ANTIMOTILITY EFFECT OF CYTOCHALASIN B
OBSERVED IN MAMMALIAN CLOT RETRACTION

DAVID SHEPRO, FRANK A. BELAMARICH, LOIS ROBBLEE, and FRANCIS C. CHAO. From
the Department of Biology, Boston University Graduate School and the Marine Biological Laboratory,
Woods Hole, Massachusetts 02543. Dr. Chao’s present address is the Blood Research Institute, Boston,
Massachusetts 02215

INTRODUCTION

A variety of agents, e.g. colchicine, that are known to affect mitosis are also of value in providing in-
formation about motility of and within the cell. Cytochalasins isolated from yeast by W. B. Turner,
and shown by Carter and others to inhibit motility and to block cell division, may be classified as
another antimotility group (1). One unique feature of this compound is that it may be spe-
cific for microfibers since cytoplasmic cleavage of mouse fibroblasts (1) and human lymphocytes (2)
is inhibited, but not nuclear division. Direct evidence on the effect of cytochalasins on micro-
tubules and microfibers or its way of action is not present.

We demonstrated that the mammalian plate-
let and nonmammalian thrombocyte contractile
mechanism as seen in clot retraction is remarkably
similar to the contractile systems controlling bio-
logical movement described for other cells (3),
and a theoretical model for contractile microfiber
formation in platelets was postulated (4). Re-
cently, we described in glycerinated platelets
the complexing of a 60 A diameter microfiber
with heavy meromyosin that produced a distinct
arrowhead pattern similar to that described for
muscle actin or with microfibers from nonmuscle
cells (5). We now wish to report the effect of cyto-
chalasin B on the contractile system of human
and other mammalian platelets as seen in clot
retraction.

METHODS

The preparation of the platelet-rich plasma (PRP)
and clot retraction was described previously (3); the
cytochalasin B was dissolved in dimethyl sulfox-
ide (DMSO) following the procedure of Carter (1). Human and calf PRP suspensions were varied with
respect to number of cells, concentration of cyto-
chalasin, and time of incubation. All suspensions
were incubated at 37° C, and concentrations ex-
pressed throughout the report are final. Cell prepar-
ations were fixed for electron microscopy by using
standard glutaraldehyde-osmium tetroxide proce-
dures.

RESULTS AND DISCUSSION

The results of incubating for 45 min 10 µg of
cytochalasin-0.01% DMSO/ml PRP are illus-
trated in Fig. 1, and they show that cytochalasin B
inhibits contraction of human PRP clots and the
onset and duration of retraction of calf PRP clots.
We assume that the difference in rates and degree
of contraction between the two species is a re-
flexion of the reduced amount of cytochalasin
per cell in the calf experiment, and permeability
specificity. An equivalent inhibition of clot
retraction was obtained with 5 µg of cytochalasin
per ml PRP, but no alteration in clot retraction
was observed with the 1 µg of cytochalasin per
ml of PRP. (Experiments with cow and rabbit
PRP and with amphibian thrombocyte-rich
plasma produced parallel inhibition of clot
retraction.) Varying the incubation time from 15
min to 1 hr had a negligible effect on the rate
and degree of clot retraction, and cytochalasin-
DMSO does not appear to affect protein synthesis
as measured by leucine-14C uptake.

These data demonstrate that cytochalasin B
produces an effect on platelet contractile system
that is similar to that observed when platelets
are incubated with 10⁻³ M colchicine or when
platelets are suspended in physiological saline
made with heavy water. Because of the tem-
porary inhibition of clot retraction by cytochal-
asin B, one assumption is that the cells are pro-
ducing sufficient contractile proteins to overcome
the action of cytochalasin, and only then does clot retraction occur at a rate equivalent to the controls. The one significant difference in the present experiment when compared with the

colchicine experiment is that the platelets treated with cytochalasin have microtubules present (Fig. 2), whereas published micrographs (3) of colchicine-treated platelets show a complete ab-

FIGURE 1 The effects of cytochalasin B (10 µg-0.01% of DMSO/ml platelet suspension) on clot retraction compared with the control that is plotted as 100%. Control clot retractor at the end of 60 min equalled 92 and 95% for human and calf platelet suspensions, respectively.
sence of microtubules. These results indirectly support our premise that platelet contractility and clot retraction are functions of the microfibers; the data also support the postulation of Carter that cytochalasin suppresses biological motility and that it may be specific for microfibers.

We are indebted to Dr. S. B. Carter and the Imperial Chemical Industries Limited (Macclesfield, England) for supplying the cytochalasin B. Upon submitting our preliminary data to Dr. Carter, we were informed by Dr. R. J. Haslam, at the same institution, that he obtained similar results as reported

FIGURE 2 Fine structure of platelets incubated in plasma containing 10 μg of cytochalasin B-0.01% DMSO/ml suspension. Aliquots were used for a platelet pellet (Fig. 2a) and clot (Fig. 2b). Note in both examples the presence of microtubules (arrow). Platelet obtained from a clot does not show typical morphological alterations associated with activation, e.g. pseudopod formation, degranulation, fibrogenesis. X45,000.
in this paper, and has just recently submitted his work for publication. We wish to acknowledge the assistance of Ann Marie Roy, and use of the electron microscope facilities of Dr. W. A. Bardawil, Saint Margaret's Hospital, Boston, Mass.

The research was supported in part by United States Public Health Service grants from the National Heart Institute (HE 05411-10, HE 10002-5), and the American Heart Association (67-690).

Received for publication 13 April 1970.

REFERENCES

1. CARTER, S. B. 1967. Nature (London). 213:261.
2. SMITH, G. F., M. A. C. REDLER, and J. A. FAUNCH. Nature (London). 216:113.
3. SHEPRO, D., F. A. BELAMARICH, and F. C. CHAO. 1969. Nature (London). 221:563.
4. CHAO, F. C., D. SHEPRO, and F. YAO. 1970. Microvascular Res. 2:51.
5. SHEPRO, D., F. C. CHAO, and F. A. BELAMARICH. 1969. J. Cell Biol. 43:29 (Abstr.)