivière, A., P. R. Verhoef-Karssen, J. J. F. M. de Wit, J. A. Loos, and H. K. Prins. 1977. Large scale preparation of leucocyte concentrates and further purification of lymphocytes and granulocytes with the IBM 2991 cell processor. *Transfusion.* **17:** 509–512.
16. de Wit, J. J. F. M., H. J. P. Henrichs, J. Odink, and H. K. Prins. 1975. Experiments on the preparation of blood components with the IBM 2991 blood cell processor. *Vox Sang.* **29:** 352–362.
17. Weening, R. S., D. Roos, and J. A. Loos. 1974. Oxygen consumption of phagocytizing cells in human leukocyte and granulocyte preparations. A comparative study. *J. Lab. Clin. Med.* **83:** 570–576.
18. Loos, J. A., F. B. Blok-Schut, B. Kipp, R. van Doorn, and L. Meerhof. 1976. Size distribution, electronic recognition and counting of human blood monocytes. *Blood.* **48:** 743–754.
19. Kwan, D., M. B. Epstein, and A. Norman. 1976. Studies on human monocytes with a multiparameter cell sorter. *J. Histochem. Cytochem.* **24:** 355–362.
20. Homan-Müller, J. W. T., R. S. Weening, and D. Roos. 1975. Production of hydrogen peroxide by phagocytizing human granulocytes. *J. Lab. Clin. Med.* **85:** 198–207.
21. Weening, R. S., R. Wever, and D. Roos. 1975. Quantitative aspects of the production of superoxide radicals by phagocytizing human granulocytes. *J. Lab. Clin. Med.* **85:** 245–252.
22. Roos, D., M. de Boer, and R. S. Weening. 1977. The role of calcium and magnesium in some functions of human neutrophils. *In* Movement, Metabolism and Bactericidal Mechanisms of Phagocytes. F. Rossi, P. L. Patriarca, and D. Romeo, editors. Piccin Editore Medical Books, Padova, Italy. 223–233.
23. Loos, J. A., and D. Roos. 1974. Ficoll-Isopaque gradients for the determination of density distributions of human blood lymphocytes and other reticulo-endothelial cells. *Exp. Cell Res.* **86:** 333–341.
24. Wong, L., and J. D. Wilson. 1975. The identification of Fc and C_3 receptor on human neutrophils. *J. Immunol. Methods.* **7:** 69–76.
25. McCord, J. M., and I. Fridovich. 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* **244:** 6049–6055.
26. 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.
27. Roos, D., M. L. J. van Schaik, R. S. Weening, and R. Wever. 1977. Superoxide generation in relation to other oxidative reactions in human polymorphonuclear leukocytes. *In* Superoxide and Superoxide Dismutases. A. M. Michelson, J. M. McCord, and I. Fridovich, editors. Academic Press Inc. Ltd., London. 307–316.
28. Curnutte, J. T., and B. M. Babior. 1975. Effects of anaerobiosis and inhibitors on O_2^- production by human granulocytes. *Blood.* **45:** 851–861.
29. Klebanoff, S. J., and S. H. Pincus. 1971. Hydrogen peroxide utilization in myeloperoxidase deficient leukocytes: a possible microbicidal control mechanism. *J. Clin. Invest.* **50:** 2226–2229.
30. Holmes, B., A. R. Page, and R. A. Good. 1967. Studies of the metabolic activities of leukocytes from patients with a genetic abnormality of phagocytic function. *J. Clin. Invest.* **46:** 1422–1432.
31. Baehner, R. L., N. Gilman, and M. L. Karnovsky. 1970. Respiration and glucose oxidation in human and guinea pig leukocytes: comparative studies. *J. Clin. Invest.* **49:** 692–700.
32. Klebanoff, S. J., and C. B. Hamon. 1972. Role of myeloperoxidase-mediated antimicrobial systems in intact leukocytes. *J. Reticuloendothel. Soc.* **12:** 170–196.
33. Cline, M. J. 1970. Bactericidal activity of human macrophages. Analysis of factors influencing the killing of *Listeria monocytogenes*. *Infect. Immun.* **2:** 156–161.
34. Briggs, R. T., M. L. Karnovsky, and M. J. Karnovsky. 1975. Cytochemical demonstration of hydrogen peroxide in polymorphonuclear leukocyte phagosomes. *J. Cell Biol.* **64:** 254–260.
35. Goldstein, I. M., D. Roos, H. B. Kaplan, and G. Weissmann. 1975. Complement and immunoglobulins stimulate superoxide production by human leukocytes independently of phagocytosis. *J. Clin. Invest.* **56:** 1155–1163.
36. Takanaka, K., and P. J. O'Brien. 1975. Mechanisms of H_2O_2 formation by leukocytes. *Arch. Biochem. Biophys.* **169:** 428–435.
37. Roos, D., J. W. T. Homan-Müller, and R. S. Weening. 1976. Effect of cytochalasin B on the oxidative metabolism of human peripheral blood granulocytes. *Biochem. Biophys. Res. Commun.* **68:** 43–50.
38. Goldstein, I. M., M. Cerqueira, S. Lind, and H. B. Kaplan. 1977. Evidence that the superoxide-generating system of human leukocytes is associated with the cell surface. *J. Clin. Invest.* **59:** 249–254.
39. Briggs, R. T., M. L. Karnovsky, and M. J. Karnovsky. 1977. Hydrogen peroxide production in chronic granulomatous disease. A cytochemical study of reduced pyridine nucleotide oxidase. *J. Clin. Invest.* **59:** 1088–1098.
40. Chance, B. 1952. The effect of pH upon the equilibria of catalase compounds. *J. Biol. Chem.* **194:** 483–496.
41. Miller, T. E. 1971. Metabolic events involved in the bactericidal activity of normal mouse macrophages. *Infect. Immun.* **3:** 390–397.
42. Cohen, A. B., and M. J. Cline. 1971. The human alveolar macrophage: isolation, cultivation in vitro and studies of morphologic and functional characteristics. *J. Clin. Invest.* **50:** 1390–1398.
43. Furth, R. van. 1970. Origin and kinetics of monocytes and macrophages. *Semin. Hematol.* **7:** 125–141.
44. Stubbs, M., A. V. Kühner, E. A. Glass, J. R. David, and M. L. Karnovsky. 1973. Metabolic and functional studies on activated mouse macrophages. *J. Exp. Med.* **137:** 537–542.
45. Wintrobe, M. M., G. R. Lee, D. R. Boggs, T. C. Bithell, J. W. Athens, and J. Foerster. 1974. *In* Clinical Hematology. 7th edition. Lea & Febiger, Philadelphia. 228–229.