Kinetics of Phagocytosis of Staphylococcus aureus by Alveolar and Peritoneal Macrophages

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The rate of uptake of radiolabeled Staphylococcus aureus by macrophages in vitro was studied by use of Lineweaver-Burk analysis. It was found that competition for ingestion by excess unlabeled particles, either staphylococci or unrelated particles, resulted in diminished uptake of the labeled particles and that opsonization of particles with specific antiserum enhanced that uptake solely by altering the maximum velocity of uptake (\(V_{\text{max}}\)). Uptake of radiolabeled staphylococci opsonized with specific antiserum was not inhibited by excess numbers of unopsonized organisms; the ingestion was inhibited by excess numbers of opsonized unlabeled organisms, and that inhibition was characterized by depression of \(V_{\text{max}}\). Inhibition of phagocytosis by iodoacetate and cytochalasin B resulted from depression in both \(V_{\text{max}}\) and Michaelis constant (\(K_m\)). In addition, the phagocytic function of macrophages improved during in vitro culture, a phenomenon which was particularly striking for alveolar macrophages. That enhancement of activity resulted from improvements in both \(V_{\text{max}}\) and \(K_m\). Addition of opsonizing antibody at any stage of in vitro maturation resulted in further increases in phagocytic uptake, increases which affected only \(V_{\text{max}}\). The in vitro maturation of phagocytic function by alveolar macrophages could be inhibited by both 2-deoxy-D-glucose and cycloheximide, but not by culture in hypoxia. The data indicate that the terms of Lineweaver-Burk analysis can be correlated with functional aspects of phagocytosis and that \(V_{\text{max}}\) represents the avidity of the macrophage surface for the particle, whereas \(K_m\) is an index of the capacity of the cell for ingestion.

Although phagocytosis was shown to be susceptible to analysis of kinetics by Lineweaver-Burk plots over a decade ago (23), there have been few subsequent studies in which the method was used in studies of ingestion (10, 20, 24) and only a single attempt (20) to relate the kinetic terms of the graphic representation to biological aspects of phagocyte function. Characterization of inhibitors as competitive or non-competitive on the basis of double-reciprocal plots has seldom been attempted, and the functional meaning of those terms in phagocytic activity has not been delineated. Stossel (20) has shown that both divalent cations and heat-labile opsonin enhance phagocytosis solely by increasing the maximum velocity of uptake (\(V_{\text{max}}\)) without altering the Michaelis constant (\(K_m\)), or "affinity," of the reaction. He concluded that these stimulators act primarily by enhancing "the work of engulfment," i.e., formation of pseudopodia and endocytic vacuoles, rather than by enhancing cell-particle affinity. It is difficult to envision the mechanisms whereby opsonization would enhance uptake without there having been enhanced cell-particle affinity expressed at the level of the membrane-associated Fc and complement receptors (7). However, the assumption that the mathematical constructs of Lineweaver-Burk plots represent the biological functions seemingly implied by the terms "maximum velocity" (rapidity of ingestion) and "affinity" (cell-particle affinity) is not based on data obtained in phagocytic systems.

It therefore seemed appropriate to undertake further experiments to attempt to define data derived from Lineweaver-Burk plots in terms of phagocytic cell function. In this report are described experiments, in which a variety of agents were used as inhibitors of phagocytosis of radiolabeled Staphylococcus aureus, that strongly suggest that maximum velocity of uptake reflects not the work of phagocytosis but the avidity of the cell surface for the particle and that the \(K_m\) (affinity), in contrast to its apparent meaning, reflects work of phagocytosis rather than cell-particle affinity. Furthermore, the utility of double-reciprocal analysis in discriminating among different modes of inhibition or en-
hancement of phagocytic function is demonstrated for both alveolar and peritoneal macrophages.

**MATERIALS AND METHODS**

Peritoneal macrophages were obtained from outbred CF-1 mice (Charles River Breeding Laboratories, Wilmington, Mass.), and alveolar macrophages were obtained by bronchial lavage from outbred Sprague-Dawley rats, using methods previously described (3, 12). Peritoneal macrophages from rats and alveolar macrophages from mice were usually unsuitable for these experiments, because freshly explanted preparations were frequently contaminated with significant numbers of other cell types. Cells were maintained as monolayers containing approximately 3 × 10^5 macrophages in Leighton tubes without cover slips in medium 199 supplemented with 20% heat-inactivated newborn calf serum, penicillin, and gentamicin (TCM), in an atmosphere of air and 5% carbon dioxide. Cells were cultured in vitro for up to 3 days before addition of particles. *S. aureus* 502A, grown in medium 199 with 1.0 μCi of [methyl-3H]thymidine (specific activity, 59 Ci/mmol; Schwarz/Mann, Orangeburg, N. Y.) per ml overnight at 37°C, was rinsed four times with sterile phosphate-buffered saline, pH 7.4, and suspended in medium 199 at a concentration of approximately 5 × 10^6 colony-forming units (CFU) per ml in antibiotic-free TCM. Bacteria so labeled and rinsed lost less than 5% of their radiolabel during a 2-h incubation in antibiotic-free TCM at 37°C; macrophages incubated for 1 h with that medium incorporated no detectable radiolabel.

Radiolabeled bacteria were added to macrophage monolayers to result in concentrations of 5 × 10^3 to 5 × 10^5 CFU/ml in antibiotic-free TCM. Phagocytosis was terminated at intervals by removing the majority of undigested organisms with four rinses of phosphate-buffered saline; residual uninternalized staphylococci were lysed by a 10-min exposure to lysozymin (Schwarz/Mann) (8 U/ml), and the tubes were rinsed an additional four times with phosphate-buffered saline. Monolayers were then digested with 0.1 N NaOH. Cell-associated radioactivity was determined by liquid scintillation counting in dioxane, protein was determined by the method of Lowry et al. (11) with crystalline egg white lysozyme as the standard, and uptake of bacteria and radiolabel was normalized for total monolayer protein. Samples of the radiolabeled inocula were processed for scintillation counting and quantitative culture to determine radioactivity per CFU.

The effects of a variety of drugs and particles, specific antiserum, and in vitro differentiation on the phagocytic activities of both alveolar and peritoneal macrophages were evaluated. Competing particulates, added at the same time as the radiolabeled staphylococci, included unlabeled *S. aureus* 502A (10^5 CFU/ml), unlabeled *Escherichia coli* (10^5 CFU/ml), and polyvinyltoluene spherules (2 μm in diameter, 10^5/ml). Soluble compounds known to be or suspected of being capable of inhibiting ingestion of *S. aureus* included: D-ribose (100 mM), D-glycerol (100 mM), iodoacetate (100 μM), cytochalasin B (10 μg/ml), crude lipoteichoic acid (150 mg/ml) extracted from *S. aureus* 502A by using published techniques (6), and purified lipoteichic acid (150 mg/ml) graciously provided by T. S. Theodore, National Institutes of Health, Bethesda, Md. Staphylococci were opsonized with specific anti- serum, which had been raised in rabbits (tube agglutination titer of 1:1,280), for 1 h at 4°C, rinsed thrice with vigorous mixing, and suspended to appropriate concentrations in phosphate-buffered saline.

In addition, the kinetics of phagocytosis by macrophages (in particular alveolar macrophages, which underwent the greatest changes) at different periods of in vitro differentiation were assessed, as were the effects of potential inhibitors of that differentiation, namely, hypoxia (O_2 pressure, 2.0 kPa), 2-deoxy-D-glucose (2-DG) (50 μM), and cycloheximide (1 μM). At that concentration, cycloheximide inhibited protein synthesis by 50 to 60% as determined by measurement of incorporation of tritiated leucine into trichloroacetic acid-precipitable macrophage protein (15). These inhibitors were added to macrophage monolayers after 1 h of in vitro cultivation and were present through a 3-day in vitro culture. At least 1 h before addition of particles, the medium was aspirated, the tubes were rinsed with warm medium 199, and fresh antibiotic-free TCM was added. Phagocytosis occurred in inhibitor-free tissue culture medium at ambient oxygen tension.

Calculated rates of uptake (CFU per milligram of monolayer protein per minute) (V) and measured concentrations (CFU per milliliter) (S) of radiolabeled staphylococci were converted to their reciprocals, and straight lines were generated by the method of least mean squares from a minimum of 10 points obtained from at least two and usually more than three separate experiments. In the figures, points represent means of values obtained from three or more separate experiments. Significance of differences between slopes and y-intercepts of lines was determined by analysis of variance; only significantly (P ≤ 0.05) different lines are considered different in the text.

**RESULTS**

*S. aureus* 502A, an unencapsulated strain, was chosen for these experiments because in preliminary experiments it was found that: the bacterium was readily labeled with [3H]thymidine, the radiolabel was stably associated with the bacteria for the duration of the experiments, any contribution of uninternalized organisms to measure uptake could be virtually eliminated by treatment of macrophage monolayers at termination of phagocytosis with lysozymin, and uptake of the organisms by macrophage monolayers was linear for at least the first 60 min after addition of particles in concentrations as high as 5 × 10^6 CFU/ml. Since with the higher inocula uptake declined slightly after 60 min of incubation, only data derived from the first 60 min of incubation were used in calculation of rates of uptake. In addition, it was observed that during 1 h of phagocytosis no more than 3% of the initial inoculum was interiorized; equating substrate concentration with initial inoculum...
thus introduced a negligible error into subsequent calculations. Estimation of macrophage numbers by use of an assay for protein has been used previously (15), and in these experiments none of the manipulations significantly altered the ratio of cell numbers to protein. The ratios of protein per cell varied between 83% (alveolar macrophages cultured for 3 days in 2-DG, cycloheximide, or nitrogen) and 109% (peritoneal macrophages cultured in TCM without additives) of the values calculated for freshly explanted mouse peritoneal macrophages; neither extreme differed significantly (P > 0.3) from unity. When rates of uptake were normalized for cell numbers by protein assay, it was found that \( V_{\text{max}} \) and \( K_m \) were constant when between 1 \( \times \) \( 10^6 \) and 6 \( \times \) \( 10^6 \) peritoneal macrophages were present.

Peritoneal macrophages. As had been shown in other systems (1-4), phagocytosis of \( S. \) \textit{aureus} by peritoneal macrophages displayed Lineweaver-Burk kinetics (Fig. 1). Solubilized surface components of staphylococci (21), such as lipoteichoic acid (150 mg/ml), D-ribose (100 mM), and D-glycerol (100 mM), did not alter the kinetics of ingestion of staphylococci by macrophages (Fig. 1A). The concentrations chosen for each agent were based on published data which indicated that adherence of organisms to mucosal cells (2, 14) and phagocytosis of \( E. \) \textit{coli} by polymorphonuclear leukocytes (F. J. Silverblatt, J. Dreyer, and S. Schauer, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N. Y., abstr. no. 212, 1977) and macrophages (1) were inhibited at similar concentrations. Higher concentrations caused marked morphological alterations in macrophages as early as 4 h after addition and were not evaluated for effects on kinetics of uptake.

Excesses of unlabeled particles, however, markedly and noncompetitively inhibited uptake of radiolabeled staphylococci (Fig. 1). When concentrations of two strains of bacteria (\( S. \) \textit{aureus} 502A and \( E. \) \textit{coli}) and of latex spherules were adjusted so that similar volumes of added competing particles were added (23), both homologous and heterologous particles noncompetitively inhibited uptake of the radiolabeled organisms to approximately the same extent (Fig. 1B). There was thus no apparent recognition of differences among these particle types; the three types of ingestible particles appeared to compete only as ingestible particles per se.

Opsonization of staphylococci with specific an-

![Figure 1](http://iai.asm.org/Downloaded_from)
tiserum did not enhance phagocytosis of the organisms by peritoneal macrophages which had been maintained in culture for 3 or more days before challenge (Fig. 2), although the same antiserum greatly enhanced uptake of the organisms by alveolar macrophages (see below). In marked contrast to the results observed during ingestion of unopsonized organisms, ingestion of opsonized organisms was not inhibited by concurrent addition of excess unlabeled particles (Fig. 2). However, inhibition was observed if opsonized unlabeled staphylococci served as competing unlabeled particles (Fig. 2), and, as was the case with competition for ingestion among unopsonized particles, the observed inhibition was noncompetitive.

Inhibition of phagocytosis of radiolabeled staphylococci by iodoacetate differed in character from the inhibition by excess particles observed in the previous experiments. Whereas addition of excess particles inhibited phagocytosis by reducing only the $V_{\text{max}}$, inhibition of phagocytosis by iodoacetate resulted in diminished $V_{\text{max}}$ and diminished $K_m$ (Fig. 3A). That the effect of iodoacetate was clearly distinct from that of competition for ingestion by excess particles is evident in Fig. 3A. Phagocytosis of staphylococci by macrophages was seen to be noncompetitively inhibited by latex spherules whether or not the phagocytosis was also partially inhibited by iodoacetate. Similarly, cytochalasin B, which inhibits phagocytosis primarily through disruption of microfilaments (5), acted as an uncompetitive inhibitor (Fig. 3B), as did cytochalasin D (4 Hg/ml) and sodium fluoride (5 mM) (data not shown).

**Alveolar macrophages.** Although the rate of ingestion of staphylococci by peritoneal macrophages increased after in vitro culture for several days, the magnitude of the change was relatively small and difficult to analyze. Alveolar macrophages, however, from both rats and mice, demonstrated marked enhancement of phagocytic activity after in vitro culture. Maximal activity was reached after 48 to 72 h of culture, and thereafter remained stable through 14 days. This enhancement of phagocytic activity accompanying in vitro culture of macrophages resulted from increases in both $K_m$ and $V_{\text{max}}$ (Fig. 4).

Oxidation of staphylococci with specific antiserum enhanced the uptake of the organisms by alveolar macrophages of any in vitro age (Fig. 4), although the difference observed with 3-day-old cells was of doubtful ($P \leq 0.1$) statistical significance. As had been noted in studies of the effects of heat-labile opsonins (20), specific opsonin enhanced phagocytosis only by increasing the $V_{\text{max}}$ without altering the $K_m$ appropriate for cells of a given in vitro age. The inhibitory effects of excess particulates, both opsonized and unopsonized, and of iodoacetate were essentially indistinguishable from those observed in the previous experiments with peritoneal macrophages (data not shown).

To provide more information with which to define kinetic data in terms of macrophage function and to gain preliminary information concerning the metabolic requirement for the in vitro development of phagocytic function, the effects of selected inhibitors of glucose metabolism (2-DG), protein synthesis (cycloheximide), and oxidative metabolism (hypoxia) were assessed. When macrophages were cultured in an hypoxic environment ($O_2$ pressure, 2.0 kPa) for 72 h, the expected maturation of phagocytic activity was noted (Fig. 5). However, prolonged in vitro cultivation of macrophages in either cycloheximide or 2-DG resulted in marked inhibition of maturation of phagocytic activity. Alveolar macrophages cultured in medium containing 2-DG (50 Hg/ml) failed to develop the expected rise in either $V_{\text{max}}$ or $K_m$ (Fig. 5); functionally, after 3 days in culture, they were indistinguishable from freshly explanted cells. The inhibitory effect of 2-DG was abolished if excess glucose (100 mM) or mannose (100 mM) was also present during maturation. Alveolar mac-

![Fig. 2. Kinetics of ingestion of specifically opsonized $[^{3}H]$thymidine-labeled S. aureus ($[^{3}H]$-SA) by mouse peritoneal macrophages in vitro. In the absence of competing particles, uptake of opsonized $[^{3}H]$-SA (---) is indistinguishable from that of unopsonized $[^{3}H]$-SA (Fig. 1). Addition of unopsonized unlabeled staphylococci (----) did not significantly alter the kinetics of uptake of unopsonized $[^{3}H]$-SA, but addition of opsonized unlabeled staphylococci (---) noncompetitively inhibited ingestion of the labeled particles.](http://iai.asm.org/)
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FIG. 3. Impairment of ingestion of [3H]thymidine-labeled S. aureus (3H-SA) by iodoacetate (10^{-4} M) and polyvinyltoluene spherules (PVT). (A) Addition of latex spherules noncompetitively inhibited ingestion of 3H-SA by macrophages not exposed to iodoacetate (-, no PVT; ----, + PVT; as in Fig. 1) and by macrophages in which ingestion was already partially inhibited by iodoacetate (○, ----, no PVT; ○, ----, + PVT). Conversely, iodoacetate uncompetitively inhibited ingestion of 3H-SA by macrophages whether or not that ingestion was also partially inhibited by PVT. (B) Addition of cytochalasin B (■, ----) uncompetitively inhibited ingestion of 3H-SA by macrophages (----, uptake by control macrophages).

FIG. 4. Improvement in phagocytic function of rat alveolar macrophages after in vitro cultivation. Kinetics of uptake of [3H]thymidine-labeled S. aureus by freshly explanted alveolar macrophages (----) were markedly slower than those of alveolar macrophages which were cultured in vitro for 3 days before addition of particles (----). Uptake of 3H-labeled S. aureus was enhanced by opsonization with specific antiserum at both times (○, no antiserum; □, + antiserum).

FIG. 5. Effects of cultivation for 3 days in hypoxia and 2-DG on maturation of phagocytic activity of alveolar macrophage. Uptake of unopsonized [3H]thymidine-labeled S. aureus by alveolar macrophages cultured in hypoxia (△, ---) was indistinguishable from that of control 3-day-old cells (----), whereas that of those grown in 2-DG (■, ---) was similar to that of freshly explanted cells (----).
Macrophages cultured for 3 days in medium containing cycloheximide at a concentration of 1 μM, a dose which inhibited protein synthesis by approximately 50%, also did not develop the expected increase in phagocytic activity (Fig. 6). The impairment of phagocytic activity in cycloheximide-treated macrophages resulted solely from inhibition of the expected improvement in $V_{\text{max}}$; the $K_m$ was similar to that characteristic of control macrophages cultured in vitro for 3 days.

The data from the experiments outlined above may be summarized as follows. (i) Ingestion of labeled unopsonized particles was noncompetitively inhibited by excess unlabeled particles; i.e., the $V_{\text{max}}$ was decreased, but the $K_m$ was not altered. (ii) Ingestion of labeled particles was noncompetitively inhibited by inhibitors of glycolysis and microfilament poisons; i.e., both $V_{\text{max}}$ and $K_m$ were decreased. (iii) The inhibition of phagocytosis of labeled particles by excess unlabeled particles and that caused by iodoacetate were independent and could be superimposed upon one another. (iv) Opsonization of particles with specific antiserum, which allows ingestion to be mediated by the Fc receptor (17), enhanced ingestion of labeled particles by increasing $V_{\text{max}}$ without altering $K_m$. (v) Phagocytosis of particles opsonized with specific antiserum was not inhibited by excess unopsonized particles, but was inhibited noncompetitively by excess opsonized unlabeled particles. (vi) Macrophages which had been cultured in vitro were more phagocytically active than were freshly explanted macrophages, an increased activity which resulted from increases in both $V_{\text{max}}$ and $K_m$.

**DISCUSSION**

The kinetics of uptake of radiolabeled staphylococci by macrophages were found to be capable of either uncompetitive inhibition (parallel shifts in both $K_m$ and $V_{\text{max}}$) or noncompetitive inhibition (shift in $V_{\text{max}}$ without shift in $K_m$); no example of competitive inhibition was found.

The presence in the medium during phagocytosis of excess unlabeled particles, either homologous or heterologous, noncompetitively inhibited phagocytosis, whereas specific antiserum, at least in alveolar macrophages, noncompetitively enhanced the process. Although it is not strictly correct to apply Lineweaver-Burk analysis to the study of competition by excess homologous particles, that was done in order that the effects could be compared with those of heterologous particles and contrasted with the lack of effect of soluble staphylococcal surface components (Fig. 1). In contrast, uncompetitive inhibition resulted when iodoacetate, fluoride, or cytochalasins were present during phagocytosis, whereas in vitro maturation, most prominently for alveolar macrophages, resulted in uncompetitive enhancement of uptake. Whereas most of the observations could be explained in isolation by a variety of alternate explanations of the plots, taken together, they suggest that the aspects of the phagocytic act which functionally correlate with $V_{\text{max}}$ and $K_m$ differ from those previously proposed (20). It is suggested that $V_{\text{max}}$ reflects the avidity of cell-particle interaction at the cell surface rather than the capacity of the cell for ingestive work and that $K_m$ is an index of the cell’s capacity for phagocytic work. These proposed interpretations are compatible with the definitions of the terms of Lineweaver-Burk plots; it should be especially noted that $K_m$, sometimes termed “affinity,” has no necessary relation to surface attractiveness of cells and particles, but indicates only the con-
centation of particles at which ingestion is half-maximal.

V_{max} was found to be increased in alveolar macrophages by opsonization of particles with specific antiserum and, probably by a dilutional effect, was reduced when ingestible unlabeled particles were present. The enhanced phagocytic activity resulting from opsonization of particles presumably resulted from mediation of attachment and ingestion by Fc receptors (7, 9, 17), a process which was noncompetitively inhibited by unlabeled opsonized, but not unopsonized, particles. This preferential ingestion of specifically opsonized particles was observed for both alveolar and peritoneal macrophages and is particularly difficult to reconcile with an interpretation of kinetic analysis which suggests that the alteration in V_{max} resulting from opsonization affects the cells' capacity for phagocytic work. Since the effects of opsonins are reflected only in alterations in V_{max}, and the above considerations suggest that the primary effects of opsonization are at the cell surface, it appears that V_{max} is best considered an index of cell-particle interaction at the cell surface.

Similarly, the data indicate that the K_m cannot be construed as representing the affinity of phagocyte for particle. Depression of K_m was found when inhibitors of anaerobic glycolysis (13) iodoacetate and fluoride and the microfilament poisons (5) cytochalasins B and D were present in the medium at concentrations chosen to result in submaximal inhibition. Although, as in any study with inhibitory compounds, it is not possible to be absolutely certain that the primary metabolic effect is the cause of the functional defect under consideration, it is reasonable to propose that similar effects on kinetics of phagocytosis imply that a similar stage in the phagocytic act was inhibited. Since uncompetitive inhibitors of phagocytosis inhibited either the generation of energy for phagocytosis or the translation of that energy into actual interiorization, it is inferred that uncompetitive inhibitors are agents which interfere with the capacity of the cell for ingestive work. And, because an inhibitor which acts primarily by reducing K_m must, of necessity, reduce V_{max}, it is further proposed that these agents are altering K_m primarily, which is then an index of the cell's capacity for phagocytic work. This last suggestion is speculative and should not be considered to be firmly established on the basis of available data.

The application of Lineweaver-Burk analysis to in vitro maturation of alveolar macrophage phagocytic function serves to further illustrate some of the points already raised. Freshly explanted alveolar macrophages phagocytose a wide variety of particles relatively poorly (4, 16, 18, 22), both in comparison with peritoneal macrophages and in comparison with alveolar macrophages that have been cultured in vitro. The improvement in phagocytosis after in vitro maturation (17, 22) results from increases in both V_{max} and K_m and can be likened to release from an uncompetitive inhibitor. Such an improvement could have resulted solely from an increase in ingestive capacity (K_m) with the increase in apparent V_{max} following as a necessary consequence, or in vitro maturation could have caused independent increases in both ingestive capacity and surface avidity. That partial inhibition of protein synthesis with cycloheximide allowed the normal increment in K_m to be manifest while substantially, if not completely, inhibiting the expected enhancement in V_{max} suggests that the improvement in function resulted from separate and presumably independent biochemical events. It also suggests that development of surface avidity during culture requires active protein, possibly glycoprotein (8), synthesis.

Chronic exposure of the differentiating macrophages to 2-DG, an inhibitor of glycolysis, abrogated the expected enhancement in both surface avidity and ingestive capacity. That the inhibition of maturation by 2-DG was mediated by inhibition of glucose metabolism is suggested by the observation that the effect of 2-DG was negated by inclusion of excess glucose in the medium. By analogy with the experiments with iodoacetate and sodium fluoride, it is postulated that the inhibition resulted from inhibition of maturation of ingestive capacity. However, the possibility of a dual action of 2-DG, affecting both glucose metabolism and glycoprotein synthesis (19), cannot be rigorously excluded at this point.

In addition, use of Lineweaver-Burk analysis allowed for clear separation of the effects of in vitro maturation and specific opsonization on alveolar macrophage function. Phagocytic activity of freshly explanted alveolar macrophages was low and was improved after either opsonization with specific antiserum or in vitro maturation of the macrophages, and the net result, that more particles were ingested per unit time, was the same after either alteration. However, specific opsonins increased particle uptake solely by increasing the surface avidity of macrophages (V_{max}), whereas in vitro maturation caused improvement in both V_{max} and K_m. The effects of the two alterations were independent in that opsonization increased V_{max} of phagocytosis by alveolar macrophages of any in vitro age. Thus, irrespective of the base-line function of the alveolar macrophages, addition of specific antiserum enhanced phagocytosis, although the in-
crease in numbers of particles ingested by 3-day-old cells was small and statistically insignificant.

Application of kinetic analysis to studies of alterations in phagocytosis in other experimental and perhaps clinical situations should allow for more accurate delineation of the modes of action of inhibitors or of the basis of functional impairment of defective phagocytes.

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LITERATURE CITED


