Cytochalasin B
III. Inhibition of Human Polymorphonuclear Leukocyte Phagocytosis[1] (35535)

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Cytochalasin B (CB), a metabolic product of *Helminthosporium dematioideum* has been shown to inhibit cytoplasmic division of mammalian (1–4) and *Xenopus laevis* eggs (5). Nuclear division proceeds normally in cells in the presence of CB. This results in disassociation of cytoplasmic and nuclear division and multinucleated cells are produced (1). Cytoplasmic division (cytokinesis) can be thought of as occurring in three phases, *i.e.*, division of the nucleus, furrowing of the membranes, and fusion of membranes. It has been demonstrated that nuclear division proceeds normally (1–4) and furrowing is not affected (5) in the presence of CB. Therefore, fusion of membranes seems to be the target of drug action. Since membrane fusion also occurs in the process of phagocytosis we were interested in testing the effect of CB on this function of polymorphonuclear leukocytes. We report here the inhibition of bacterial phagocytosis by human polymorphonuclear leukocytes in the presence of Cytochalasin B.

Materials and Methods. Cytochalasin B. The CB was stored in a stock solution of 1 mg/ml in dimethylsulfoxide (DMSO). Dilutions were made in Hank's balanced salt solution (BSS). Phagocytosis assays utilized a modified Maaløe method previously described (6, 7). This consisted, essentially, of mixing 5×10^6 human leukocytes, separated from venous blood by dextran sedimentation and differential centrifugation with bacteria at a 1:1 bacteria:leukocyte ratio. Leukocytes and bacteria were suspended in the BSS containing 0.1% gelatin. Opsonin was provided by adding pooled adult human serum at a final concentration of 10%. A total volume of 1 ml was incubated in 12–75-mm plastic tubes (Falcon Plastics) and rotated end over end at 10 rpm on a Roto rack (Fisher Scientific Co.). The phagocytic mixtures were sampled immediately after mixing and after 30-, 60-, and 120-min incubation by placing 0.001 ml in 1 ml of distilled water and adding 0.1 ml to Penassay agar (Difco Labs) for pour plates and colony counts. After 120-min incubation, the phagocytic mixtures were centrifuged at 180*g* to separate nonphagocytized from leukocyte-associated bacteria. The supernatant was sampled and discarded and the cell pellet was washed in heparinized saline, centrifuged, suspended to original volume, and sampled for bacterial colony count. In certain experiments, 100 units of penicillin G and 100 μg of streptomycin/ml were added to the phagocytic mixtures after 30-min incubation to separate extracellular from intracellular bacteria.

Results. Phagocytic mixtures containing CB at a concentration of 10 μg/ml showed little phagocytic activity (Fig. 1). There were nearly the same number of colonies of *S. aureus* 502A and *E. coli* K-12 after 60- and 120-min incubation as at 0 time in the presence of 10 μg/ml of CB. A concentration of 1 μg/ml of CB or suspending media containing 1% DMSO had, essentially, no effect on leukocyte function.

The *in vitro* leukocyte–bacteria phagocytosis system depends on interaction of bacteria,

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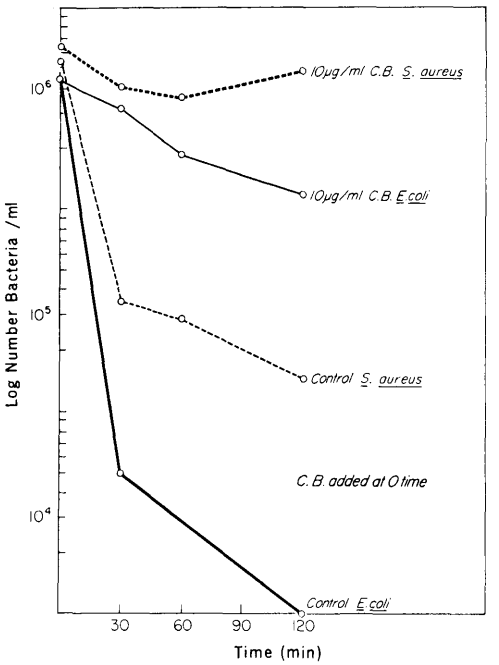

FIG. 1. Effect of 10 µg/ml of Cytochalasin B on phagocytosis of *S. aureus* 502A and *E. coli* K-12 by human polymorphonuclear leukocytes.

opsonins, and polymorphonuclear leukocytes. Therefore, in order to attribute drug inhibition to an effect on leukocytes it was necessary to rule out effects on bacteria and/or opsonin. Preincubation of *S. aureus* 502A and *E. coli* K-12 with concentrations of CB as high as 200 µg/ml had no effect on bacterial growth. CB had no effect on opsonic activity in experiments in which opsonin, bacteria, and CB were preincubated (Fig. 2). The bacteria in these experiments were phagocytized and killed at a normal rate. Effects of CB on bacteria or opsonin or a combination of bacteria and opsonin are therefore not sufficient to explain the action of the drug.

The effect of CB on leukocytes was tested in two ways: first by preincubating leukocytes with CB and second by dye uptake studies. When the polymorphonuclear leukocytes were washed after a 30-min incubation in CB and used in phagocytosis tests, phagocytosis and killing of bacteria occurred at a normal rate. In addition, phagocytic mixtures were exposed to aqueous trypan blue after 120-min incubation in the presence of CB. There was no evidence of increased uptake of trypan blue in cells exposed to CB. These experiments imply that CB is not irreversibly toxic for polymorphonuclear leukocytes.

Experiments were done to determine the rate of action of CB on leukocyte function (Fig. 3). As shown, there was sudden cessation of bacterial killing immediately after CB was added to the phagocytic mixtures.

The location of viable bacteria in the presence of CB in the phagocytic mixtures was determined in order to distinguish between inhibition of engulfment of bacteria and intracellular survival of bacteria. Penicillin and streptomycin do not enter polymorphonuclear leukocytes (8) and an addition of these antibiotics resulted in rapid reduction of viable bacteria in phagocytic mixtures containing CB (Fig. 4). This result shows the extracellular location of bacteria and suggests that CB affects the ability of leukocytes to engulf bacteria. Since it might be suggested that CB or DMSO could affect the permea-

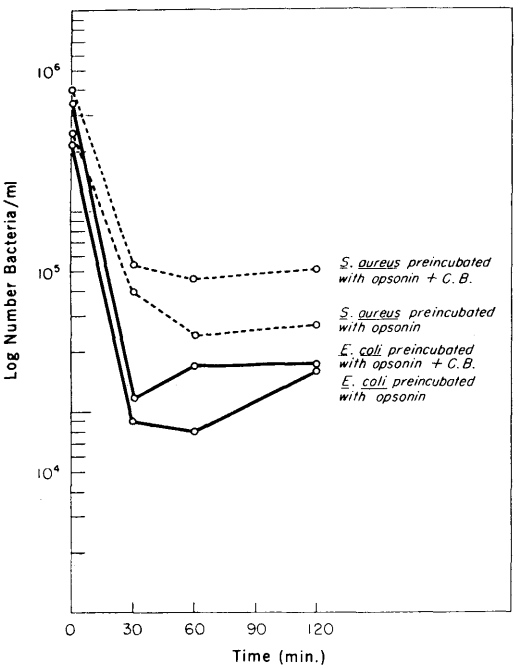

FIG. 2. Preincubation of *S. aureus* 502A and *E. coli* K-12 with Cytochalasin B and with normal serum as opsonin.

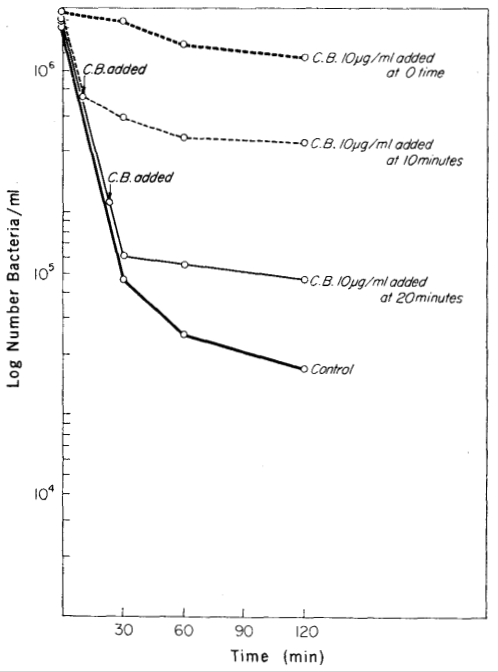

FIG. 3. Effect of adding Cytochalasin B at various times during phagocytosis.

bility of leukocyte membrane and allow antibiotics to penetrate the leukocyte, polymorphonuclear leukocytes from a patient with chronic granulomatous disease (CGD) were studied. Bacteria survive intracellularly in the leukocytes of patients with this syndrome (7). As shown in Fig. 4, there was no evidence that antibiotics killed intracellular bacteria in leukocytes of patients with CGD in the presence of CB. When these antibiotics were added to phagocytic mixtures with CB and normal leukocytes, only extracellular bacteria were killed and the effect of CB appeared to be on the process of engulfment of bacteria rather than on intracellular bacterial killing.

Discussion. Our results indicate that CB affects leukocyte phagocytosis by inhibiting bacterial uptake. CB has no effect on opsonization of bacteria. The effect of the drug on the leukocytes is rapid and relatively complete and reversible.

Cytochalasin B could inhibit phagocytosis by one of three mechanisms: (i) prevent attachment of opsonized bacteria to the cell surface, (ii) prevent movement of the cell around an attached bacterium, or (iii) prevent the fusion of membrane surfaces after the membrane has moved around the adherent bacterium. Direct microscopic examination of phagocytizing PMN's and thin sections of similar cells suggest that bacteria do attach to the PMN surface normally. The results reported here do not distinguish between the latter two possibilities.

There are structural similarities between the phagocytic process and the cytokinetic process. For example, the furrowing which occurs during cytokinesis could be analogous to movement of the leukocyte membrane around a bacterium during phagocytosis. The fusion of the furrowed membrane during cytokinesis and the fusion of membrane surfaces during phagocytosis are also similar.

Microfilaments have been associated with cell movements (13) and furrowing in eggs (15–17) and mammalian cells (1, 3). A number of investigators have reported that CB affects microfilaments. Schroeder (9) observed that CB at concentrations of 2×10^{-7} *M* stopped furrowing in sea urchin eggs and

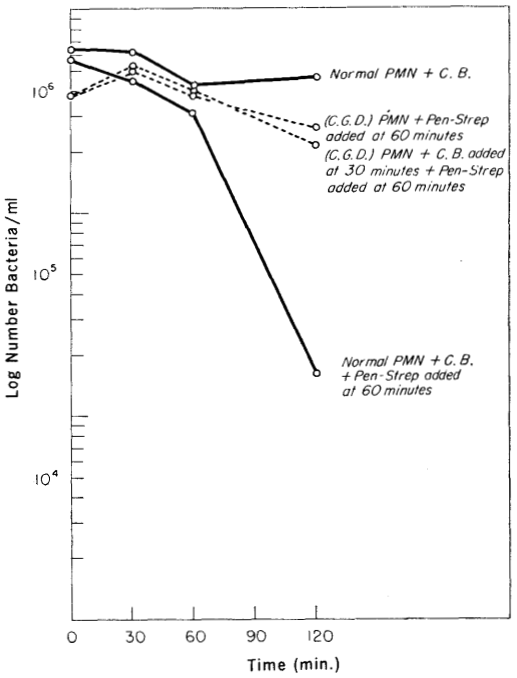

FIG. 4. Comparison of chronic granulomatous disease PMN and normal PMN in the presence of Cytochalasin B and penicillin streptomycin.

caused the disappearance of microfilaments. Wessels and co-workers (10–12) and one of us (Ladda, R. L., and Estensen, R. D., manuscript in preparation) have observed aggregation and disorganization of microfilaments of mammalian cells at doses comparable to those used in our experiments. Further, CB is known to inhibit cell motility and ruffling movements of cell membranes (1). If membrane movement is necessary for engulfment of bacteria, then the evidence would suggest that CB acts by interfering with microfilament function. As a consequence CB may prevent membrane fusion or vesicle formation because portions of membrane fail to move and come close enough to fuse.

As was indicated above, several investigators have implicated microfilaments in furrowing. In contrast to Schroeder's observations of sea urchin eggs, we have found that furrowing is not inhibited in *X. laevis* eggs. In fact, furrows are initiated and progress normally with subsequent "reversal" (5). Similar observations have been made on mammalian cells (1, 3). This suggests that microfilaments may not be the only cellular element involved in cytokinesis. CB may directly affect the membrane itself or other membrane-associated elements. If cytokinesis and phagocytosis are analogous in this regard, then inhibition of phagocytosis would be directly attributable to failure of membrane fusion.

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