MODIFICATION OF ZYMOSAN-INDUCED RELEASE OF LYSOSOMAL ENZYMES FROM HUMAN POLYMORPHONUCLEAR LEUKOCYTES BY CYTOCHALASIN B

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ABSTRACT

During the process of phagocytosis, polymorphonuclear leukocytes (PMN) release lysosomal enzymes into the extracellular medium. When the antibiotic cytochalasin B (CB) is present in the incubation medium along with phagocytable particles, enhanced recovery of enzyme activities from the incubation medium has been observed. These findings have led to the interpretation that CB enhances lysosomal enzyme release. Our results contradict this interpretation. The lysosomal enzymes acid phosphatase and β-galactosidase are unstable after they are released from cells. During the first 5–15 min of phagocytosis, significant amounts of both acid phosphatase and β-galactosidase can be recovered from the extracellular medium. After this, the recovery of enzyme from the medium declines, presumably because the rate of loss of lysosomal enzyme activity exceeds the rate of release at later time periods. In the presence of CB, the appearance of lysosomal enzymes in the extracellular medium of cells exposed to zymosan is retarded for 5–10 min, after which it begins and then continues for approximately 20 min. At the end of a 30-min incubation period, therefore, in the absence of CB, extracellular levels of lysosomal enzymes (especially those which are unstable) are declining toward low levels while, in the presence of CB, extracellular enzyme levels are continuing to rise. We also measured the lysosomal enzyme remaining within cells after exposure to zymosan. CB retarded the disappearance of enzyme from cells and resulted in significantly less total cell enzyme loss. Thus, in the presence of CB, a greater proportion of the lysosomal enzyme lost from cells is recovered in the extracellular medium. In contrast to the previous conclusions that CB enhances lysosomal enzyme release, our results indicate that CB delays and decreases the zymosan-stimulated release of lysosomal enzymes from PMN. Since CB inhibits phagocytosis by PMN, our results indicate that the antibiotic modifies the mechanism of release of lysosomal enzymes, resulting in zymosan stimulation of their release independently of phagocytosis.

INTRODUCTION

Lysosomal enzymes are released from polymorphonuclear leukocytes (PMN)[1] when the cells are stimulated in any of several ways. Reports from several laboratories have indicated that the amounts of lysosomal enzyme recovered from the incubation medium of cells stimulated for prolonged periods are increased when cytochalasin B (CB) is also present. CB enhances the recovery from extracellular medium of lysosomal enzymes released from rabbit PMN exposed to *Escherichia coli* (1), from rabbit and human PMN adherent to immune complexes attached to surfaces too large to be phagocytosed (2), from rabbit PMN exposed to latex particles coated with rabbit gamma globulin (3), and from human PMN exposed to zymosan particles (4, 5) or immune complexes on a nonphagocytable Millipore filter (4). These effects of CB on lysosomal enzyme release occur in spite of inhibition of phagocytosis and of increased carbohydrate metabolism associated with particle uptake by PMN. The experiments reported here were done to characterize the effect of CB on lysosomal enzyme release and elucidate the mechanism of the effect of the antibiotic.

MATERIALS AND METHODS

Zymosan (Sigma Chemical Co., St. Louis, Mo.) was suspended in saline and boiled. The suspension was cooled to 37°C, and an equal volume of plasma from the donor of cells used in the experiment was added. After the mixture had been incubated at 37°C for 1 h, the zymosan was collected by centrifugation, washed twice with Krebs-Ringer phosphate (KRP) buffer (6), and suspended in the buffer for incubation. A stock solution (1 mg/ml) of CB (Imperial Chemical Industries) was prepared in dimethylsulfoxide and diluted with buffer to give the desired experimental concentrations. [*Carboxyl*-^{14}C]inulin was purchased from Amersham/Searle Corp. (Arlington Heights, Ill.). Trace amounts (0.5 μCi/0.5 ml) were used in the incubation mixtures.

Human peripheral blood leukocytes were isolated from normal male donors. 1 vol of 6% dextran was added to 5 vol of heparinized blood to facilitate sedimentation of erythrocytes. The plasma was removed and centrifuged at 250 *g* for 10 min. The white cell pellet which was obtained was suspended in 10 ml of plasma and mixed with 30 ml of 0.87% NH_4Cl to lyse residual erythrocytes (7). The mixture was centrifuged, and white cells were washed twice in KRP buffer containing 0.01% gelatin, and suspended in buffer before addition of cells to incubation tubes. The final suspension contained 1–2 $\times$ 10^7 cells/0.2 ml (80–95% PMN).

When cells were preincubated with CB, 0.2 ml of cell suspension was added to tubes containing 0.2 ml of CB to give the desired final concentration. After preincubation, 0.1 ml of zymosan suspension was added and the incubation continued. In other experiments, 0.2 ml of cell suspension was added to tubes containing 0.3 ml of buffer with the appropriate additions. Glucose, when present (see figures), was used at a final concentration of 5 mM. All incubations were done in triplicate. At the end of the desired incubation time, tubes containing cells were placed in an ice bath, and 1 ml of KRP buffer containing nonradioactive inulin, 1 mg/ml, was added. The cells were transferred to 5-ml siliconized conical centrifuge tubes with two 1-ml washes of inulin in buffer and centrifuged at 250 *g* for 10 min. The supernate was saved for the determination of enzyme activities, and the cell pellet saved for the determination of either [^{14}C]inulin content or cell-associated enzyme activity.

The uptake of [^{14}C]inulin (presumably into phagocytic vesicles) served as an index of phagocytosis (5, 8). Inulin can also enter cells in pinocytic vesicles. In these experiments, [^{14}C]inulin taken up by cells not exposed to zymosan was less than 10% of the amount that entered cells incubated with zymosan, 1 mg/ml, for 30 min.

In studies of the effects of CB on the stability of lysosomal enzymes, cells were incubated with zymosan (3 mg/ml) for 10 min in the absence of CB or for 20 min with CB (10 μg/ml). Cells were separated from incubation medium by centrifugation. 0.5-ml aliquots of medium were pipetted into tubes containing 0.01 ml of either KRP buffer or buffer containing CB (final concentration 40 μg/ml) and incubated for 20, 40, or 60 min. In a similar experiment, cells were first incubated with zymosan (1 mg/ml) for 30 min and then separated from medium by centrifugation. The cell pellet was suspended in KRP buffer containing 0.2% Triton and lysed by sonication. The lysate was then diluted with buffer to give enzyme activities similar to those in the incubation medium from which cells had been removed. Aliquots of medium or diluted cell lysate were incubated as before, either with or without CB (20 μg/ml) for up to 90 min. Incubations were stopped by the addition of 6 vol of chilled buffer. The contents of the incubation tubes were frozen, stored, and thawed at a later time for enzyme assays.

The activity of β-glucuronidase was measured as previously described (7), using phenolphthalein glucuronide as substrate. For acid phosphatase determinations, *p*-nitrophenyl-phosphate served as substrate, and for β-galactosidase determinations *p*-nitrophenyl-galactoside was used. Measurements of lactic dehydrogenase in extracellular medium (9) showed that less than 2% of the total activity of that enzyme was released in all experiments, indicating negligible cell death (10). For all

[1] *Abbreviations used in this paper:* CB, cytochalasin B; KRP, Krebs-Ringer phosphate; LDH, lactic dehydrogenase; PMN, polymorphonuclear leukocytes.

determinations done on incubation medium, blanks measured on tubes removed at zero time were subtracted from other values. Total enzyme activity and cell-associated enzyme activity were determined in cell suspensions lysed by Triton X-100 (10).

RESULTS

In order to explore the specificity of the effect of CB upon zymosan-stimulated lysosomal enzyme release, we measured the effect of the antibiotic on the release of β-glucuronidase, β-galactosidase, and acid phosphatase. In a series of preliminary experiments, cells obtained from 17 different donors were incubated on 20 occasions either in buffer alone or in the presence of zymosan, 1 mg/ml (Fig. 1). Enzyme activity in the medium was determined after 30 min of incubation. Significantly greater amounts of all three enzymes were recovered from the medium when cells were incubated with zymosan. However, whereas an average of 8.9% of the total cellular β-glucuronidase was recovered in the incubation medium, only 3.6% of β-galactosidase and 4.6% of acid phosphatase were recovered. To test the effect of CB, cells were preincubated with the antibiotic for 30 min, and [^{14}C]inulin in either buffer or buffer containing zymosan was then added (Fig. 2). After an additional 30 min of incubation, enzyme activities in the medium were measured and cells were prepared for analysis of [^{14}C]inulin. Cells preincubated with CB took up less inulin than control cells, reflecting the inhibition of phagocytosis by the antibiotic (11, 12). Only negligible amounts of β-galactosidase and acid phosphatase were recovered from the medium when cells were incubated with zymosan alone. However, when cells were incubated with both zymosan and CB, 6–60 μg/ml, the recovery of activities of these enzymes

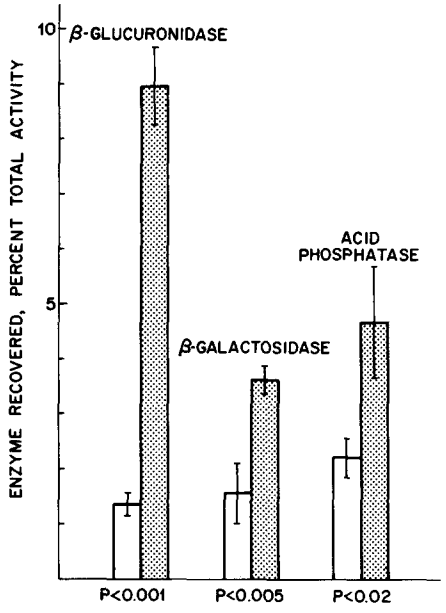

FIGURE 1 Recovery of lysosomal enzyme activity from incubation medium of PMN exposed to zymosan. PMN were isolated from the blood of 17 normal human donors on 20 occasions and incubated for 30 min either in buffer alone (clear bars) or with zymosan, 1 mg/ml (stippled bars), as described in Materials and Methods. At the end of 30-min incubation, cells were separated from incubation medium which was saved for determination of enzyme activities. The height of each bar represents the mean of 20 experiments. The lengths of the lines represent two standard errors of the means. The significance (Student's *t* test) of the differences between groups is indicated.

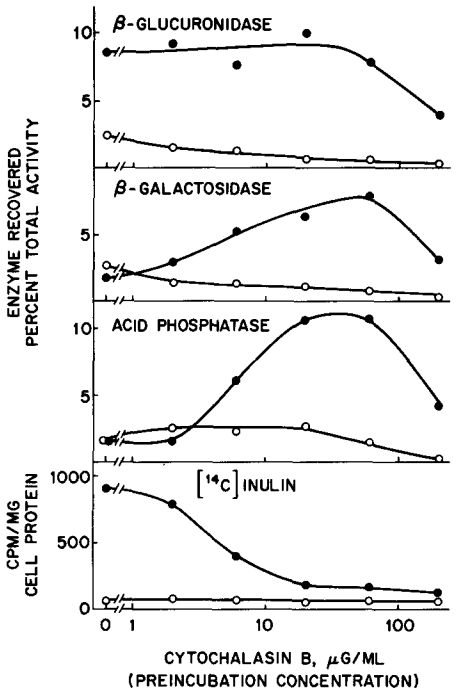

FIGURE 2 Effect of preincubation with CB on phagocytosis and recovery of lysosomal enzymes in incubation medium. Cells were preincubated for 30 min in a total volume of 0.4 ml without or with CB at the concentration indicated. Zymosan, 0.1 ml (●, to give a final concentration of 1 mg/ml), or buffer, 0.1 ml (○), was added and incubation continued for an additional 30 min. Inulin uptake and activities of lysosomal enzymes in the incubation medium were measured as described in Materials and Methods.

from the media was increased by comparison with cells incubated with zymosan alone. Preincubation of cells with CB had a negligible influence on the recovery of β-glucuronidase from cells incubated with zymosan, except at the highest concentration tested, 200 μg/ml, where recovery of all three lysosomal enzymes declined.

CB inhibits the uptake of glucose by human PMN (13). We previously suggested that the effects of the antibiotic on phagocytosis and lysosomal enzyme release were not coupled to this effect on glucose metabolism, since phagocytosis and β-glucuronidase released in response to zymosan were not altered by the omission of glucose from the medium (5). The data shown in Fig. 3 confirm and extend this observation. Glucose had no effect upon [^{14}C]inulin uptake by cells preincubated with CB and then exposed to zymosan. Likewise, incubation of cells with glucose did not alter the recoveries from the medium of β-galactosidase, acid phosphatase, and β-glucuronidase activities.

It has been suggested that CB augments the release of lysosomal enzymes by removing a barrier to their release (1, 2, 4). The antibiotic might, therefore, enhance the rate of release of lysosomal enzymes from cells exposed to zymosan. To test this possibility, we preincubated cells for 30 min in the absence or presence of CB, 20 μg/ml (Fig. 4). Zymosan, in an amount to give a final concentration of 1 mg/ml, was then added. Incubations were stopped at varying times up to 30 min after the addition of zymosan. During the preincubation period there was a small release of lysosomal enzyme activity, which was inhibited by CB. After exposure to zymosan, acid phosphatase, β-galactosidase, and β-glucuronidase activities promptly appeared in the medium. β-Glucuronidase continued to appear for 20 min at which time extracellular levels reached a plateau. The activities of acid phosphatase and β-galactosidase in the medium reached maxima at 5 min after exposure to zymosan. The activities of both enzymes in the medium then declined. The release of each lysosomal enzyme activity from cells preincubated with CB was retarded for 5 min after exposure to zymosan. After this lag period, the enzymes were released during the remainder of the 30-min incubation. The uptake of [^{14}C]inulin was inhibited in cells preincubated with CB, as expected from previous studies.

The data shown in Fig. 4 suggest that CB does not enhance the release of lysosomal enzymes, but rather retards their release for a period of time after exposure of cells to zymosan. This interpretation is complicated, however, by the observations that small amounts of lysosomal enzymes were released during the period of preincubation without zymosan and that CB inhibited this release. Therefore, we measured the time-course of the effect of CB without preincubation (Fig. 5). In the presence of zymosan alone, the activities of acid phosphatase and β-galactosidase increased in the medium in the first 15 min of incubation, after which they declined slightly. In the presence of

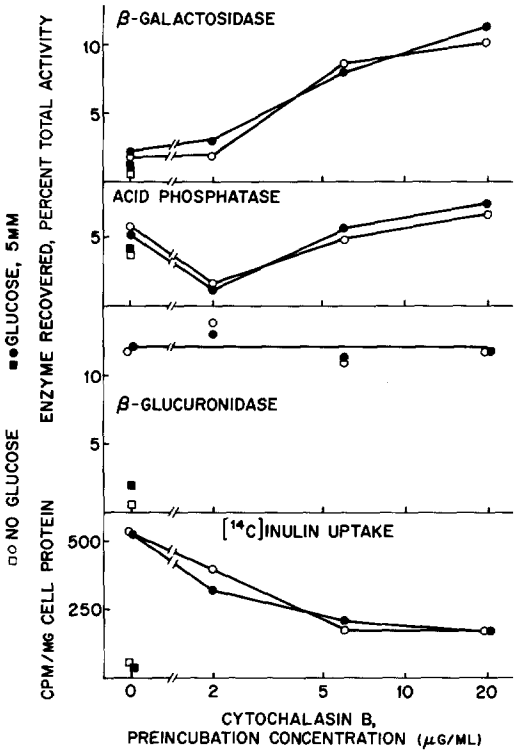

FIGURE 3 The effects of glucose and preincubation with CB on phagocytosis and the recovery of lysosomal enzymes from medium of PMN exposed to zymosan. Cells were preincubated with CB for 30 min at the concentrations indicated. A suspension of zymosan, 0.1 ml (to give a final concentration of 1 mg/ml), and of glucose, either 0 (○) or 5 mM (●), was then added. At the end of an additional 30-min incubation period, lysosomal enzyme activity in medium and [^{14}C]inulin uptake by cells were measured as described in Materials and Methods. For reference, values from cells incubated without zymosan or CB and with (■) or without (□) glucose during the final 30 min are indicated.

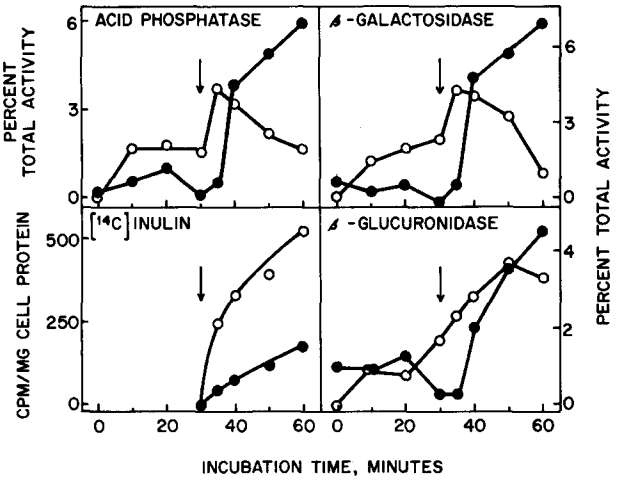

FIGURE 4 Time-course of recovery of lysosomal enzymes in incubation medium, and of phagocytosis by cells exposed to zymosan after preincubation with CB. Cells were incubated in a total volume of 0.4 ml in the presence (●) or absence (O) of CB, 20 μg/ml. After 30 min, 0.1 ml of zymosan (to give a final concentration of 1 mg/ml) was added to each tube (arrow) and incubation continued for an additional 30 min. Tubes were removed at intervals, and measurements of inulin uptake and lysosomal enzyme activity in medium were done as described in Materials and Methods.

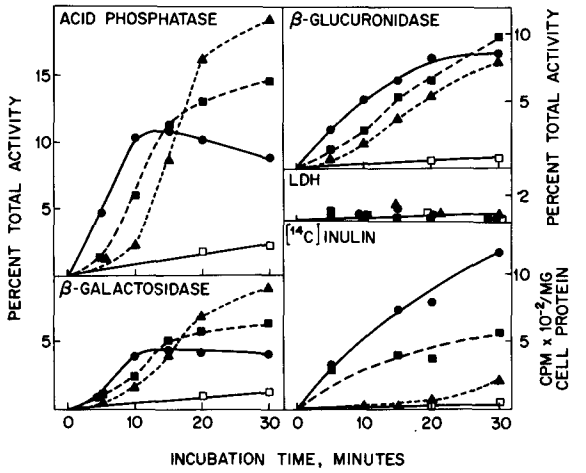

FIGURE 5 Time-course of the effect of CB on phagocytosis and on recovery of lysosomal enzymes and LDH from incubation medium on exposure of cells to zymosan. The incubation medium contained: no addition (□); zymosan, 1 mg/ml (●); zymosan plus CB, 3 μg/ml (■) or 30 μg/ml (▲). Tubes were removed at intervals during the incubation for measurement of lysosomal enzymes in incubation medium and uptake of [^{14}C]inulin into cells, as described in Materials and Methods.

zymosan and 30 μg/ml of CB, the release of these enzymes was initially retarded. The medium activities then rose and continued to rise for the remainder of the incubation period. The curves of enzyme activity in the medium vs. time for cells incubated with zymosan and CB, 3 μg/ml, were intermediate between those described above. In the presence of zymosan alone, β-glucuronidase activity in the medium increased for the first 20 min of incubation and then reached a plateau. CB, 30 μg/ml, again initially retarded the zymosan-stimulated increase in medium enzyme activity. The extracellular enzyme activity then rose and, by the end of the 30-min period, reached a level

comparable to that of cells incubated with zymosan alone. Again, cells incubated with zymosan and CB, 3 μg/ml, released β-glucuronidase activity with an intermediate time-course. Cells incubated with neither zymosan nor CB were shown, as previously, to release only small amounts of lysosomal enzymes during a 30-min incubation period. No significant amounts of the cytoplasmic enzyme lactic dehydrogenase (LDH) were released under any conditions of incubation. Phagocytosis was inhibited by CB, as demonstrated previously.

Lysosomal acid phosphatase (α-naphthyl-phosphate phosphatase) has been shown to be labile in solution after release into the incubation medium (14). Our results suggest that β-galactosidase as well as acid phosphatase (measured using *p*-nitrophenyl-phosphate as substrate) is similarly unstable (Figs. 4 and 5). The effects of CB noted above could have resulted from an inhibitory effect of the antibiotic on the decay of lysosomal enzyme activities in extracellular fluid. Results of a series of experiments indicate that CB does not, however, stabilize lysosomal enzymes. Lysosomal enzyme activities were released from cells incubated with zymosan or with zymosan plus CB (Fig. 6). Cells were removed by centrifugation. Medium recovered from cells incubated with zymosan alone was divided, and incubated in the presence or absence of CB. Medium obtained from cells exposed to both zymosan and CB was incubated in the continued presence of CB. The activity of β-glucuronidase was stable for up to 60 min under all conditions tested. The activities of acid phosphatase and β-galactosidase declined with time. CB did not retard the rate of loss of enzyme activity. In fact, acid phosphatase and β-galactosidase activities released from cells in the presence of CB appeared to decay at a faster rate than when they were released in the presence of zymosan alone.

In another experiment (Fig. 7), cells were incubated with zymosan (1 mg/ml) for 30 min, and then separated from medium by centrifugation. The medium was then incubated as before, with or without CB. The cell pellet was sonicated, diluted, and incubated with or without CB. β-Glucuronidase was the most stable of the enzyme activities in the cell lysate preparation, followed by acid phosphatase, then β-galactosidase. Likewise, in the preparation obtained from the supernate, the stability of β-glucuronidase far exceeded that of the other two enzymes. CB did not significantly alter the stability of any of the enzymes in either preparation.

The experiments cited thus far indicated that CB does not, as previously suggested, enhance the rate of release of lysosomal enzymes from cells exposed to particles, but rather retards enzyme release. They also indicated that CB does not alter the stability of lysosomal enzymes after their release into the medium. It has been demonstrated that release of lysosomal enzymes from phagocytosing cells is associated with a decrease in the amount of cell-associated enzyme (14). To elucidate the mechanism of action of CB further, we incubated cells in the presence of zymosan with or without added antibiotic (Fig. 8). After varying times of incubation, cells and medium were separated by centrifugation and enzyme activity in the two fractions determined. Cell-associated activities of both β-galactosidase and β-glucuronidase fell promptly when cells were exposed to zymosan and they continued to fall for approximately 30 min. Cell-associated enzyme activity was lost more

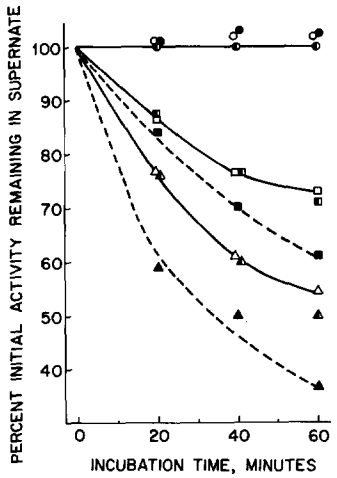

FIGURE 6 Effect of CB on stability of lysosomal enzymes released from cells. Cells were incubated with zymosan and then separated from the incubation medium which was divided into aliquots and incubated without (open symbols) or with (half-closed symbols) CB (40 μg/ml). Cells were also incubated in the presence of zymosan plus CB (10 μg/ml) and separated from the medium. Aliquots of this medium were then incubated (closed symbols, dashed lines). Incubation of medium rich in lysosomal enzymes was stopped by freezing the tubes which were then stored for assay, after thawing, of β-glucuronidase (○), acid phosphatase (□), and β-galactosidase (△) activities.

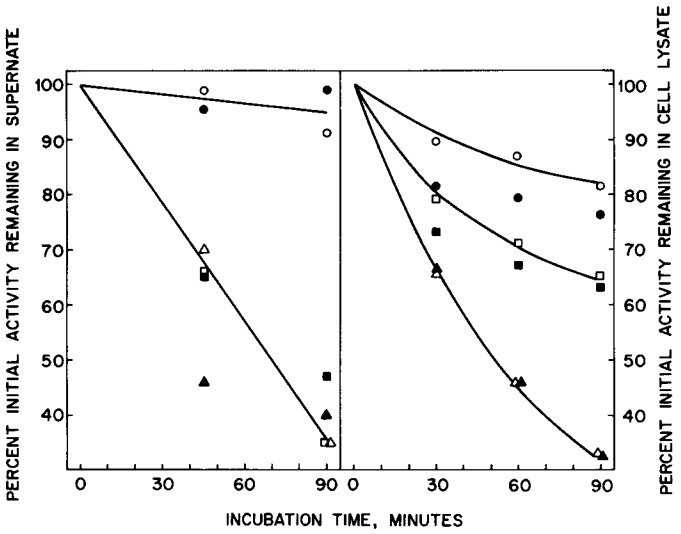

FIGURE 7 Effect of CB on stability of lysosomal enzymes released from cells by stimulation with zymosan and by sonication. Cells were incubated with zymosan (1 mg/ml) for 30 min and then separated from incubation medium by centrifugation. The cell pellet was lysed by freeze-thawing in the presence of Triton, and the lysate was diluted. Aliquots of incubation medium and of cell lysate, rich in lysosomal enzyme activities, were incubated for periods of time up to 90 min in the absence (open symbols) or presence (closed symbols) of CB (20 μg/ml). At the end of the appropriate incubation period, the contents of the tubes were frozen and later thawed and assayed for β-glucuronidase (○), acid phosphatase (□), and β-galactosidase (△) activities.

slowly from cells exposed to both zymosan and CB, and the extent of loss was less than that from cells exposed to zymosan alone. The effects of CB on recovery of lysosomal enzyme activities in the medium were similar to those described in earlier experiments.

DISCUSSION

It has been amply demonstrated that PMN undergoing phagocytosis in vitro release lysosomal enzymes into the incubation medium (1–5, 10, 14). However, when cells are incubated with the antibiotic CB, which inhibits phagocytosis, the activity of lysosomal enzymes recovered in the medium after 30–60 min of exposure to phagocytable particles equals or exceeds that recovered in the medium of cells incubated with zymosan alone (1–5). Thus, phagocytosis is not required for enzyme release. These findings have also been interpreted as indicating that CB enhances the rate of release of lysosomal enzymes. This interpretation is open to question, however, since measurements of enzyme activities in extracellular medium were made at only one time point after exposure to phagocytable particles.

Wright and Malawista (14) observed that the activities of some lysosomal enzymes (acid phosphatase and cathepsin) increased only slightly in the incubation medium when PMN ingested heat-killed bacteria. The fall in cell-associated activities of these enzymes was significant and far exceeded the enzyme activity which appeared in the medium. These findings were explained by the further observations that acid phosphatase and cathepsin activities were unstable when preparations containing these enzymes were incubated in extracellular medium. We have similarly observed that only relatively small amounts of the lysosomal enzyme β-galactosidase can be recovered from extracellular medium 30 min after exposure of PMN to zymosan. This finding can also be attributed to instability of the enzyme activity in incubation medium. Significant acid phosphatase and β-galactosidase activities were recovered from the medium, however, when cells were preincubated with CB before exposure to zymosan. One possible explanation for this finding is that CB in some way stabilized the enzymes released into the extracellular medium. It was not possible to demonstrate, however, that the stabilities of either acid

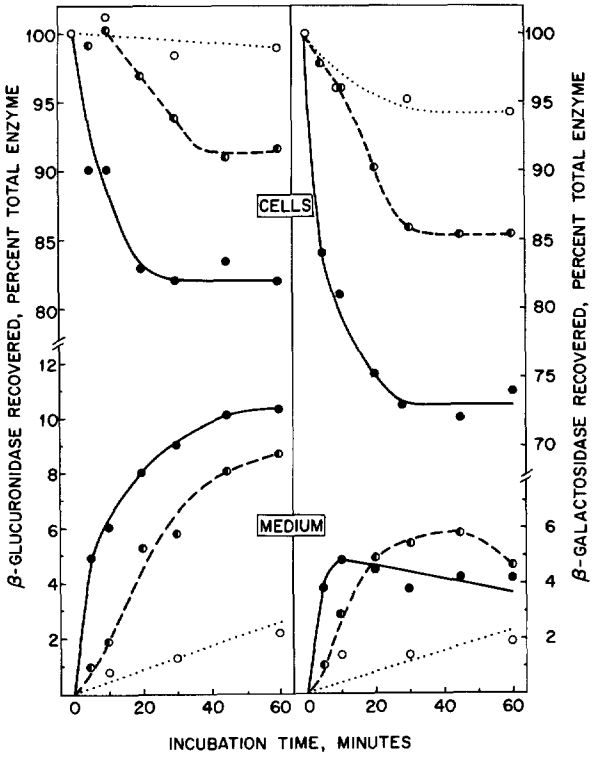

FIGURE 8 Time-course of influence of CB on recovery of lysosomal enzymes from cells and medium after exposure to zymosan. Cells were incubated in the presence of buffer alone (○), zymosan, 1 mg/ml (●), or zymosan plus CB, 10 μg/ml (◐). Tubes were removed at intervals during the incubation for measurement of lysosomal enzyme activity in incubation medium and in cells, as described in Materials and Methods.

phosphatase or β-galactosidase were altered when these enzymes were released in the presence of CB, or when CB was added to the incubation medium containing these enzymes released from cells either by incubation with zymosan or by sonication (Figs. 6, 7).

CB interferes with energy metabolism in PMN by inhibiting the uptake of glucose (13). The inhibitory effect of the antibiotic on phagocytosis could possibly be coupled to this effect on glucose metabolism. Inhibition of phagocytosis could also result in enhanced lysosomal enzyme release by the process of "regurgitation during feeding" if CB inhibited closure of the mouth of the phagocytic vesicle (2, 15). Our data suggest, however, that inhibition of phagocytosis and enhanced lysosomal enzyme release are not dependent on an effect of CB on glucose uptake. The concentration-response curves for the effects of CB on zymosan-induced inulin uptake and lysosomal enzyme release were almost identical in the presence or absence of glucose (Fig. 3).

The effect of CB on PMN function is not the result of a lethal toxic effect, for the effects of CB on PMN are reversible when cells are washed free of the antibiotic (16); also, there is a lack of nonspecific loss of enzymes, such as the cytoplasmic enzyme LDH, from cells incubated with CB (1–4) (Fig. 5).

Davies et al. (1) reported that CB stimulated the release of the lysosomal enzymes β-glucuronidase, β-galactosidase, and acid protease from rabbit PMN incubated in the absence of particles. However, we (Fig. 2) as well as others (2, 4) have found that CB does not stimulate release of lysosomal enzymes by "resting" PMN. This suggests that CB acts by altering the mode of response of PMN to particles to which they are exposed.

A hypothesis has been proposed independently by a number of investigators to explain the effects of CB on zymosan-stimulated lysosomal enzyme release from PMN (1, 2, 4). In a number of cells, CB binds to membrane-associated microfilaments and interferes with their function. It has been

suggested, therefore, that the network of microfilaments in PMN acts as a barrier to the release of lysosomal enzymes. By interfering with the network, CB could enhance the release of lysosomal enzymes. Our results are not consistent with this hypothesis. If CB were to remove a barrier to the release of lysosomal enzymes, then cells preincubated with CB and exposed to zymosan should release enzyme at a faster rate than cells preincubated without CB. This is not the case. Cells exposed to CB before stimulation by zymosan release lysosomal enzymes only after a lag period, whereas cells preincubated in the absence of CB promptly release lysosomal enzymes when exposed to zymosan (Fig. 3). Secondly, if the antibiotic removed a barrier to enzyme release, it might be expected that more enzyme would be lost from cells in the presence of CB than in its absence. This also is not the case; the total amount of enzyme lost from cells is greater when PMN are incubated with zymosan alone than when they are incubated with zymosan plus CB (Fig. 8).

We would like to offer the following explanation for the effect of CB on lysosomal enzyme release: When PMN are exposed to zymosan (in the absence of CB), the particle is phagocytosed. During the process of phagocytosis, lysosomal enzymes are released into the developing phagocytic vesicle. A substantial amount of enzyme activity enters the extracellular medium through the unclosed mouth of the vesicle (regurgitation during feeding [10]). As phagocytosis is completed, secretion of some lysosomal enzyme into the developed phagocytic vesicle continues. This enzyme activity is rapidly lost, either because of its instability in the extracellular medium contained within the vesicle or because of its degradation by enzymes such as neutral protease, contained in lysosomes and presumably also secreted into the vesicle. Under these conditions of exposure to zymosan, a small amount of lysosomal enzyme is also secreted directly across the cell membrane. The amount of enzyme recovered from the extracellular medium is less than the fall in enzyme activity in the cells because of the loss of enzyme activity in the phagocytic vesicle and extracellular medium. When cells are exposed to CB (either before or simultaneously with exposure to zymosan), phagocytosis is markedly inhibited. Thus lysosomal enzyme is released only across the cell membrane, primarily at the site of attachment of particles to the cell (4, 17). There is no appreciable loss of enzyme activity into phagocytic vesicles. Therefore, the amount of enzyme recovered from the extracellular medium more closely approximates the amount lost from cells. According to this explanation, it is not necessary to postulate that CB removes an inhibitory influence of membrane-associated microfilaments on lysosomal enzyme release. We suggest that the conclusion from previous studies that microfilaments directly modulate lysosomal enzyme release requires reconsideration.

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REFERENCES

1. DAVIES, P., R. I. FOX, M. POLYZONIS, A. C. ALLISON, and A. D. HASWELL. 1973. The inhibition of phagocytosis and facilitation of exocytosis in rabbit polymorphonuclear leukocytes by cytochalasin B. *Lab. Invest.* **238**:16.
2. HENSON, P. M., and Z. G. OADES. 1973. Enhancement of immunologically induced granule exocytosis from neutrophils by cytochalasin B. *J. Immunol.* **110**:290.
3. HAWKINS, D. 1973. Neutrophilic leukocytes in immunologic reaction in vitro: Effect of cytochalasin B. *J. Immunol.* **110**:294.
4. ZURIER, R. B., S. HOFFSTEIN, and G. WEISSMANN. 1973. Cytochalasin B: Effect on lysosomal enzyme release from human leukocytes. *Proc. Natl. Acad. Sci. U. S. A.* **70**:844.
5. SKOSEY, J. L., D. CHOW, E. DAMGAARD, and L. B. SORENSEN. 1973. Effect of cytochalasin B on response of human polymorphonuclear leukocytes to zymosan. *J. Cell Biol.* **57**:237.
6. COHEN, P. P. 1957. *In* Manometric Techniques. W. W. Umbreit, R. H. Burris, and S. F. Stauffer, editors. Burgess Publishing Company, Minneapolis. 3rd edition. 149–150.
7. BAEHNER, R. L., M. J. KARNOVSKY, and M. L. KARNOVSKY. 1969. Degranulation of leukocytes in chronic granulomatous disease. *J. Clin. Invest.* **48**:187.
8. BERGER, R. R., and M. L. KARNOVSKY. 1966. Biochemical basis of phagocytosis. V. Effect of phagocytosis on cellular uptake of extracellular fluid and on the intracellular pool of α-glycerophosphate. *Fed. Proc.* **25**:840.
9. WACKER, W. E. C., D. E. ULMER, and B. L. VALLEE.

1956. Metalloenzymes and myocardial infarction. *New Engl. J. Med.* **225**:449.

10. WEISSMANN, G., R. B. ZURIER, P. J. SPIELER, and I. M. GOLDSTEIN. 1971. Mechanisms of lysosomal enzyme release from leukocytes exposed to immune complexes and other particles. *J. Exp. Med.* **134**(3, Pt. 2):149 s.
11. DAVIS, A. T., R. ESTENSEN, and P. G. QUIE. 1971. Cytochalasin B. III. Inhibition of human polymorphonuclear leukocyte phagocytosis. *Proc. Soc. Exp. Biol. Med.* **137**:161.
12. ZIGMOND, S. H., and J. G. HIRSCH. 1972. Effects of cytochalasin B on polymorphonuclear leukocyte locomotion, phagocytosis, and glycolysis. *Exp. Cell Res.* **73**:383.
13. ZIGMOND, S. H., and J. G. HIRSCH. 1972. Cytochalasin B inhibition of 2-deoxy-D-glucose transport into leukocytes and fibroblasts. *Science (Wash. D. C.).* **176:** 1432.
14. WRIGHT, D. G., and S. E. MALAWISTA. 1972. The mobilization and extracellular release of granular enzymes from human leukocytes during phagocytosis. *J. Cell Biol.* **53**:788.
15. HENSON, P. M. 1971. The immunological release of constituents from neutrophil leukocytes. II. Mechanisms of release during phagocytosis, and adherence to nonphagocytosable surfaces. *J. Immunol.* **107**:1547.
16. MALAWISTA, S. E., J. B. L. GEE, and K. G. BENSCH. 1971. Cytochalasin B reversibly inhibits phagocytosis. Functional, metabolic, and ultra-structural effects in human blood leukocytes and rabbit alveolar macrophages. *Yale J. Biol. Med.* **44**:286.
17. HENSON, P. M. 1973. Mechanisms of release of granule enzymes from human neutrophils phagocytosing aggregated immunoglobulin: An electron microscopic study. *Arthritis Rheum.* **16**:208.