MYOSIN-LIKE TACTOIDS IN TRYPSIN-TREATED BLOOD PLATELETS

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INTRODUCTION

In a recent study of amphibian smooth muscle it was found that on exposure to trypsin solutions some of the cells develop large populations of thick filaments which have the size and shape of artificially prepared aggregates of smooth muscle myosin and which also exhibit an axial periodicity consistent with that derived from X-ray diffraction studies of myosin (7, 8).

Since blood platelets are also known to be contractile and to contain an actomyosin-like protein, thrombosthenin (2), the equivalent experiment was carried out on them in an effort to induce aggregation of their myosin-like component into a form capable of being visualized and characterized *in situ*. Under the conditions used, thick filaments do in fact appear in some of the platelets; however, these inclusions are considerably

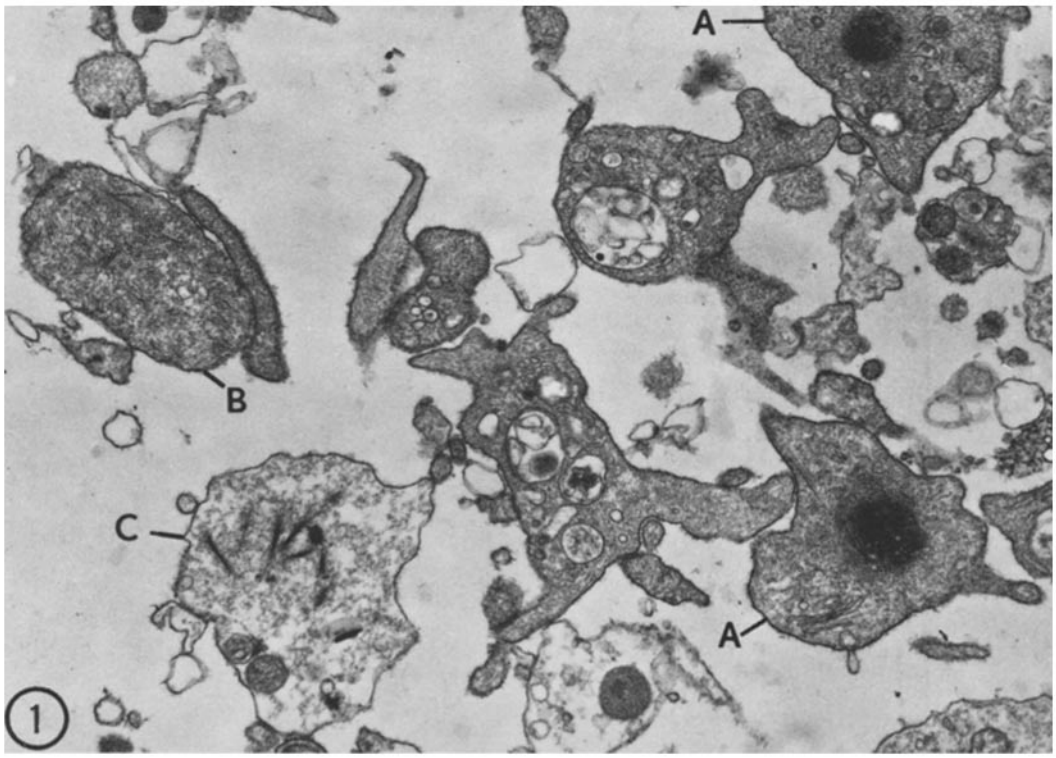
FIGURE 1 Survey view of trypsin-treated blood platelets. Two of the platelets (*A*) exhibit a dense filamentous ball; one platelet (*B*) contains several dispersed spicular thick filaments; and one platelet (*C*) contains several larger caliber thick filaments. × 18,000.

FIGURE 2. Detail of filamentous ball from a platelet in dendritic form. Many ~90-A filaments are visible and some of these (arrows) are beaded or interconnected at a period of ~115 A. × 111,000.

FIGURE 3 Detail of slightly damaged platelet containing tactoidal inclusions ~325 A in maximum diameter. Crossbanding is faintly visible in these filaments. × 62,000.

FIGURE 4 Detail of moderately damaged platelet containing thick filaments ~415 A in diameter. Crossbanding is again faintly visible. × 104,000.

FIGURE 5 Several overlapping crossbanded tactoids ~340 A in diameter appear to have fused along their sides (left). Amorphous inclusions occur at the right. × 62,000.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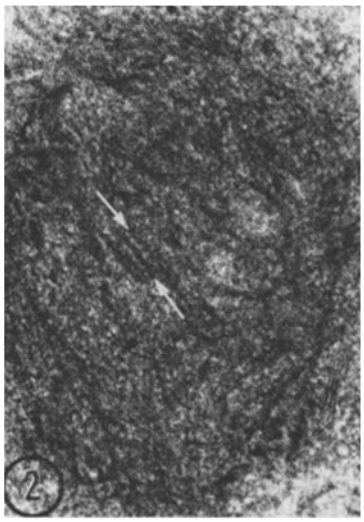
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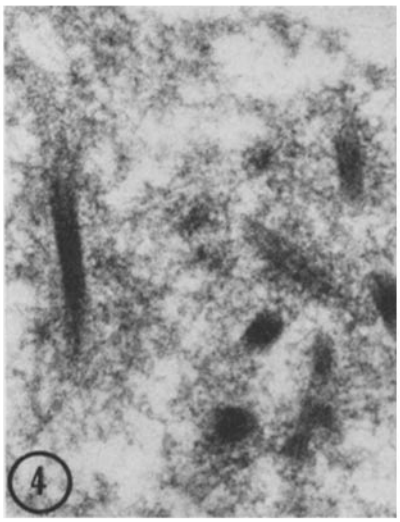
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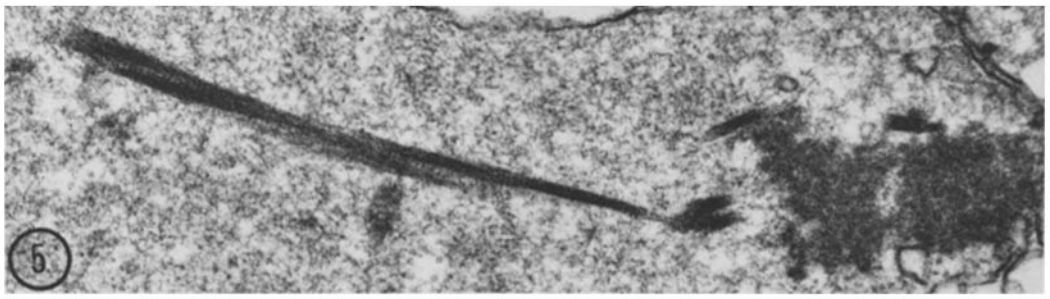
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more irregular in size and shape than the thick filaments that occur in trypsin-treated smooth muscle cells, and they exhibit crossbands which repeat at a smaller period. The identity of these inclusions is discussed in relation to thrombosthenin and other platelet proteins.

MATERIAL AND METHODS

Human blood-platelet concentrate in acid citrate dextrose (ACD) solution was distributed into polystyrene tubes and centrifuged at 3100 rpm in a Sorvall SS centrifuge for approximately 5 min. The supernatant was discarded and replaced with Ringer's solution plus 0.02 M Tris-HCl buffer (pH 7.4) plus either salt-free trypsin (2 × or 3 × crystalline) in a concentration of 2 mg/ml or diisopropyl fluorophosphate (DFP)-inactivated trypsin in the equivalent concentration. The tubes were kept at room temperature for approximately 2 hr, after which the test solutions were replaced with 2.8% glutaraldehyde in PO_4 buffer (pH 7.4) for about 1 hr. In some instances the platelets were left in pellet form throughout the procedure after the initial centrifugation. In others, the platelets were resuspended in the test solution and at each subsequent step. Rinsing, postfixation with OsO_4, and further processing for electron microscope examination were carried out by conventional methods. Some of the specimens were soaked in 0.5% uranyl acetate at pH 5 before dehydration. Sections stained with uranyl acetate and lead hydroxide were viewed in a Philips EM 300 electron microscope. The platelets used were obtained from three different donors during January and February of 1970 by the New York Blood Center.

RESULTS

A field of trypsin-treated platelets contains cells in a wide range of conditions varying from severely damaged at one extreme to virtually unaffected at the other and including many in intermediate states (Fig. 1). Platelets that are clearly damaged, but have not yet disintegrated, frequently contain tactoidal inclusions whose diameter varies greatly from cell to cell but is rather constant within any one cell (Figs. 3 and 4). These inclusions are ~200–800 A across, the larger caliber ones occurring in the more severely damaged platelets. At high magnification, finer, longitudinally oriented subunits can be visualized within the inclusions (Fig. 8). In certain cases, damaged cells contain "super aggregates," which, when sectioned obliquely, may exhibit staircase-like arrays of the longitudinal subunits (Fig. 7).

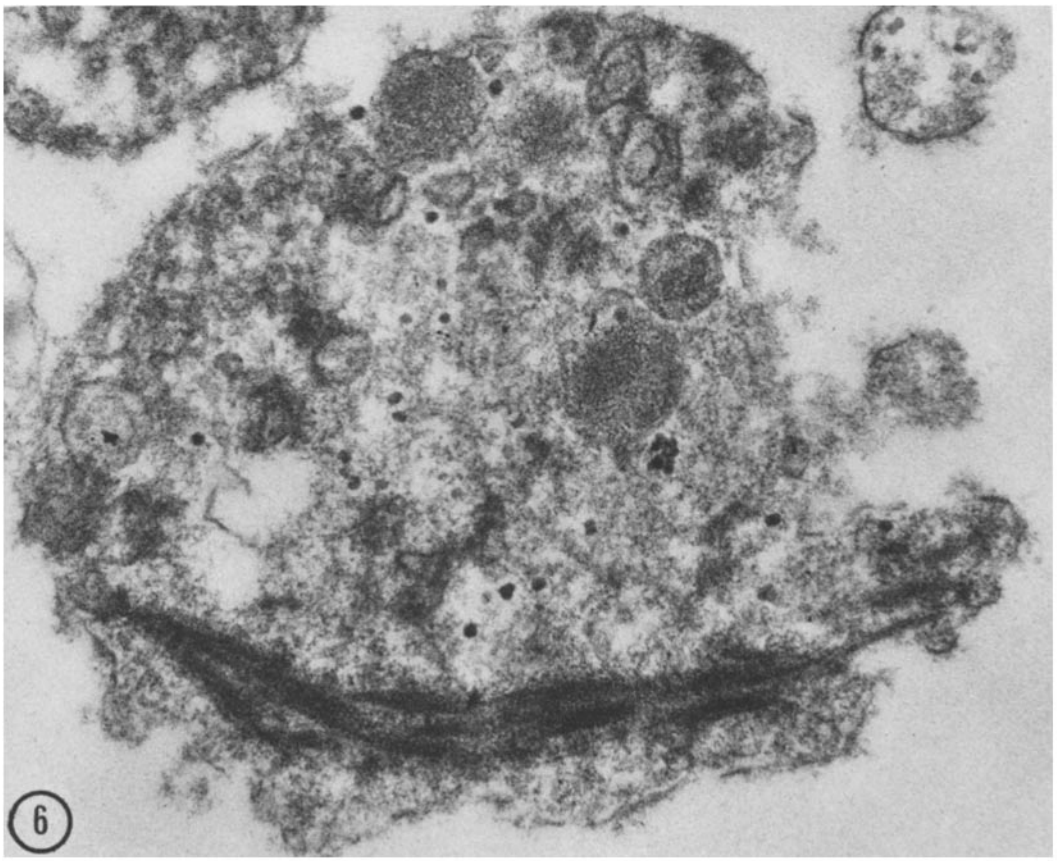
The most striking feature of these tactoids is their crossbanding (Figs. 4–8). Dark and light bands alternate with a repeat period of ~115 A. This axial period approximates that of the beaded ~90-A filaments which occur within platelets in the dendritic form (Fig. 2). Periodic banding is much more obvious in the larger caliber aggregates than in the smaller ones, and in some instances (e.g. Fig. 4) dark crossbands seem to extend beyond the visible edge of the inclusion producing the appearance of serrations or projections. Since the aggregates are not homogeneously dense across their width, however, (Fig. 8) the location of the edge is ambiguous in preparations of this kind. Apparent notching of the edges was also noted in the tactoids found in smooth muscle cells under the same conditions (Fig. 8 inset in reference 8).

In addition to these banded intracellular inclusions, which tend to be elongated and straight, some of the damaged platelets also contain dense amorphous masses which do not have a crystalline appearance (Fig. 5). Such amorphous dense material also appeared together with thick filaments in trypsin-treated smooth muscle cells (8). Undamaged platelets contain prominent microtubules, but these are not present in cells that have been damaged and contain the tactoidal inclusions. Extracellularly, fibrin threads, approximately 500 A in diameter, are present in some control preparations. These threads also exhibit a crossband pattern which, however, has a larger repeating period than that seen in the

FIGURE 6 Damaged blood platelet showing interruptions in its limiting membrane. A group of tactoids ~375 A in diameter is present at the bottom. Crossbanding at a period of ~115 A is distinct in this case. × 80,000.

FIGURE 7 Transverse and oblique views of "super aggregates." Their width in this case is ~1500 A. × 84,000.

FIGURE 8 Detail of crossbanded inclusion ~600 A wide showing longitudinally oriented subunits. × 205,000.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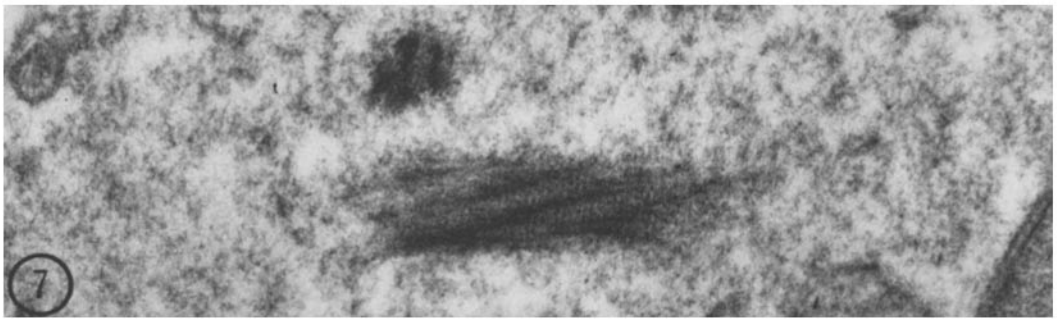
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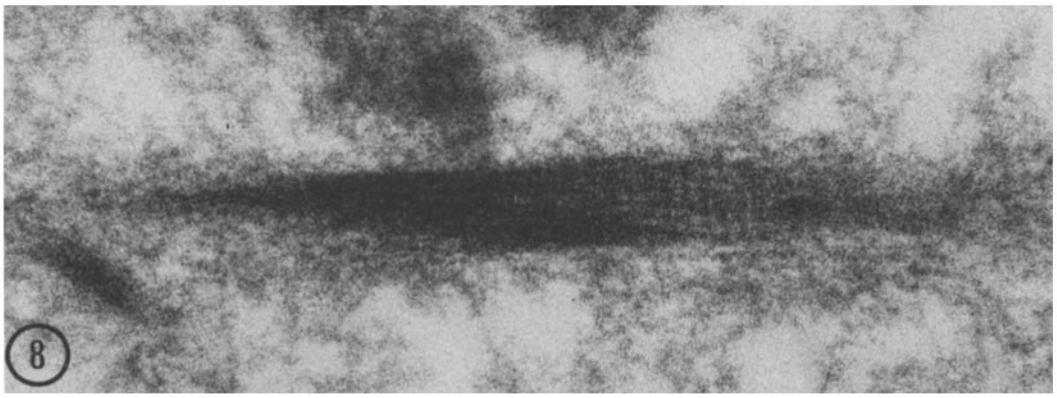
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intracellular tactoids, and they also have a much greater tendency to bend and branch.

Control preparations exhibit numerous platelets in the dendritic form containing beaded ~90-A filaments plus occasional damaged platelets containing tactoidal inclusions. The latter occur much less commonly than in the trypsin-treated samples.

DISCUSSION

Blood platelets are one of a growing number of cells which are distinct from muscle but which nevertheless contain counterparts to both actin and myosin. In platelets as in smooth muscle cells, however, the contractile proteins ordinarily exhibit no orderly arrays comparable to the double array of filaments in cross-striated muscles.

The present study shows that tactoidal thick filaments can be found in platelets, as in smooth muscle, after trypsin treatment, in cells that exhibit signs of damage. The thick filaments that form in smooth muscle cells are strikingly similar to artificially prepared aggregates of smooth muscle myosin, and it therefore seems very likely that they are composed of myosin (8). The platelet tactoids, however, are much more variable in size, and the repeat period they exhibit is distinctly smaller than that of muscle myosin; their identity is therefore not certain.

Light meromyosin (LMM) derived from skeletal muscle tends to form larger and more irregularly sized aggregates than myosin itself (4). The aggregates reported here might thus be composed wholly or partly of platelet myosin tails (cf. reference 5). Platelets are also known to contain fibrinogen (6). The axial period of fibrin threads is usually reported as 230 A, or approximately double that of the intracellular tactoids described here (3). However, according to one view (1), the fibrinogen molecule is roughly 375 A long, is composed of three ellipsoid subunits in a chain, and aggregates in a "staggered" manner. Such a model could account for an axial period either one-third or two-thirds the monomeric length. Other intracellular proteins, such as microtubule protein, could in principle form spindle-shaped crystals as well, and so indeed could extracellular proteins that might enter damaged cells. These last possibilities seem unlikely, however, in view of the absence of such inclusions in the various nonmuscle cells that were exposed to trypsin in earlier studies of the intestinal wall.

It is concluded that the inclusions reported here probably consist of either platelet myosin, light meromyosin, or a combination of the two, or, as a second possibility, of a form of fibrin in which the axial period is half the usual figure. In an independent study, Zucker-Franklin has found similar thick filaments in blood platelets which have been treated with adenosine triphosphate (9).

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