Cytochalasin B: Inhibition of D-2-Deoxyglucose Transport into Leukocytes and Fibroblasts

Abstract. *Cytochalasin B inhibits transport of* D-*2-deoxyglucose and of glucosamine into leukocytes, but does not impair uptake of leucine by these cells. This inhibitory action is rapid and reversible, and results in suppression of glycolysis. Cytochalasin B also blocks transport of* D-*2-deoxyglucose, but not of leucine, into mouse L strain fibroblasts.*

Cytochalasin B (CB), a metabolite of the mold *Helminthosporium dematioideum*, exerts an inhibitory effect on various cell functions, including cytokinesis, membrane movements (*1*), locomotion in fibroblasts (*1*) and polymorphonuclear leukocytes (*2*), phagocytosis in leukocytes (*2, 3*), pinocytosis in hepatoma cells (*4*), and morphogenesis in a number of tissues (*5*). The mechanisms of action of CB have not been determined. In some instances the drug alters the arrangement of peripheral 4- to 5-nm filaments (*5, 6*). Spudich and Lin (*7*) have reported that CB depresses the viscosity of f-actin and actomyosin, and also depresses the activity of myosin adenosine triphosphatase activated by actin.

Cytochalasin B has no significant inhibitory action on certain cell metabolic activities, such as respiration (*4*), and incorporation of labeled precursors into protein, nucleic acids, or lipids (*8*). However, Sanger and Holtzer found recently that CB inhibits incorporation of glucosamine, but not of leucine, in myoblasts (*9*), and we have noted an inhibition of glycolysis in polymorphonuclear leukocytes (*2*). We now report that CB inhibits the transport of glucose and glucosamine into cells.

Our previous studies showed that lactate production and glucose utilization in horse and rabbit leukocytes were reversibly inhibited, 70 to 90 percent, by 10 μg of CB per milliliter of medium. The inhibition could be detected with concentrations of CB as low as 0.3 μg/ml. Cytochalasin B suppressed glycolysis by the Embden-Meyerhof pathway, and the hexose monophosphate shunt, as demonstrated in studies on glucose labeled with ^{14}C at the C-1 or the C-6 position. The interference with both shunt and glycolysis, and the observation that glucose utilization and CO_2 production from labeled glucose were inhibited slightly more than lactate production, suggested that CB interfered with an early step in glucose metabolism, for example, with glucose transport or phosphorylation. Action of the drug on one of these processes might allow breakdown of intracellular glycogen stores, which would provide glucose-1-phosphate for continuing lactate production at a low level.

We therefore examined the transport into horse leukocytes of a tritium-labeled, nonmetabolizable analog of glucose, D-2-deoxyglucose (DOG), with the use of the rapid sampling technique of Hawkins and Berlin (*10*). Populations of horse leukocytes which normally contained 75 percent polymorphonuclear leukocytes, 20 percent lymphocytes, and 5 percent monocytes were allowed to attach themselves to cover slips. They were incubated first with or without CB, and then were exposed to [^{3}H]-DOG for 30 to 120 seconds. The first incubation was carried out in a medium free of glucose in order to minimize the physiological difference between the two samples. The exposure to [^{3}H]DOG was stopped by dipping the cover slips in beakers of ice-cold saline. The cover slips were broken into scintillation vials, and the material was solubilized with NaOH, neutralized with HCl, and counted in Bray's solution in a Nuclear-Chicago counter at an efficiency of about 37 percent. The uptake of [^{3}H]-DOG in the presence of 10 mM unlabeled DOG was shown to be linear for at least 90 seconds at 37°C.

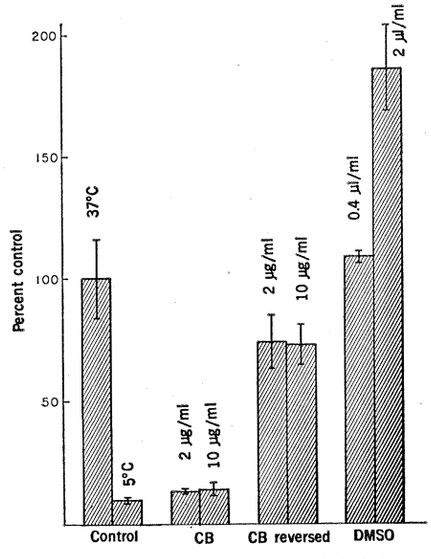

Fig. 1. Inhibition of uptake of DOG by CB. Uptake was measured during 45 seconds in cells adherent to glass cover slips (*10*). All test samples contained 10 mM unlabeled DOG and [^{3}H]DOG (2 μc/ml) (New England Nuclear, specific activity 4.7 mc/mmole). The results, presented as a percentage of the control uptake ± standard error of the mean, show that CB, at 2 or 10 μg/ml, suppressed uptake of DOG similar to that in the control specimen at 5°C. The CB inhibition was reversed when the cell sheets, exposed for 1 hour to CB at concentrations of 2 or 10 μg/ml, were rinsed, placed in drug-free medium, and tested for uptake 30 minutes later (CB reversed). The effects of DMSO on uptake of DOG were examined at DMSO concentrations of 0.4 and 2 μl/ml, amounts of DMSO contained in the CB preparations of 2 and 10 μg/ml, respectively.

Figure 1 shows the effects of CB on uptake of [^{3}H]DOG by horse cells during a 45-second exposure in the presence of 10 mM unlabeled DOG. Cells treated with CB concentrations of 2 or 10 μg/ml were severely inhibited in their uptake of DOG, the uptake by CB-treated cells being only slightly greater than that of cells maintained at 5°C. Protein determinations indicated that the adhesion of the cells to the cover slip was not altered by incubation in CB. The effect of CB on DOG transport was very rapid; the addition of CB simultaneously with the [^{3}H]DOG at the beginning of a 30-second exposure resulted in an uptake of isotope that was less than half that of the control uptake, an indication that the inhibition had occurred within 15 seconds. The inhibition of glucose transport by CB was reversible; cover slips treated for 1 hour with CB, then rinsed and incubated with fresh media (free of CB) for 30 minutes, showed about 70 percent recovery of transport activity.

Because the CB solution had been prepared in dimethylsulfoxide (DMSO), we studied the effects of this material on glucose transport. At a DMSO concentration of 2 μl/ml, an amount comparable to that present in CB concentrations of 10 μg/ml, there was no inhibitory effect on uptake; in fact, this concentration of DMSO stimulated glucose transport nearly twofold (Fig. 1).

Figure 2 shows Lineweaver-Burke plots of the uptake of [^{3}H]DOG into leukocytes in the presence and in the absence of CB (2 μg/ml). The maximum velocity of uptake by the control cells was approximately 7×10^{-4} mmole/min per 10^6 cells, nearly ten times greater than that of cells treated with CB (2 μg/ml). These kinetics suggest that CB acts as a noncompetitive inhibitor of glucose transport.

Uptake of [^{14}C]glucosamine (5 μc/ml)

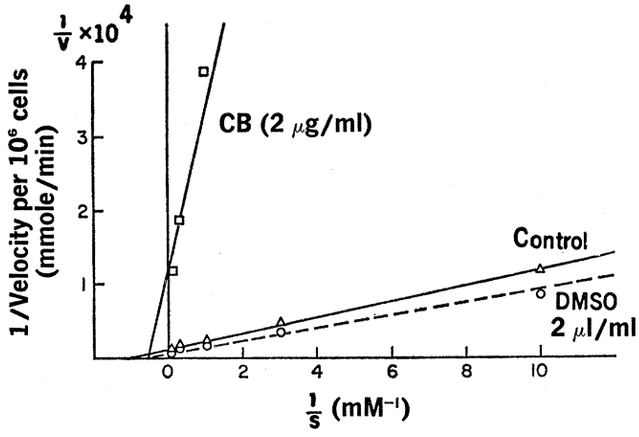

Fig. 2. Lineweaver-Burke plots of the influence of CB on rates of uptake of DOG by horse leukocytes. Cells adherent to cover slips were incubated for 30 to 60 minutes in glucose-free medium containing CB (2 μg/ml), DMSO (2 μl/ml), or no addition. The uptake of [³H]DOG at 37°C was then measured during a 45-second period after the addition of [³H]DOG (1 or 2 μc/ml) and 0.1, 0.3, 1, 3, or 10 m*M* unlabled DOG (the 1/S points at 3 and 10 m*M* are not shown on the graph of CB, but were used in the determination of the line drawn by least squares). Determinations for uptake by cells at 0°C were subtracted from the determinations at 37°C to correct for adsorption and nonfacilitated diffusion.

and D-3-*O*-[¹⁴C]methylglucose (2 μc/ml) were also inhibited by CB (10 μg/ml). For these assays, we incubated cells without glucose and tested them in glucose-free Gey's medium containing 2 m*M* unlabeled glucosamine or 10 m*M* unlabeled 3-*O*-methylglucose.

In contrast, [³H]leucine transport (2 μc/ml) by cells both incubated in, and then tested in, minimum essential medium (Eagle) was not inhibited by CB (10 μg/ml). The uptake in all cells over a 2-minute test period was linear.

We have also examined the effects of CB on leucine and DOG uptake in another type of cell, an L strain mouse fibroblast that had been maintained in tissue culture (*11*). The uptake of DOG by L cells was inhibited by CB in a manner similar to that observed in horse leukocytes (Fig. 3). Leucine uptake by L cells was unaffected by CB (10 μg/ml). Thus, the results obtained with fibroblasts were similar to those in leukocytes.

If the effect of CB on glycolysis were entirely due to blockade of glucose transport, then leukocytes incubated in media containing no glucose would be expected to produce similar amounts of lactate, whether or not CB was added. This, in fact, seemed to be the case; in the absence of glucose, untreated cells and cells treated with CB (10 μg/ml) produced 14 percent and 17 percent, respectively, of the lactate produced by control cells in media containing 10 m*M* glucose. In the same experiment, the amount of lactate produced by cells treated with CB (10 μg/ml) in the presence of glucose was 23 percent that of the control cells.

Furthermore, if CB were suppressing glycolysis by blocking glucose transport into leukocytes, then homogenates should be able to metabolize glucose equally well in the presence and the absence of CB. A leukocyte homogenate was prepared by the method of Beck and Valentine (*12*); particulate material was removed after centrifugation at 5000*g* for 10 minutes. The lactate production was shown to be proportional to the amount of supernatant added, and was also dependent on the presence of added glucose. Cytochalasin B did not inhibit the production of lactate in this homogenate system (Table 1). Hexokinase activity in the supernatant of the leukocyte homogenate was assayed independently (*13*), and was found to be unaffected by concentrations of CB up to 50 μg/ml.

We have thus demonstrated that CB blocks transport of glucose into leukocytes and fibroblasts, which leads to a suppression of glycolysis. Cytochalasin B also blocks transport of glucosamine into leukocytes, an action that may be responsible for the suppression of glycoprotein synthesis by this drug in other cell types (*9*). The inhibition of locomotion of polymorphonuclear leukocytes by CB is clearly not based on the lowered glycolytic rate that results from the inhibition of glucose transport, since these cells are able to move and phagocytose normally for at least 1 hour after being placed in glucose-free media (*14*). It seemed possible, in fact, that the reduced glycolysis in CB-treated cells might be partly a result, rather than a cause, of impaired locomotion, because active cells probably metabolize at a more rapid rate than do sessile ones. However, CB, at concentrations of 10 μg/ml, reduced lactate production by horse erythrocytes by approximately 70

Table 1. Lactate production by a leukocyte homogenate. The homogenate contained the supernatant equivalent to 3×10^7 cells. The control, CB, and DMSO samples all contained glucose.

Sample	Lactate (μg/ml) produced at	
	10 min	20 min
DMSO, 0.2 percent	47	107
CB, 10 μg/ml	53	105
Control	37	100
Control, no glucose	14	19

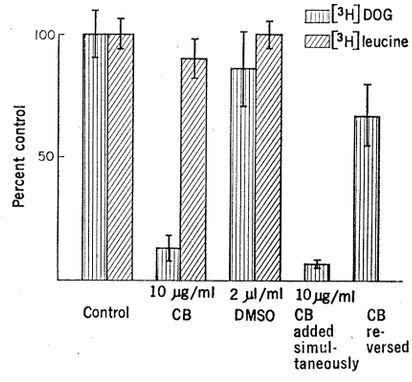

Fig. 3. Inhibition by CB of uptake of DOG, but not of leucine, by mouse L strain fibroblasts. Specimens were incubated for 30 to 60 minutes in minimal essential medium (Eagle) containing 10 m*M* unlabeled leucine, with or without CB or DMSO, before replacement with fresh medium containing 0.4 m*M* unlabeled leucine and [³H]leucine (2 μc/ml) at the beginning of the 45-second period of observation. The results are presented as percentages of the control (no CB) transport values for DOG (vertically striped bars) and for leucine (diagonally striped bars) ± standard error of the mean. At concentrations of 10 μg/ml, CB suppressed the uptake of DOG, but had no significant effect on the uptake of leucine. At concentrations of 2 μl/ml, DMSO did not alter the transport of either of these substances in the L cells. The inhibitory action of CB was rapid; its simultaneous addition with labeled DOG at the beginning of the 45-second measurement period resulted in inhibition as great as that seen when the cell sheets were incubated with CB for 30 to 60 minutes prior to the test period. The inhibitory effects of CB on L cells were reversible as seen by the recovery of nearly normal DOG transport capacity after the cells were washed and tested in CB-free media (CB reversed).

percent (*15*); CB thus also interferes with glycolysis in nonmotile cells.

The various inhibitory effects of CB on leukocyte metabolism and function exhibit a similar dose-response relation, and they are all rapid and reversible, suggesting a single site of action for the drug with multiple effects. The findings reported here are compatible with the site of action of CB being at the plasma membrane, leading to blockade of certain transport systems, with resulting metabolic changes in the cell. The mechanisms responsible for inhibition by CB of cell functions such as locomotion and endocytosis are as yet undetermined; these effects, which have similar time and dose characteristics, may be related directly or indirectly to the action of CB on transport, or they may reflect multiple sites of drug action on membranes, microfilaments, or other structures.

Note added in proof: While the present work was in press, a report appeared showing inhibition by cytochalasins of deoxyglucose transport into certain cells in culture (*16*).

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Histological Changes in Lobsters (Homarus americanus) Exposed to Yellow Phosphorus

Abstract. *An industrial discharge of yellow phosphorus killed fish and crustaceans in Long Harbor, Placentia Bay, Newfoundland, in 1969. During subsequent toxicity studies various organs from lobsters killed by exposure to suspensions of yellow phosphorus were examined for histological damage. Antennal gland and hepatopancreas both showed degenerative changes, and cellular damage in the latter was extensive.*

In 1969 an industrial discharge of yellow phosphorus caused fish and crustacean mortality in Long Harbor, Newfoundland (*1*). In an ensuing study on lobsters (*Homarus americanus*) the toxicity of yellow phosphorus was determined (*2*). At that time no attempt was made to determine the exact cause of death, but it appeared that massive hemolymph clotting caused death by asphyxiation. An analysis of tissue samples from these lobsters killed by phosphorus has revealed a pronounced histological change in the hepatopancreas and variable destruction of parts of the antennal gland. This report details these findings.

Laboratory-conditioned juvenile lobsters of both sexes, in stage D_0-D_1 of the molt cycle, were held in tanks (1 by 1 by 0.3 m) containing 200 liters of continuously aerated seawater and various concentrations of phosphorus-contaminated mud obtained from the pollution site in Long Harbor. Tissue from the hepatopancreas, heart, carapace, intestine, gill, gastrolith plate, and supraesophageal ganglion was removed from 16 moribund lobsters contaminated by phosphorus and two controls that were killed by asphyxiation in oxygen-free seawater. Tissues were fixed in Bouin's fluid and stored in 70 percent ethyl alcohol. Carapace sections were stained with Mallory's trichrome; other tissue was stained with Delafield's hema-

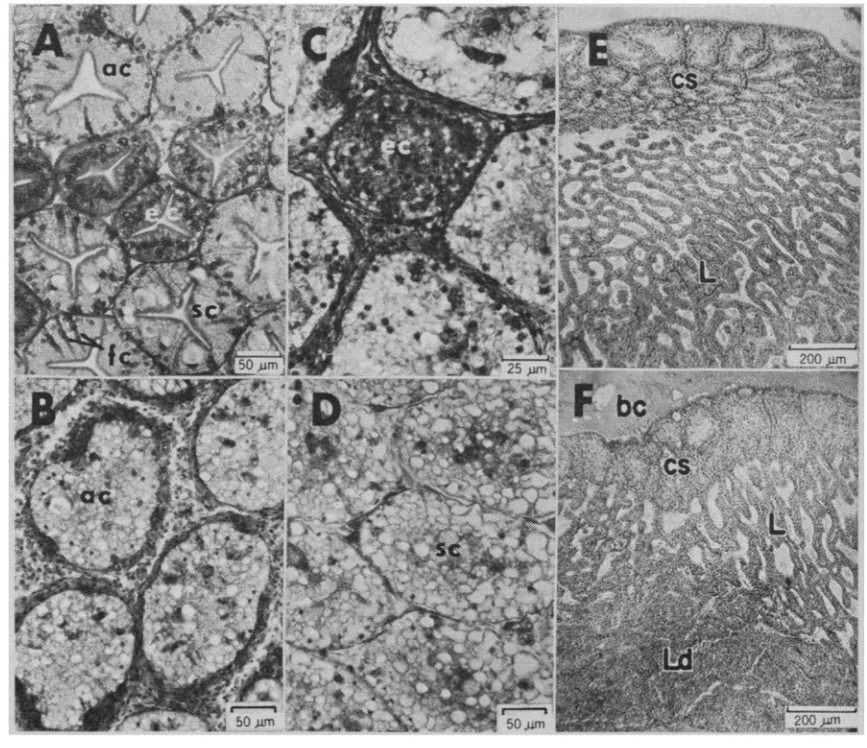

Fig. 1. Comparison of normal tissue from hepatopancreas and antennal gland with similar tissue from lobsters exposed to yellow phosphorus. (A) Sections of normal hepatopancreatic tubules showing four cell types: absorptive (*ac*), embryonic (*ec*), fibrillar (*fc*), and secretory (*sc*). (B to D) Effect of phosphorus on absorptive, embryonic, and secretory cells. Note loss of cellular integrity, extensive vacuolization, and obliteration of tubule lumen. (E) Normal antennal gland tissue showing labyrinth (*L*) and coelomosac (*cs*). (F) Antennae gland from lobster exposed to yellow phosphorus. Note degenerated region of the labyrinth (*Ld*), breakdown of the coelomosac basement membrane, and clotted blood (*bc*).