# Inhibition of Glucose Transport in the Human Erythrocyte by Cytochalasin B†

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ABSTRACT: Cytochalasin B inhibits glucose transport in the human erythrocyte. Half-maximal inhibition is at $\sim 5 \times 10^{-7}$ M cytochalasin B. Both kinetic data and evidence obtained from chemical modification experiments with 1-fluoro-2,4-dinitrobenzene and *N*-ethylmaleimide indicate that inhibition is noncompetitive in nature.

Cytochalasin B (Aldridge *et al.*, 1967; Carter, 1967) inhibits a wide variety of cell movements. It has been proposed that this effect is due to a change in the morphology of a specific class of 50-Å microfilaments (for a review see Wessells *et al.*, 1971). Recently several laboratories have reported that cytochalasin B also inhibits sugar transport in many cells (Kletzien *et al.*, 1972; Estensen and Plagemann, 1972; Mizel and Wilson, 1972; Zigmund and Hirsch, 1972; Cohn *et al.*, 1972). The concentrations of the drug required to affect the two processes are approximately the same, suggesting that the two effects may conceivably be related. However, further work is necessary to determine whether or not this is the case.

Of the eucaryotic sugar transport systems, that of the human erythrocyte is one of the most accessible and best characterized. It is a facilitated diffusion system, unaffected by energy poisons or ionic gradients, and capable of transporting many monosaccharides across the plasma membrane (for reviews, see LeFevre, 1961; Stein, 1967; Miller, 1969). The effects of cytochalasin B on this system have been studied and it is reported here that cytochalasin B is a potent noncompetitive inhibitor of glucose transport in the human erythrocyte. The results are of potential interest with respect to both the mechanism of the transport process and the mode of action of cytochalasin B.

## Materials and Methods

Cytochalasin B was purchased from the I.C.I. Research Laboratories, Alderly Park, Cheshire, England, and was dissolved in dimethyl sulfoxide to give a 10 mg/ml solution. Phloretin, obtained from K&K Laboratories, was used as a 10 mM solution in ethanol. 1-Fluoro-2,4-dinitrobenzene ($N_2$ph-F)[1] and *N*-ethylmaleimide (MalNEt) were from Mann and Sigma, respectively. D-Glucose was from Calbiochem.

Red blood cells drawn into acid–citrate dextrose were most generously provided by Miss Helen Holland of the Blood Research Institute, Boston, Mass. They were routinely washed free of the buffy coat in 0.9% NaCl before use.

Glucose transport activity was assayed by the volumetric method of Sen and Widdas (1962a) with modifications. Briefly, cells were equilibrated with 0.1 M glucose in 10 mM potassium phosphate–145 mM NaCl (pH 7.4) for 30 min at 37°. An aliquot, 0.1 ml, of an ~20% by volume cell suspension was added to 10 ml of the potassium phosphate–NaCl buffer (pH 7.4) in a 2-cm glass cuvet (Carl Zeiss, part no. 55-80-72-002) containing a small stirring bar. The cuvet was placed in a water jacketed compartment maintained at 37° and equipped with a stirring motor. The compartment was in the light path of a Zeiss PMQ II spectrophotometer set at 700 nm and equipped with a recorder (Estezline Angas Speed Servo, Model S601S). As sugar leaves the cells, the cells shrink, and this was recorded as a change in transmittance. The exit time, $t$, which is inversely proportional to the initial velocity of sugar efflux (Sen and Widdas, 1962a), was obtained by extrapolating the initial slope of the tracing to the equilibrium value. The kinetic parameters of transport can be obtained by assaying in the presence of increasing sugar concentrations in the assay buffer. When the exit time is plotted against the external sugar concentration, the intercept on the ordinate gives the minimum exit time at zero glucose concentration, and the intercept on the abscissa, $-K_m$ (Sen and Widdas, 1962a). All assays were performed at 37°.

The effect of cytochalasin B on glucose transport was determined by equilibrating the cells with 0.1 M glucose as described above and then with the appropriate concentration of inhibitor for 10 min at 37°. The cells were then assayed for sugar efflux in buffers also containing the proper concentrations of inhibitor. In order to remove cytochalasin B, the cells were washed three times in 20 vol of an inhibitor-free solution of 0.1 M glucose.

Reactions with 2 mM $N_2$ph-F or 10 mM MalNEt were carried out as a function of time in isotonic sodium phosphate buffer (pH 7.5) at 25° with cells preequilibrated at 37° with glucose, inhibitors, or buffer. $N_2$ph-F was added as a 0.4 M solution in acetone and MalNEt as a 1 M solution in 50% ethanol–$5 \times 10^{-3}$ N HCl. Reactions were stopped by pipetting 1 vol of the reaction mixture into 3 or 4 vol of ice-cold buffer, centrifuging briefly, and washing the cells once with phosphate-buffered saline. Cells were then equilibrated with 0.1 M glucose for >16 hr at 5° and assayed for transport activity as described above.

## Results

When efflux of glucose from red blood cells is assayed in the presence of cytochalasin B, extensive inhibition is found. Inhi-

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[1] Abbreviations used are: $N_2$ph-F, 1-fluoro-2,4-dinitrobenzene; MalNEt, *N*-ethylmaleimide.

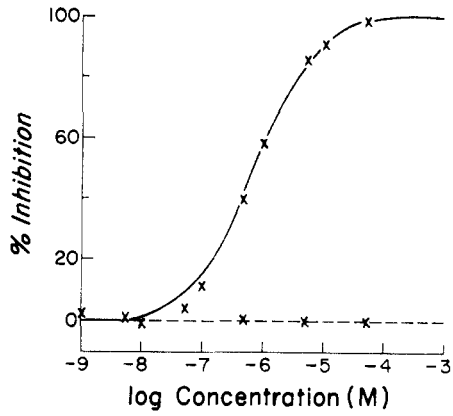


FIGURE 1: Inhibition of sugar transport as a function of the concentration of cytochalasin B. Red cells were first equilibrated with 0.1 M glucose for 30 min at 37° and then with the appropriate concentration of cytochalasin for 10 min at 37°. They were assayed at 37° for sugar efflux in the potassium phosphate–NaCl buffer (pH 7.4) containing the proper concentration of the inhibitor. The solid line represents the inhibition in the presence of cytochalasin B; the dashed line represents inhibition after cells are washed free of the inhibitor. The ordinate indicates the per cent inhibition of maximal transfer rate.

bition is half-maximal at $5 \times 10^{-7}$ M cytochalasin B, and at all concentrations it is readily reversible by washing cells in inhibitor-free solutions (Figure 1).

The effects of cytochalasin B on the kinetic parameters of sugar transport are presented in Figure 2. At three concentrations of inhibitor, only the $V_{max}$ of transport is decreased, while $K_m$ remains unchanged. Cytochalasin B therefore inhibits sugar transport noncompetitively, in agreement with its reported effect on lymphocytes (Zigmund and Hirsch, 1972) and HeLa cells (Mizel and Wilson, 1972). The effects expected for competitive inhibition are also shown in Figure 2, using phloretin as the inhibitor (Miller, 1969; Sen and Widdas, 1962b; LeFevre, 1954). The results are clearly different.

If cytochalasin B is in fact a noncompetitive inhibitor of sugar transport, it should have predictable effects on the covalent modification of the transport system with $N_2$ph-F and MalNEt. Since both these reagents inhibit transport irreversibly by decreasing $V_{max}$ without changing $K_m$ (Sen and

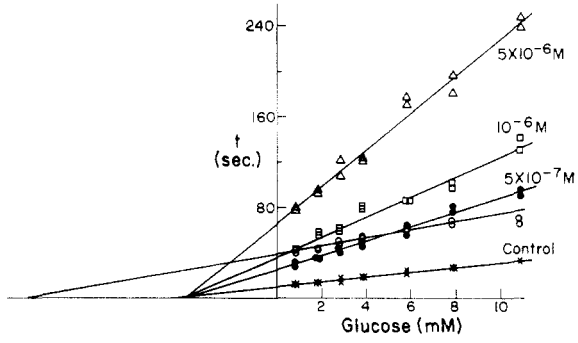


FIGURE 2: The effects of cytochalasin B on the kinetic parameters. Cells were first equilibrated with 0.1 M glucose for 30 min at 37°. They were then equilibrated for 10 min at 37° either with $5 \times 10^{-6}$ M phloretin (○), or with cytochalasin B at $5 \times 10^{-7}$ (●), $10^{-6}$ (□), or $5 \times 10^{-6}$ M (△). They were assayed by the method of Sen and Widdas (1962a) in the potassium phosphate–NaCl buffer (pH 7.4) at 37° in the presence of inhibitor to determine the kinetic parameters. The control (X) was free of inhibitor. The ordinate represents the exit time. The decrease in $V_{max}$ seen in the presence of phloretin is expected for a competitive inhibitor under these assay conditions (for a discussion, see Miller, 1969).

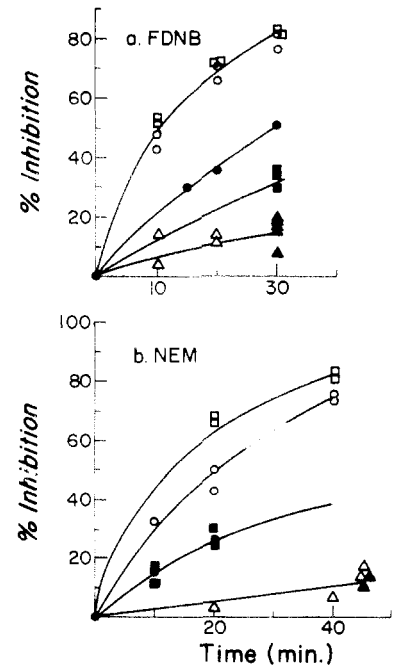


FIGURE 3: The effects of cytochalasin B on the reactions of $N_2$ph-F and MalNEt with red cells. Cells were equilibrated with 0.1 M glucose for 30 min at 37° when appropriate and then with $5 \times 10^{-5}$ M cytochalasin B or $10^{-4}$ M phloretin for 10 min at 37°. The cell suspensions were then brought to 25° and the reagents were added to a final concentration of 2 mM for $N_2$ph-F and 10 mM for MalNEt. Reaction conditions were: (○) in the presence of glucose; (●) in the absence of glucose; (△) plus cytochalasin B in the presence of glucose; (▲) cytochalasin B alone; (□) plus phloretin in the presence of glucose; (■) phloretin alone. All reactions were done in the isotonic sodium phosphate buffer (pH 7.5), 25°. Assays were done in the potassium phosphate–NaCl buffer (pH 7.4) at 37°.

Widdas, 1962a; Dawson and Widdas, 1963), they are also noncompetitive inhibitors. Thus, cytochalasin B would be expected to decrease the rate of reaction of $N_2$ph-F and MalNEt with the site on the transport system which is necessary for translocation, and this effect should be independent of glucose concentration. Figure 3 shows that these predictions are correct. A comparison with the effects of phloretin on these reactions (Figure 3) indicates once again that the two inhibitors have different effects on the transport system.

When the cells are washed free of cytochalasin B and of $N_2$ph-F or MalNEt, and then exposed again to $N_2$ph-F or MalNEt in the absence of cytochalasin B, transport is again rapidly inactivated indicating that the cells are fully sensitive to $N_2$ph-F or MalNEt.

## Discussion

The finding that cytochalasin B is a noncompetitive inhibitor of glucose transport is in agreement with published results. Two other laboratories, using lymphocytes (Zigmund and Hirsch, 1972) and HeLa cells (Mizel and Wilson, 1972), have also found that cytochalasin B is a noncompetitive inhibitor. One, however, has reported that it is a competitive inhibitor (Estensen and Plagemann, 1972). The reason for this discrepancy is not clear, but may be due in part to the leakiness of the hepatoma cells used in that study. The report of Cohn *et al.* (1972) that cytochalasin B ($2.8 \times 10^{-5}$ M) does not affect total sugar utilization by erythrocytes is not at variance with the results reported here. Glucose transport in human red cells proceeds $10^3$ times faster than does utilization (Murphy, 1960; Wood *et al.*, 1968), and would have to be inhibited more

than 99% before utilization would be affected. Their finding corroborates previous reports that, at concentrations inhibitory to transport, cytochalasin B has no independent effect on glycolytic activity (Estensen and Plagemann, 1972; Mizel and Wilson, 1972; Zigmund and Hirsh, 1972).

Although it is not known whether or not cytochalasin B interacts directly with the sugar transport system, it is a valuable probe for this system. Cytochalasin B is the only reversible noncompetitive inhibitor known, and it protects transport activity against inactivation by $N_2$ph-F and MalNEt better than any compound so far examined[2] (Krupka, 1971). These reagents appear to react with an amino group essential for sugar transport.[2] Since cytochalasin B affects only $V_{max}$, and since it competes with these reagents, the amino group is probably related to sugar translocation rather than to binding. Further use of cytochalasin B in conjunction with modifying reagents should furnish information about the other groups involved in sugar binding and translocation.

Because cytochalasin B protects transport activity against chemical modification by $N_2$ph-F and MalNEt so well, it has also been used in attempts to identify the sugar "carrier," using differential double-labeling techniques. These attempts have been unsuccessful, and the results indicate that the number of transport systems per cell is of the order of $10^4$ or smaller.[3]

It is to be noticed that cytochalasin B, at concentrations similar to those that inhibit sugar transport, also reversibly inhibits a variety of cell movements, presumably dependent on a specific class of 50-Å microfilaments (Wessells *et al.*, 1971). The relationship, if any, between these two effects of cytochalasin B is not clear. However, since macromolecular biosynthesis in several tissues is unaffected by cytochalasin B (Estensen and Plagemann, 1972; Cohn *et al.*, 1972; Spooner and Wessells, 1970; Yamada *et al.*, 1970), it is most unlikely that inhibition of cell movement is secondary to inhibition of sugar transport.

On the other hand, it is certainly possible that cytochalasin B interacts with a unique membrane component (protein) which can modulate the function of those components involved in cell movements and affect the membrane transport systems. For example, cytochalasin B might bind to a membrane protein that is part of a membrane-bound actomyosin-like system (Guidotti, 1972) which interacts with components responsible for the movement of receptors in membranes (dePetris and Raff, 1972) and of the cell itself (Wessells *et al.*, 1971). Such a cytochalasin B binding component by regulating the local state of the membrane could affect particular transport systems in addition to its effect on cell movements.

[2] R. Bloch, manuscript to be submitted for publication.

[3] R. Bloch, unpublished observations.

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