ANTIMOTILITY EFFECT OF CYTOCHALASIN B OBSERVED IN MAMMALIAN CLOT RETRACTION

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INTRODUCTION

A variety of agents, e.g. colchicine, that are known to affect mitosis are also of value in providing information 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 specific 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 microtubules and microfibers or its way of action is not present.

We demonstrated that the mammalian platelet and nonmammalian thrombocyte contractile mechanism as seen in clot retraction is remarkably similar to the contractile systems controlling biological movement described for other cells (3), and a theoretical model for contractile microfiber formation in platelets was postulated (4). Recently, 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 cytochalasin 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[1] was dissolved in dimethyl sulfoxide (DMSO) following the procedure of Carter (1). Human and calf PRP suspensions were varied with respect to number of cells, concentration of cytochalasin, and time of incubation. All suspensions were incubated at 37° C, and concentrations expressed throughout the report are final. Cell preparations were fixed for electron microscopy by using standard glutaraldehyde-osmium tetroxide procedures.

RESULTS AND DISCUSSION

The results of incubating for 45 min 10 μg of cytochalasin-0.01% DMSO/ml PRP are illustrated 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 reflection 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-^{14}C 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^{-2} M colchicine or when platelets are suspended in physiological saline made with heavy water. Because of the temporary inhibition of clot retraction by cytochalasin B, one assumption is that the cells are producing sufficient contractile proteins to overcome

[1] Several cytochalasins differ in potency but have similar antimotility activity.

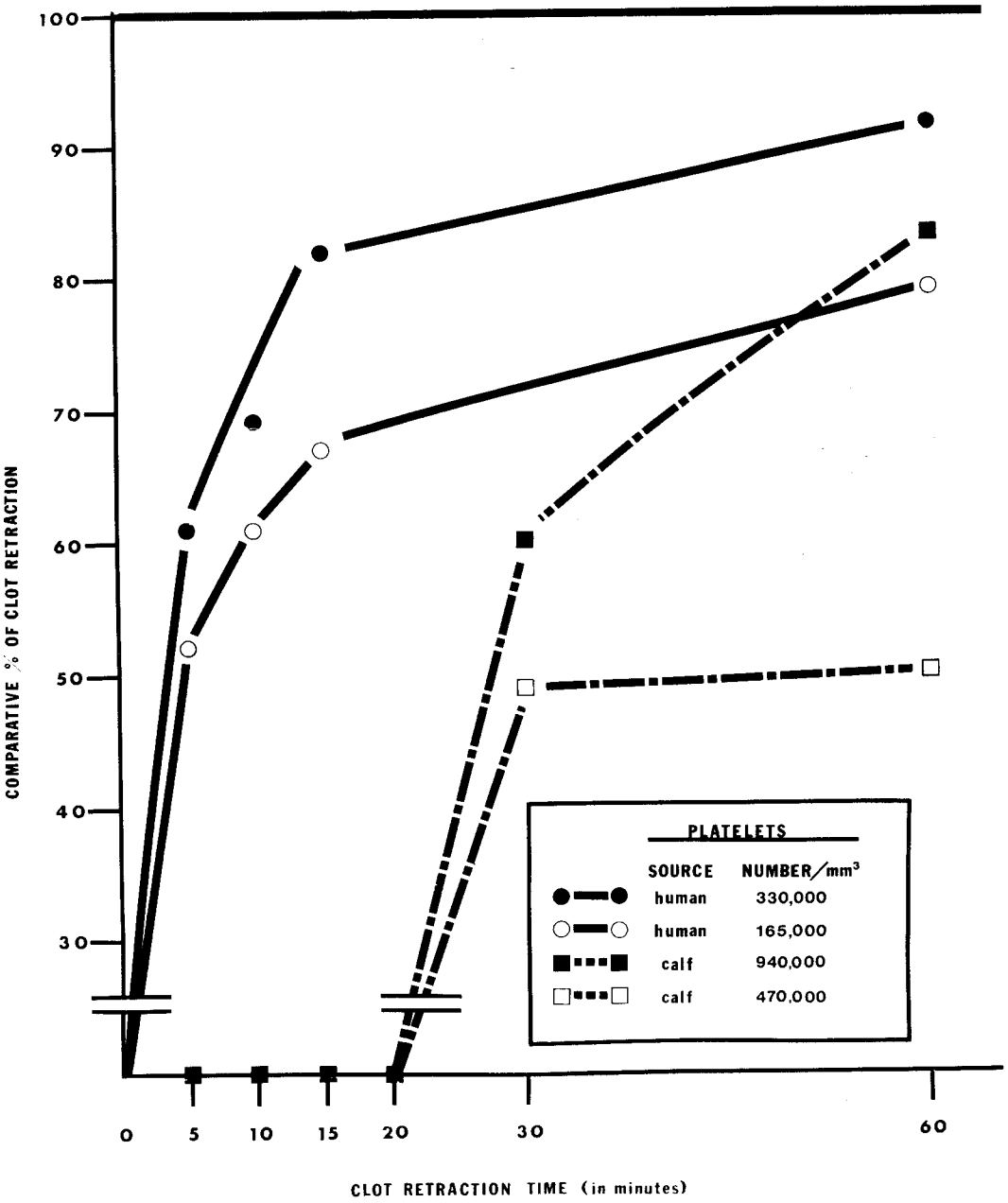

FIGURE 1 The effects of cytochalasin B (10 μg–0.01% of DMSO/ml platelet suspension) on clot retraction compared with the control that is plotted as 100%. Control clot retractions at the end of 60 min equalled 92 and 95% for human and calf platelet suspensions, respectively.

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-

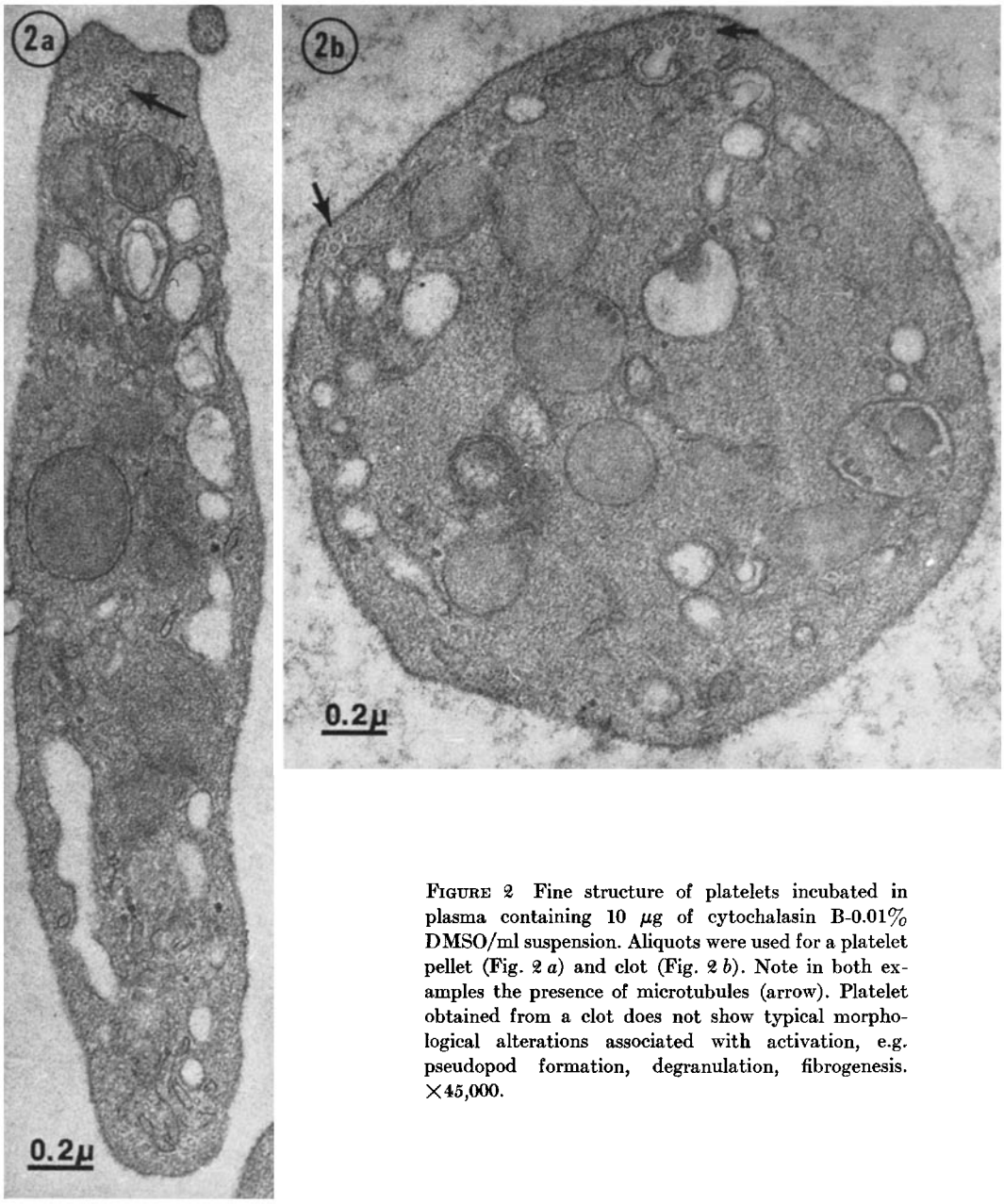

FIGURE 2 Fine structure of platelets incubated in plasma containing 10 μg of cytochalasin B-0.01% DMSO/ml suspension. Aliquots were used for a platelet pellet (Fig. 2 *a*) and clot (Fig. 2 *b*). Note in both examples the presence of microtubules (arrow). Platelet obtained from a clot does not show typical morphological alterations associated with activation, e.g. pseudopod formation, degranulation, fibrogenesis. ×45,000.

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.

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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 Margarets Hospital, Boston, Mass.

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