Arrangements of Actin Filaments in the Cytoskeleton of Human Platelets

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Assembly of actin molecules into filaments is closely associated with platelet shape change and exercise of contractile function. Since assembled actin filaments serve both as a framework for distortion of discoid shape and for generation of contractile force, it is important to determine the organizations of actin filaments capable of serving the two opposed functions of distortion and contraction. The present study has used negative staining alone and after combined glutaraldehyde fixation and detergent extraction to examine the arrangements of actin filaments in the cytoskeletons of surface activated platelets. Actin filament assembly developed as one of the earliest manifestations of platelet activation. Small protuberances containing random networks of actin filaments extending beyond the circumferential microtubules appeared to be an initial step in the response to stimulation. Transformation into dendritic forms was associated with development of parallel bundles of actin filaments organized into paracrystalline lattices with a periodicity of 5.5 nm at an angle of 60 degrees with the long axis of the pseudopod. Parallel bundles of actin filaments formed the concave borders of late dendritic forms and expanded to become the convex margin of most spread cells, suggesting a possible role in the spreading process. Other bundles of actin filaments resembled stress fibers radiating through the cytoplasm into pseudopods or organized in a variety of other apparently stable configurations. More loosely associated masses of actin filaments formed concentric layers around constricted rings of microtubules or a random network in the peripheral cytoplasm of spread cells. The arrangements of newly assembled actin filaments suggest their involvement in cell deformation, as well as contractile events. (Am J Pathol 1984, 117:207-217)

PLATELETS are, in essence, circulating muscle cells.[1-4] Most of their responses to stimulation, including shape change, internal transformation, secretion, hemostatic plug formation, and clot retraction, are regarded as manifestations of contractile activity.[5] The cells contain membrane complexes,[6] the equivalent of muscle sarcoplasmic reticulum, and calcium flux appears to serve as a final common pathway through which most agonists cause activation.[7] In addition, platelets possess substantial amounts of protein closely resembling the actin, myosin, tropomyosin, and other molecules that make up the contractile system in skeletal and smooth muscle cells.[8]

However, resting platelets do not contain the highly organized arrays of thick and thin fibers found in skeletal muscle; nor do they possess the sheets of assembled filaments observed in smooth muscle.[9] Instead, the contractile protein molecules appear to be primarily in a globular form.[8-10] After platelet stimulation globular actin rapidly assembles into masses of filaments. The masses and bundles of actin filaments serve as a framework to distort cell shape and to generate contractile force.[11,12] How the actin filament system organizes in order to serve the two apparently opposed functions of deformation and contraction is uncertain.

In the present study I have used the whole-mount method to study platelets at intervals after activation by carbon-coated formvar surfaces of electron microscope grids.[13,14] Platelet samples were either negatively stained without fixation or exposed to fixation and detergent extraction first and then negatively stained. The assembly of actin filaments into parallel bundles in pseudopods and radiating from or around the cell center, in a peripheral weave at the platelet margin and as

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a random meshwork in the cytoplasm, suggest that contractile elements can serve as stress fibers to stabilize asymmetric form or act as a force-generating system, depending upon the organization they assume.[15-17]

Materials and Methods

General

Blood for this study was obtained from well-characterized normal donors after informed consent. Samples obtained by venepuncture were mixed immediately with citrate-citric acid-dextrose, pH 6.5 (9.3 mM sodium citrate, 7.0 mM citric acid, and 140 mM dextrose) in a ratio of 9 parts blood to 1 part anticoagulant.[18-21] Platelet-rich plasma (C-PRP) was separated from whole blood by centrifugation at 100*g* for 20 minutes at room temperature.

Special Reagents

Taxol,[22,23] a microtubule-stabilizing agent, has been employed in several previous investigations from our laboratory.[24-26] The drug protected platelet microtubules from disassembly by cold and antimitotic agents but had no effect on any aspect of the physiologic platelet response. It was combined in half the experiments of the present study to minimize damage caused by air-drying. Taxol was obtained from the Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. The agent was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM. A volume of 10 μl added to 1 ml of C-PRP yielded a final concentration of 10^{-4} M. Samples of C-PRP were combined with taxol for 30 minutes before use.

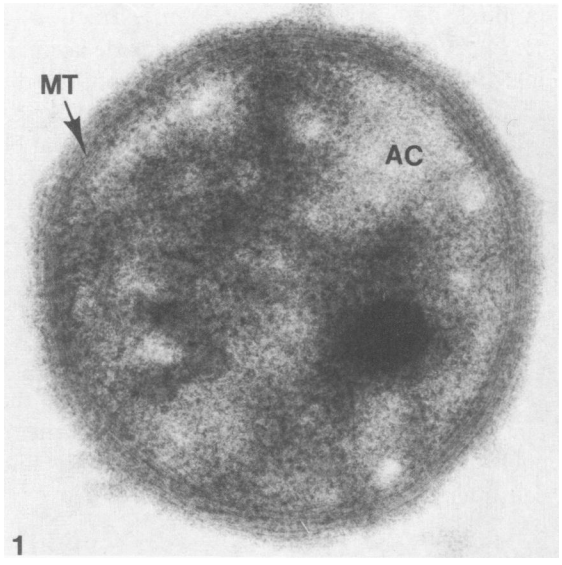
Experimental Studies

Incubated samples and control C-PRP were fixed in suspension for study in thin section according to methods described previously.[18-20] Drops of taxol treated and control C-PRP were placed on carbon stabilized, formvar-coated grids for 1, 10, 15, 30, and 60 minutes. Previous studies had shown that these intervals were adequate for maximal activation, and further contact time did not alter the frequency of various platelet forms.[13,14] On occasion, the grids were also coated with polylysine by flotation on drops containing 0.1% reagent, but in general, the additional layer was unnecessary.

Preparation of Whole Mounts

After incubation in moist chambers the grids were prepared for ultrastructural evaluation according to the procedure described by Small,[27] as modified from Hoglund et al,[28] or by negative staining alone. For the Small method the triton X-100 glutaraldehyde mixture consisted of 0.5% triton X-100 and 0.25% glutaraldehyde. Detergent extraction and fixation were carried out at room temperature. Grids carrying spread platelets were washed briefly in Tris-buffered saline followed by a cytoskeleton buffer (NaCl, 127 mM; KCl, 5 mM; Na_2HPO_4, 1.1 mM; KH_2PO_4, 0.4 mM; $NaHCO_3$, 4 mM; glucose, 5.5 mM; $MgCl_2$, 2 mM; EGTA, 2 mM; PIPES, 5 mM; pH 6.0–6.1). After washing in the cytoskeletal buffer, the cells were transferred to the triton X-100 glutaraldehyde mixture for 1 minute. After a brief wash in cytoskeleton buffer, the grids were stored on coverslips on the same buffer containing 2.5% glutaraldehyde for 2 hours before negative staining for electron microscopy. Staining in sodium silicotungstate was carried out at room temperature. Grids were rinsed two times in distilled water and transferred sequentially through 4 drops of bacitracin (40 mg/ml in water; Sigma Chemical Co., St. Louis, Mo) in a plastic Petri dish and drained briefly on the edge with filter paper. They were then passed through 4 drops of 3% sodium silicotungstate and then finally drained of excess stain and allowed to air-dry. Negative staining alone was accom-

Figure 1 — Discoid platelet from a sample of C-PRP fixed with glutaraldehyde and simultaneously extracted with detergent 1 minute after contact with a carbon-stabilized formvar grid and subsequently exposed to negative staining. The discoid cells are relatively thick and, therefore, somewhat resistant to transmission of the conventional electron beam. However, a circumferential microtubule (*MT*), coiled on itself several times, is apparent at the periphery of this cell. Extraction of internal membranes by detergent has left only vague outlines of internal organelles. Amorphous cytoplasm (*AC*) fills the interior of the platelet. (× 24,500) **Figure 2** — Discoid platelet prepared in a similar manner. A few randomly dispersed filaments (*F*) can be seen in the cytoplasm interior to the circumferential microtubule (*MT*). (× 57,000) **Figure 3** — Negatively stained platelet. The cell in this illustration was not fixed or exposed to detergent before negative staining. The cell has collapsed on the grid during air-drying. Its cytoplasm is filled with membranous channels (*MC*) of the open canalicular and dense tubular systems, as well as the membranes enclosing granules (*G*). They obscure the presence of microfilaments that might be present in platelet cytoplasm. A coiled band of microtubules is evident at the cell periphery. (× 14,700) **Figure 4** — Platelet activation. Some platelets begin to show signs of physical alteration within 1 minute after exposure to grid surfaces. A small protuberance (*P*) of cytoplasm extending beyond the circumferential microtubule (*MT*) on this discoid cell is one of the earliest signs of activation. A random network of filaments (*F*) is present in the protuberance and developing in the cytoplasm. The cell was negatively stained after simultaneous detergent extraction and fixation. (× 22,000) **Figure 5** — Platelet activation. This cell was prepared as in Figure 4, 10 minutes after exposure to the grid surface. Several protuberances (*P*) are evident, extending beyond the circumferential microtubule (*MT*). A random network of filaments (*F*) fills the protuberances and platelet cytoplasm. (× 22,000) **Figure 6** — Negatively stained platelet following activation. The platelet here was exposed to the grid for 10 minutes and then negatively stained without prior fixation or detergent extraction. Filaments (*F*) fill the protuberance (*P*) extending beyond the circumferential microtubule (*MT*). The subfilaments making up the microtubule are revealed in negatively stained samples which have not been fixed first. (× 70,000)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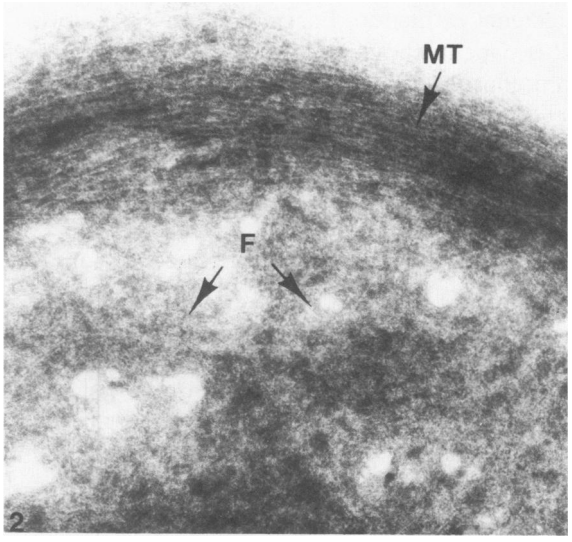
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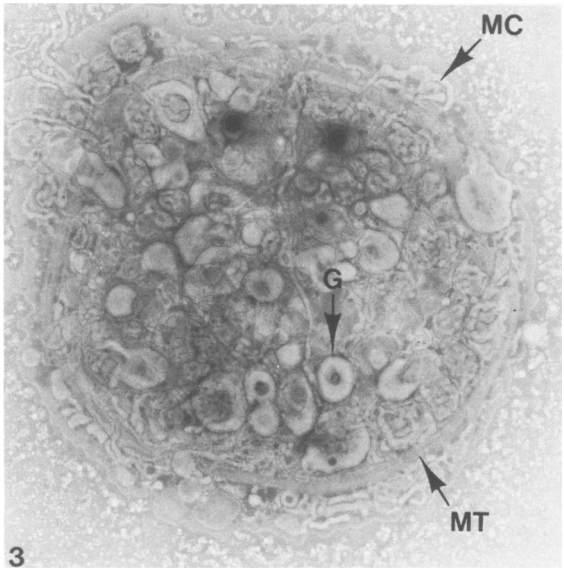
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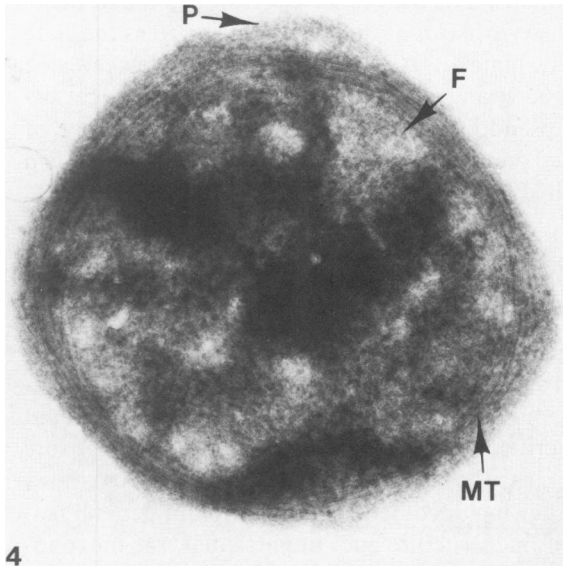
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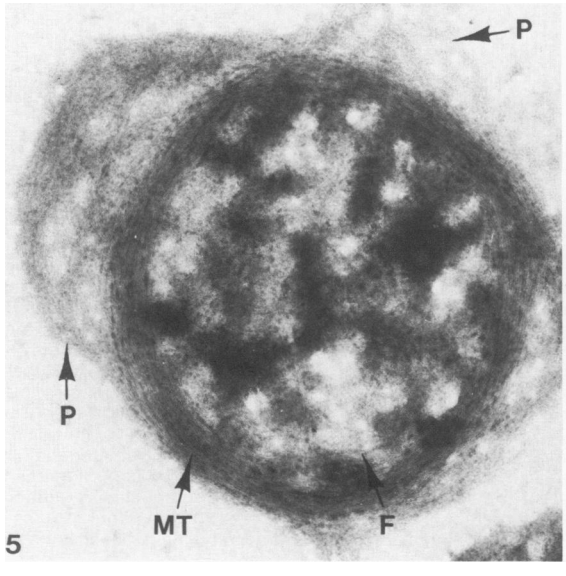
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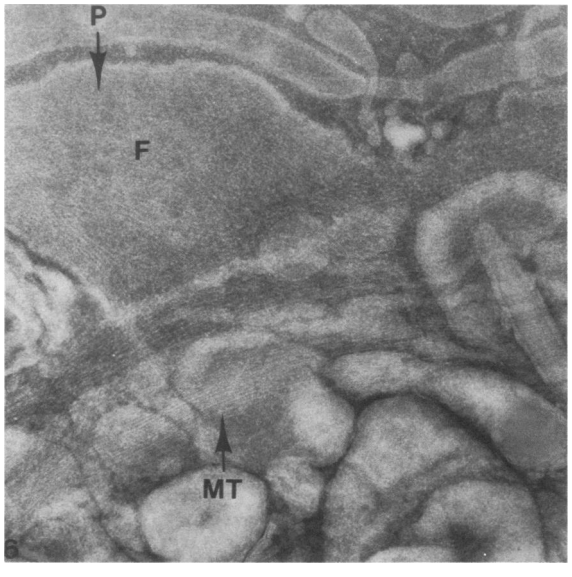
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plished by omitting the step of simultaneous fixation and detergent extraction. Excess plasma was removed with filter paper without permitting the grid to dry. The stain procedure was then carried out as described above.

Results

General Features of the Platelet Cytoskeleton

The fine structure of platelets activated by contact with the surfaces of carbon- or polylysine-coated grid surfaces has been described by many workers.[8,9,29-31] After settling for 15 minutes to an hour, the physical changes in platelets become reasonably stable.[13,14] About 10% of the cells retain their discoid form. The majority are relatively spherical, with a variable number of pseudopods, and are referred to as "early dendritic forms." In time these cells flatten and extend long pseudopods. The 80–100-kv electron beam can penetrate the cytoplasm of this type, referred to as the "late dendritic platelet." About 25% of the platelets revealed advanced changes. Cytoplasm filled in the spaces between pseudopods, which yielded the appearance of flattened pancakes. The thin films of platelet cytoplasm are called "spread forms."

Negatively Stained and/or Detergent-Extracted Platelets

General features of platelets which were negatively stained after simultaneous detergent extraction and fixation were similar to those which others have prepared by the critical-point method with or without exposure to detergent.[29-31] Major differences were apparent, however. They included better preservation of general features of fine structure and, in particular, the morphologic features of fibrous and filamentous elements.

Discoid Platelets

A circumferential microtubule wound into several closely associated coils was apparent at the extreme periphery of discoid platelets prepared by the negative-stain whole-mount method after simultaneous glutaraldehyde fixation and detergent extraction (Figure 1). The relative opacity of the cells made accurate estimates of the number of coils in the microtubule difficult. Examples in which they could be observed clearly contained 6–12 microtubule rings in the marginal bundle. Platelets resembling nearly perfect disks or ovals with the circumferential microtubule at the very periphery contained very few filaments (Figure 2) or other definable structures in their cytoplasm. Platelets prepared by negative staining without prior fixation or detergent extraction were rarely perfect disks (Figure 3). A circumferential microtubule consisting of closely associated, parallel subfilaments was evident near the margin of the cells. Masses of canaliculi and membrane enclosed storage organelles of various kinds filled the cell interiors.

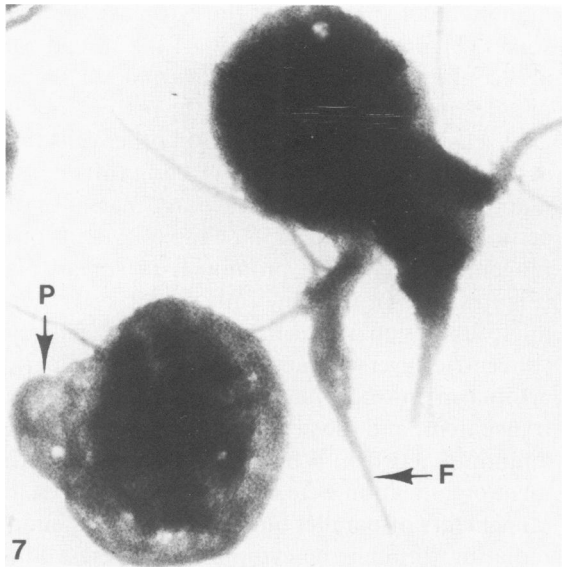
Initial Shape Change

The first physical change suggestive of surface activation in discoid platelets was the development of cytoplasmic expansion beyond the coil of microtubules (Figures 4 and 5). In some cells the expansion consisted of a narrow belt of cytoplasm (Figure 4). In others the expansions resembled irregular protuberences (Figure 5). The cytoplasm of the protuberances contained an irregular network of 5–6-nm filaments. A similar network of irregularly associated microfilaments was apparent in the cytoplasm of platelets possessing surface protuberences. Expansions of cytoplasm beyond the microtubule coils were also apparent in cells exposed to negative staining alone (Figure 6). They also contained a random network of 5–6-nm filaments. Lipid membrane elements filling the cytoplasm of platelets prepared in this manner obscured the organization of the 5–6-nm filaments deeper in the cells, though they were clearly present.

Early Dendritic Forms

The development of long, filiform processes signaled the next stage of platelet activation (Figure 7). Conversion to dendritic forms may have developed in some platelets before contact with the grid. This is suggested

Figure 7 – Platelet activation. Platelets from a sample exposed to the grid surface for 15 minutes before fixation, detergent extraction, and negative staining. The cell on the left reveals an early stage of protuberance (*P*) development similar to those shown in the previous illustrations. A platelet on the right is an early dendritic form. Numerous filipodia (*F*) extend from the irregular cell body to the grid surface. (×9500) **Figure 8** – Platelet activation. The negatively stained cell in this example was not exposed to detergent. It resembles the platelet on the left in the previous illustration. Organelles remain concentrated within the central zone of the cell and encircled by microtubule (*MT*) coils as the protuberances spread into a veil. (×13,000) **Figures 9 and 10** – Filaments are organized into parallel bundles in the pseudopods (*Ps*) of dendritic platelets prepared by fixation, detergent extraction, and negative staining. As shown in **Figure 9**, they remain in parallel association even when the pseudopod splits into two processes. **Figure 10** demonstrates the parallel association of filaments in the pseudopods. Removal of the constraining membrane by detergent may have loosened their association. All the filaments in platelet pseudopods are 5–6 nm in diameter and have the sawtooth substructure (↑) characteristic of actin microfilaments. (**Figure 9**, ×80,000; **Figure 10a**, ×80,000; **b**, ×80,000) **Figures 11 and 12** – Negatively stained dendritic forms prepared without exposure to triton. The parallel actin filaments appear more closely and regularly associated in pseudopods when the glycocalyx (*G*) and cell membrane (*CM*) are not exposed to detergent extraction (**Figure 11**). At higher magnification, the substructure of the actin is apparent as in **Figure 12**. Parallel actin filaments form a paracrystalline lattice (↑) with a regular periodicity of 5.5 nm at an angle of 60 degrees with the long axis of the pseudopod (**Figure 12**).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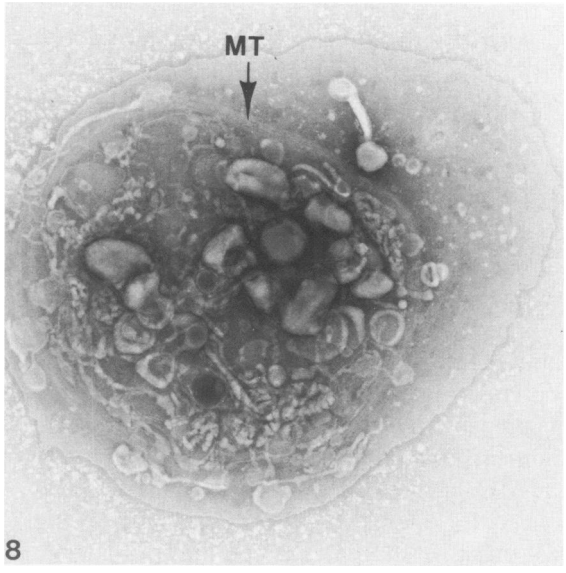
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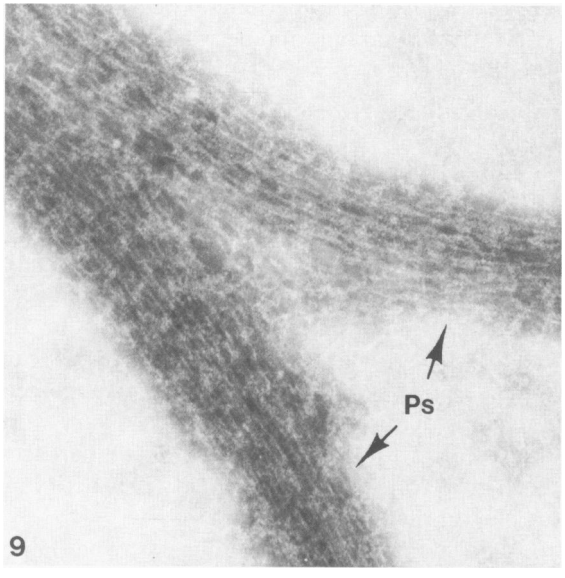
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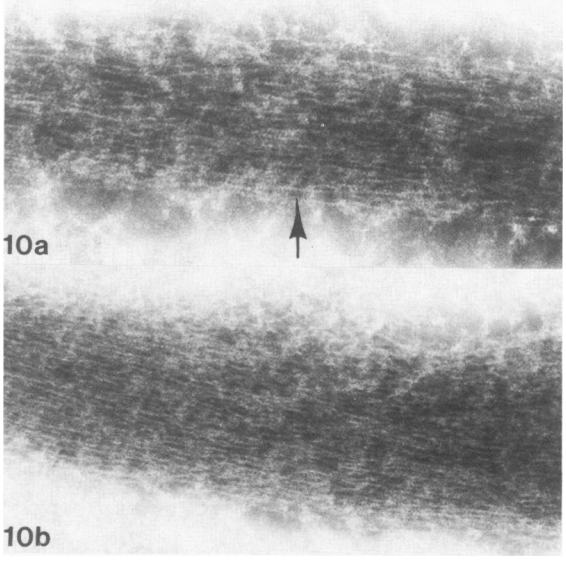
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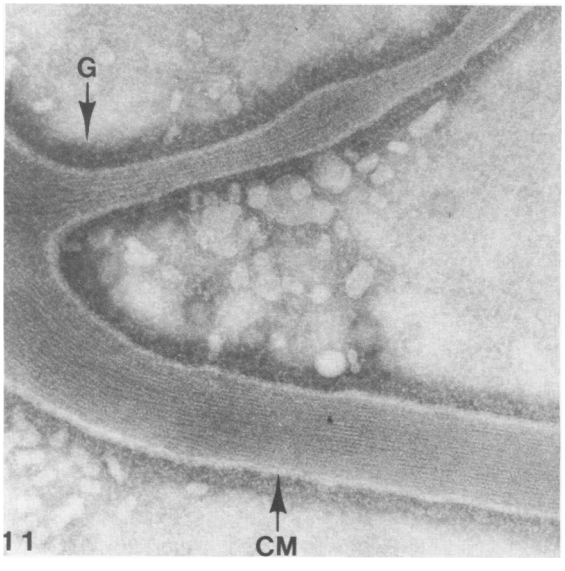
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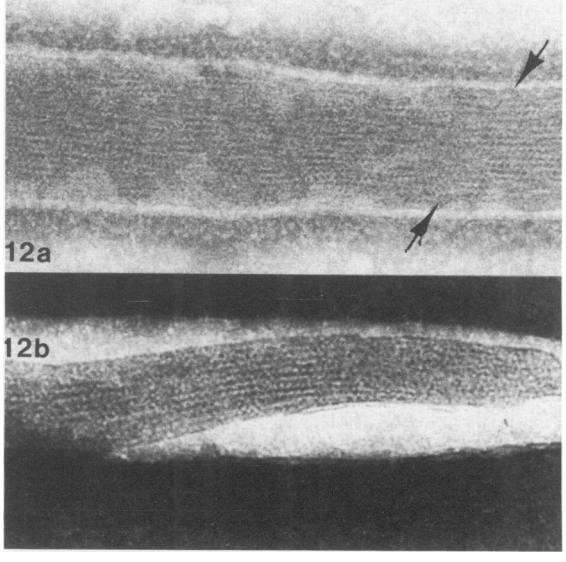
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because the body of many early dendritic platelets was globular and impenetrable by the electron beam (Figure 7), while others contacting the grid in a discoid state remained relatively transparent during transformation (Figure 8). The early dendritic forms were the first stage in which parallel associations of actin microfilaments were observed. Long filiform processes or pseudopods were filled with 5–6-nm filaments in close parallel relationships (Figures 9 and 10). The microfilaments appeared relatively irregular in platelets exposed to triton X-100 before negative staining, possibly because detergent removes the barrier of the cell membrane, resulting in collapse of filament organization. In dendritic platelets prepared by negative staining alone the actin microfilaments were in precise parallel register and revealed a periodicity of 6.0 nm at an angle of 60 degrees to the long axis (Figures 11 and 12). The highly regular organization suggested a paracrystalline arrangement, rather than chance associations. High magnification revealed the sawtooth substructure in each microfilament characteristic of actin (Figures 9–12).

Late Dendritic Forms

Dendritic platelets which had spread sufficiently to permit visualization of their cytoplasm were characterized as late forms. It is possible, however, that they were discoid on contact with the grid and transformed into thin films with long pseudopods on activation, rather than becoming globular. Coiled bundles of microtubules were regularly present in these cells, usually in the central zone. The dendritic cells resembled holly leaves and were filled with microfilaments (Figure 13). Bundles of parallel filaments followed the cell margin and extended into pseudopods.

Spread Platelets

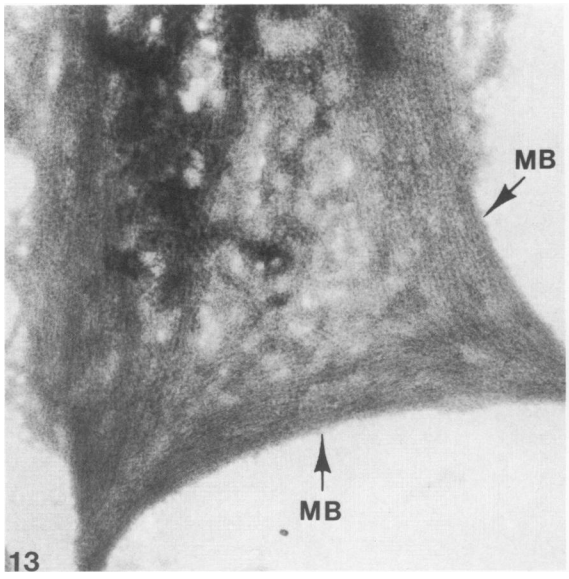
Transition from the dendritic state to spread forms was characterized by extension of the cytoplasm to fill spaces between pseudopods. Actin filament organization appeared to depend on the extent of transformation to the fully spread state. Bundles of parallel actin filaments radiating from the central zone into pseudopods were probably the cores of filiform processes remaining after spreading of the cytoplasm between the spikes (Figure 14). The margin of the spread platelets also contained prominent bundles of actin filaments in parallel association resembling the peripheral weave in glial cells[28] (Figures 15 and 16). In other cells the actin at the cell periphery was organized in tufts perpendicular to the margin (Figure 17). Large masses of parallel actin filaments arranged in triangles, V's, or other arrangements were also prominent in spread platelets (Figures 18–24). In many fully spread cells the coiled microtubules in the central zone were encircled by concentric layers of actin filaments. The peripheral cytoplasm in such platelets was relatively electron-transparent, and the actin filaments were organized in a random network. While other patterns of actin filament organization were occasionally observed, the arrangements of parallel bundles found in pseudopods, radiating through the cytoplasm like stress fibers, or along the cell wall in a peripheral weave and as concentric layers or a random network in the cytoplasm predominated.

Discussion

The present investigation has employed negative staining alone and after combined glutaraldehyde fixation and detergent extraction for examination of the arrangements of microfilaments in surface-activated platelets. Results of the study demonstrate that activation of the cells on carbon-coated grids causes a massive assembly of actin filaments. Arrangements assumed by actin in stimulated platelets vary from one area of the cytoplasm to another. The differences may be critical to the several complex roles actin appears to play in platelet physiology.

Actin filament assembly appears to be one of the earliest physical signs of platelet activation.[12] It is often difficult to resolve fine structural details in the cytoplasm of fixed, detergent-extracted, and negatively stained discoid platelets because of their relative thickness and cytoplasmic density. As a result, the differences in the state of actin have been determined primarily by biochemical and biophysical methods. On the basis of

Figure 13 – Late dendritic platelet. This cell and the platelets in all subsequent illustrations were prepared by fixation, detergent extraction, and negative staining. Bundles of parallel actin filaments form marginal bands (*MB*) along three borders of a dendritic cell and extend into pseudopods. (× 14,700) **Figure 14** – Dendritic platelet. Parallel bundles of actin filaments extend from the central region of this platelet and radiate into pseudopods. Randomly associated actin filaments (RA) are prominent in the cytoplasm, and a marginal band (*MB*) of parallel associated actin filaments is developing between pseudopods. (× 45,500) **Figures 15 and 16** – Marginal bundle (*MB*) of actin filaments. The development of parallel associated bundles of actin (*MB*) filaments oriented perpendicular to pseudopods suggests a possible role in platelet spreading. The conversion from concave margins in dendritic cells (Figures 13 and 14) to convex borders in spreading platelets (**Figure 16**) suggests that actin assembly at the periphery may spread the cell by filling in spaces between pseudopods, much as a tent is raised between tentpoles. Randomly arranged actin filaments (*RA*) are inside the marginal band of parallel filaments in spread platelets (**Figures 15 and 16**). (**Figure 15**, × 45,500; **Figure 16**, × 45,000) **Figure 17** – Margin of spread platelets. Most spread platelets have marginal bands of parallel actin filaments at their periphery, such as the cells in Figures 15 and 16. Some, however, have margins containing tufts (*T*) of actin filaments perpendicular to the cell border. (× 55,000) **Figure 18** – Stress fibers. Parallel bundles of actin filaments are present in the cytoplasm, as well as in pseudopods and marginal bands at the cell periphery. Some of the bundles resemble the stress fibers (*SF*) found in many cell types. They are surrounded by random networks of actin filaments (*RA*) in spread platelets. (× 52,000)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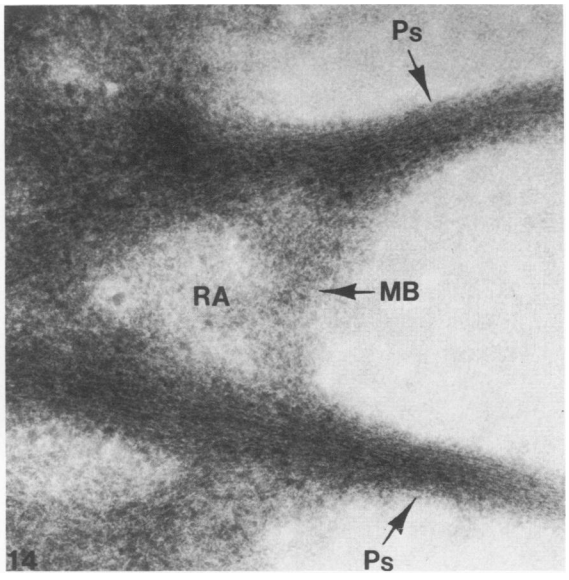
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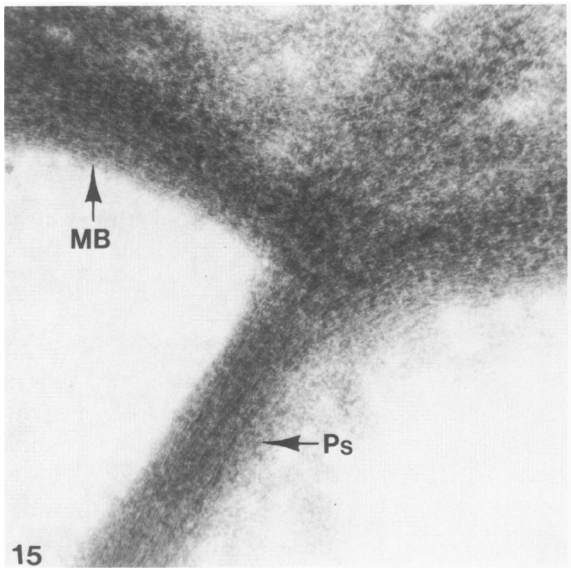
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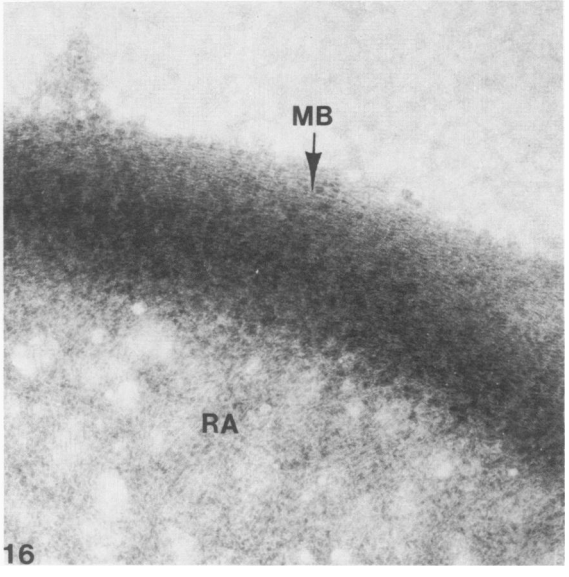
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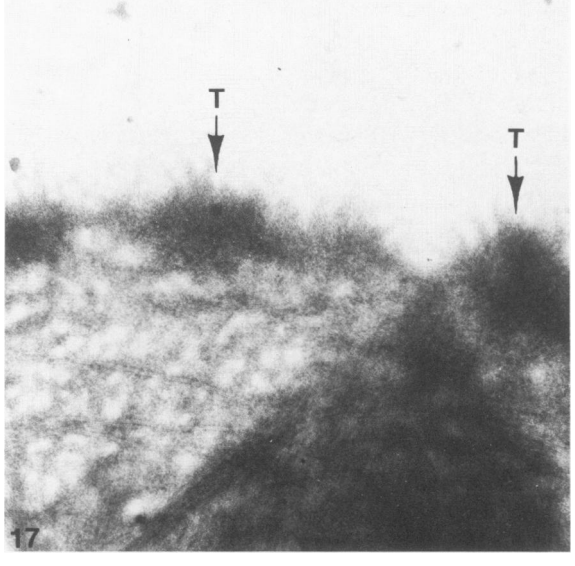
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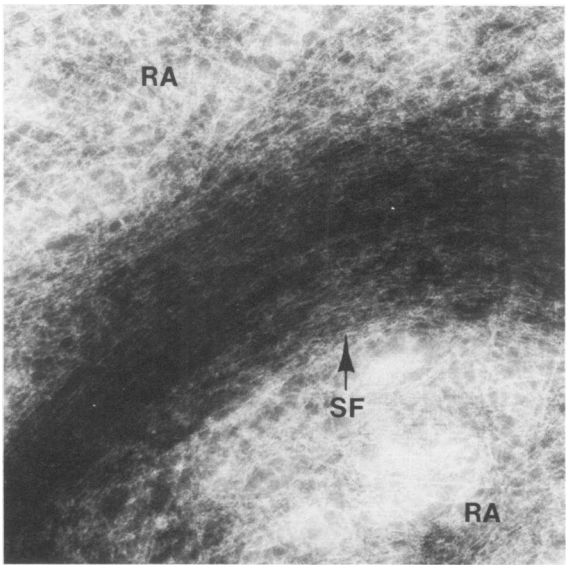
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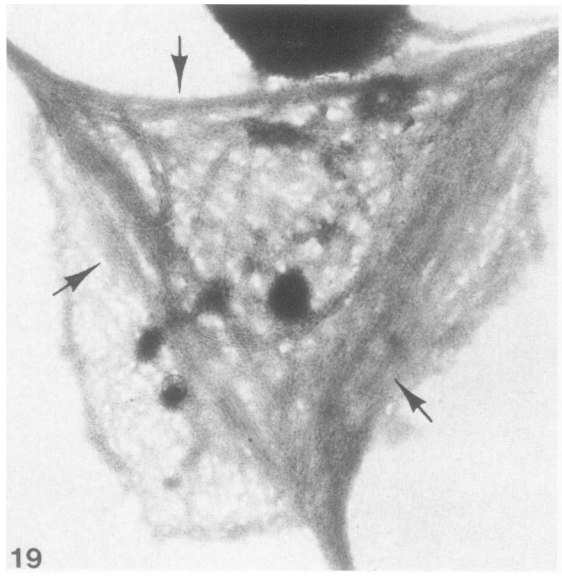
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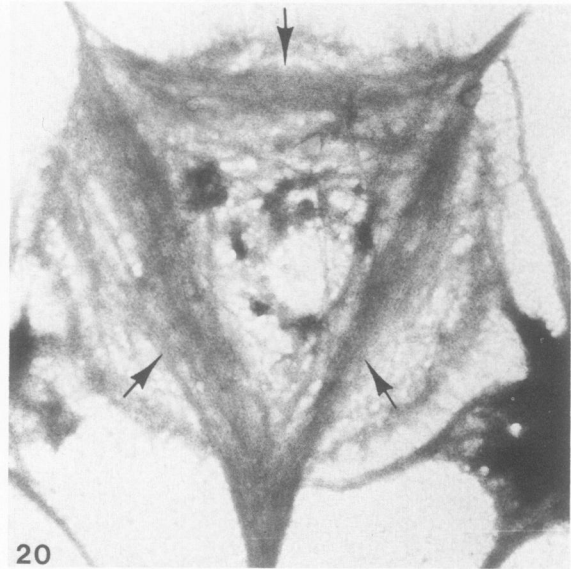
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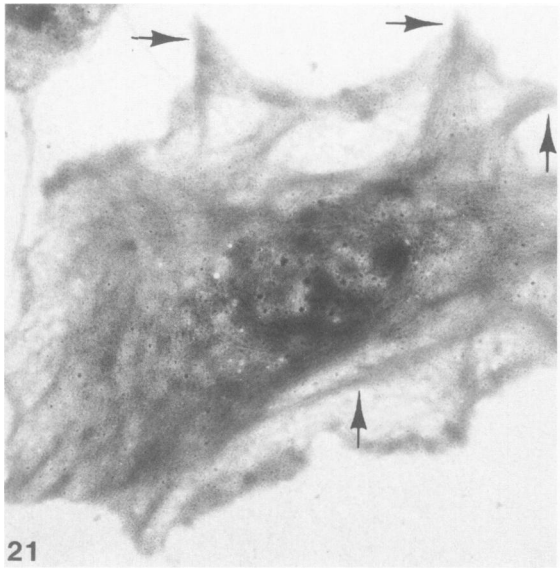
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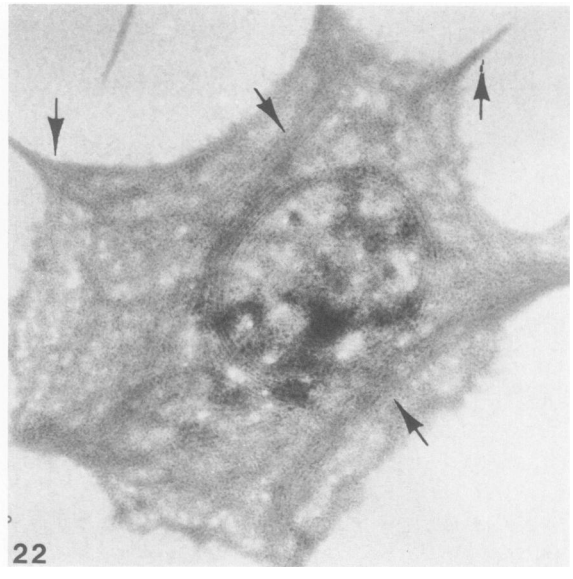
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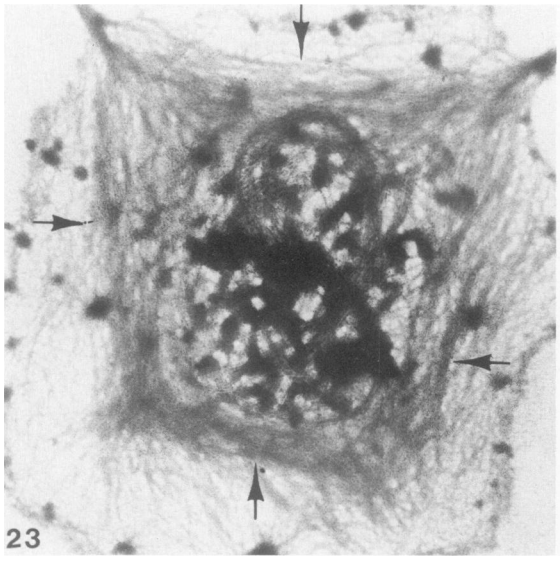
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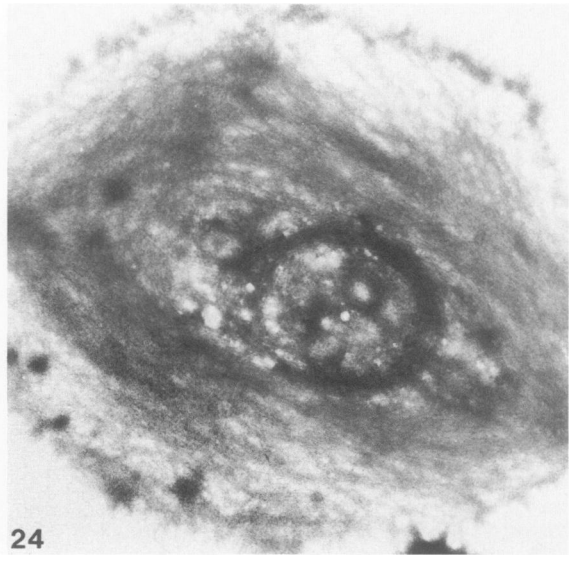
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investigations with local anesthetics, Nachmias concluded that little if any actin in quiescent platelets was F actin.[9,12] Carroll et al also concluded that only about 10% of the actin in resting platelets was assembled into filaments.[32] At the other extreme, Rosenberg and her colleagues[33] reported that 90% of the actin in control cells existed in the filamentous form. Fox and Phillips[8] have found that 40% of the actin exists in a filamentous state in discoid platelets. Intermediate values have also been reported by others.[34,35]

The findings of the present study cannot resolve the controversy regarding the state of actin in resting platelets. Some very short filaments (5–10 nm) might not be detected by negative stain or be buried in the amorphous but detergent-resistant cytoplasm. As a result, electron microscopy might miss a significant number of filaments that would be detected by the DNAase assay employed by others.[8,34] However, the difference in the actin content between discoid and activated platelets in the present study was pronounced, and would favor the low or intermediate values for resting cells suggested by others.[8,9,12,34,35]

The initial assemblies of actin are irregular networks just outside the circumferential bundle of microtubules in small protrusions extending from the discoid cell. Actin filaments are also present deeper inside the cytoplasm. These changes precede the development of early dendritic forms, which lose their discoid shape, become irregular, and extend long filiform processes. Unfortunately, the conventional electron microscope does not usually penetrate the bodies of globular platelets. The organization of actin filaments, however, was visible inside the pseudopods of dendritic platelets. Actin was organized in parallel bundles in pseudopodal extensions of detergent-extracted platelets. However, the filaments were loosely associated, and the irregularities may have been related to removal of the surface membrane. In negatively stained platelets which had not been extracted with triton X-100 during fixation, the actin filaments were in rigid parallel association. A side-by-side physical relationship was suggested by a regular periodicity of 6 nm at an angle of 60 degrees with the long axis of the pseudopod. The interaction between actin filaments may give the developing bundles sufficient cohesive strength to drive the cell membrane ahead of it into long, filiform processes.

Late dendritic forms revealed an organization of actin filaments at the cell margin similar to the peripheral weave observed in glial cells.[28] The parallel arrangements of actin followed the concavities of the dendritic platelet and extended into filiform extensions. Spreading of dendritic platelets eliminated concavities and filled in spaces between pseudopods. The outer third of the cytoplasm appeared thinned out in spread platelets, and the peripheral weave was more prominent. Organization of actin filaments into parallel bundles at the cell margin suggested a possible role in the spreading process. However, the filaments in the peripheral weave of other platelets were present in tufts perpendicular to the cell margin. As a result, the mechanism by which actin filament assembly fosters platelet spreading remains uncertain.

Stress fibers[15–17] are a third form of parallel actin filament association in platelets. Most of them radiate from the center toward the periphery and into pseudopods and represent the residual cores of filiform processes. Others resemble serpentine coils or are present in single masses, V-shaped arrangements, or triangles. Stress fibers may produce shape change and help to stabilize the irregular form of activated dendritic and spread platelets, as well as participate in contractile events.

Irregular networks, as noted above, were the first arrangements observed in stimulated platelets. In early dendritic cells the masses of actin filaments were concentrated. As a result, irregular or random arrangements were difficult to identify in the central zone. However, the random organization was apparent in the periphery of late dendritic forms and fully spread cells. Actin filaments were also arranged in concentric layers around the central region, where microtubule coils were the dominant feature. Recent studies have shown that the circumferential microtubule is constricted in activated platelets.[14] It is possible that these loose arrangements of actin appearing more concentrated in the central zones of spread platelets are involved in constricting the microtubule band.[36]

Investigations of the platelet cytoskeleton and organization of actin filaments have been carried out by other workers.[8–12,29–31] However, the methods employed and the results obtained have differed significantly. In general, others have preferred to use critical-point drying after simultaneous fixation and exposure to triton X-100. A brief exposure to osmic acid has occasionally been included in the preparative procedure, although most have examined platelets critical-point-dried after glutaraldehyde fixation without exposure to osmium or

Figures 19 and 20 – Parallel bundles in spread platelets. Masses of parallel actin filaments are prominent in the cytoplasm of spread cells. Two cells shown here contain several large bundles of parallel actin filaments arranged in triangular patterns. The bundles extend into pseudopods at the apices of the triangles. (**Figure 19**, × 13,500; **Figure 20**, × 10,500) **Figures 21 and 22** – Parallel bundles in spread platelets. Bundles of parallel actin filaments (↑) radiating through the cytoplasm into pseudopods are irregularly dispersed in these cells. (**Figure 21**, × 10,500; **Figure 22**, × 13,500) **Figures 23 and 24** – Parallel bundles in spread platelets. In many cells the masses of parallel actin filaments (↑) are arranged in loose, concentric layers around constricted rings of microtubules. (**Figure 23**, × 9000; **Figure 24**, × 11,000)

detergent. Results obtained with these methods have suggested that platelets contain a microtrabecular cytoskeleton similar to that observed in fibroblasts and a variety of other cell types.[37] Microfilaments observed in critical-point-dried platelets have revealed wide variations in size. Recently, Lewis suggested that there were as many as six different classes of microfilaments in activated platelets, varying from 4–5 up to 20–22 nm in diameter.[38] A roughly parallel association of these filaments has been noted in pseudopods, but not at the platelet margin or in the cytoplasm. The techniques used by these workers[29–31] have generally failed to preserve the constricted coil of microtubules seen regularly in thin sections of activated platelets with or without prior incubation with the microtubule-stabilizing agent, taxol.[26,38]

For reasons that are still uncertain, negative staining alone or after combined glutaraldehyde fixation and detergent extraction preserves morphologic features of the platelet cytoskeleton that are not seen or appear distorted in critical-point-dried platelets. Discoid platelets attached to the grid surface without signs of activation appeared more concentric and less altered than after critical-point drying.[13] The circumferential bundle of microtubules was well maintained, and very few microfilaments were evident in the cytoplasm of discoid cells. Initial signs of activation, such as surface irregularity or early pseudopod development, were associated with the rapid assembly of actin into irregular filamentous networks, paracrystals, large bundles resembling stress fibers, and loosely arranged concentric layers. Though the organization varied, the filaments were all 5–6 nm in diameter. To a certain extent, the arrangements observed were artificial, because removal of internal and external membrane systems by exposure to detergent and collapse of the extracted cell on the grid during air-drying undoubtedly influence the appearance observed in the electron microscope. Yet the excellent preservation of microtubules and microtubule coils, the constancy of microfilament diameters, and agreement between observations on samples prepared by negative staining after simultaneous fixation and exposure to detergent with platelets exposed to negative staining alone suggest that the results are an improvement over those obtained by critical-point drying.[27,28]

In conclusion, the present study has demonstrated that assembly of actin filaments is associated with the earliest physical responses of surface-activated platelets. Formation of a random network appears to be the first stage of actin assembly but is quickly followed by the development of close parallel associations. In pseudopods the aligned microfilaments form a paracrystalline lattice, which suggests physical associations. Nachmias[9] has suggested previously that actin filaments in filiopodia are cross-linked, and tropomyosin has been demonstrated in them by immunofluorescence. However, the frequency of the periodic interval and its angular relationship to the long axis of pseudopods found in the present study do not resemble those previously described.[10] It is reasonable to suggest, however, that cross-links may stabilize the parallel associated actin filaments, which results in the cohesive force necessary to deform the platelet membrane.[39]

Actin filament bundles parallel to the cell margin may foster extension of the cytoplasm between filiform processes during spreading. Yet the presence of actin filaments organized in tufts perpendicular to the cell margin in some spread platelets suggests the need for some caution in regard to this interpretation. The organization of actin filaments into parallel associations resembling stress fibers and into massive bundles suggests that shape change is to a large extent dictated by actin filament assembly. How this highly organized framework involved in deforming the platelet also serves in contractile activities remains to be determined. It is possible that the random network and loosely associated concentric layers of actin are involved in internal contraction, while the parallel bundles serve primarily as a cytoskeletal support system. Experiments to clarify the nature of the dual functions of assembled actin are in progress.

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