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CYTOSKELETAL CHANGES DURING ADHESION AND RELEASE: A COMPARISON OF HUMAN AND NONHUMAN PRIMATE PLATELETS

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Abstract

The organization of cytoskeletal proteins in whole-mount adherent platelets from African green monkeys and normal human volunteers has been studied by SEM, high vacuum electron microscopy (HVEM) and conventional (120 kV) electron microscopy. We describe three distinct organizational zones, the Central Matrix, the Trabecular Zone and the Peripheral Web in spread platelets from both sources. The Central Matrix is an ill-defined superstructure of 80-100 Å filaments of short length which enshrouded the granules, dense bodies, mitochondria and elements of the open-channel and dense-tubular systems. The latter, identified through the use of peroxidase cytochemistry with the whole mounts, is an anastomosing network of elongate saccules having diameters of 600-1200 Å. The Trabecular Zone, which encircles the Central Matrix, contains 165, 80-100 and 30-50 Å filaments in an open lattice of irregular lattice spacing. The outermost region of the cells, the Peripheral Web, is comprised of 70 Å filaments organized in a honeycomb lattice with center to center spacing in the range 150-300 Å. This pattern for the spread cells is not consistently observed in cells during the early stages of adhesion; therefore, correlations of SEM and TEM observations are made for the various stages of adhesion/activation.

KEY WORDS: Adhesion, Monkey Platelets, Human Platelets, Cytoskeleton

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Introduction

Platelet adhesion to and activation upon contact with foreign surfaces has long been recognized as a major factor in numerous physiologic and pathologic processes. The physiologic role of platelet activation is best exemplified in the function these cellular elements play in primary hemostasis or the arrest of bleeding following vascular injury. Under normal circumstances platelets circulate in a vascular compartment and are isolated from extravascular tissue by a continuous layer of endothelial cells which line the luminal aspect of blood vessels. Following blood vessel injury, most typically due to a traumatic event, the endothelial cell continuity is disrupted exposing the complex array of basement membrane and subendothelial macromolecules including elastin, fibronectin, glycosaminoglycans, collagen and the von Willebrand protein. This subendothelial matrix is highly stimulatory for platelets; and contact with it leads to platelet activation, adhesion and ultimately the release of platelet granule constituents, among which are potent stimulators of the coagulation process and vasoconstrictors. The accumulation of platelets at the site of injury and the action of released platelet macromolecules at the site of the lesion lead to the initial arrest of bleeding. Platelet adhesion, activation and release in addition to being a desirable physiologic process also has been implicated in numerous pathologic conditions; and recent investigations in numerous laboratories have documented the involvement of platelets and platelet release products in vascular disorders ranging from atherosclerosis and pulmonary occlusion to coronary artery spasm leading to myocardial infarction.

Since platelet adhesion has many negative as well as positive ramifications, a full understanding of the adhesion process is essential if control of the pathologic aspects is to be achieved. Among the many events associated with activation leading to release is the rapid polymerization of fibrous proteins to form a cytoskeletal network within the activated platelets (6,11,14,16). In addition

to structural elements such as microtubules (10), biochemical studies have identified within the cytoskeleton several major components of a contractile apparatus including actin, actin-binding protein, and myosin (11, 16). In addition to these major constituents, platelets contain several minor and regulatory proteins including tropomyosin, profilin, alpha-actinin, vinculin, gelsolin and the actin regulatory protein P235 (11,17).

Although much is known about the biochemical makeup of the platelet cytoskeleton, relatively little work has been done to describe the structural organization of this system during the adhesion/activation process (7,8,12,15). Therefore, to better understand the cytoskeletal events which occur during adhesion and lead to the release of granule constituents, we have undertaken a series of experiments involving the adhesion of platelets from the African green monkey (Cercopithecus aethiops) to artificial surfaces (7,8,10). The present studies are an extension of earlier papers to include a description of the general cytoskeletal matrix and its relationship to internal membranes as the cells undergo adhesion. In addition, the studies compare the events in platelets from the African green monkey to those which occur in human platelets.

Materials and Methods

Human blood platelets were obtained by venipuncture from normal volunteers at the University of Colorado Health Center in Boulder. Potential donors were screened for aspirin and oral contraceptive use and informed consent was obtained prior to venipuncture. Platelets from African green monkeys (Cercopithecus aethiops) were obtained from animals maintained in the Animal Resource Unit of the Atherosclerosis Research Center at Bowman Gray School of Medicine. The blood samples were drawn into plastic syringes and anticoagulated with either heparin (10 units/ml) or sodium citrate (3.8%; 1 part to 9 parts of blood). Platelets obtained from platelet rich plasma by centrifugation in plastic tubes were washed by repeated centrifugation and resuspension in a balanced salts solution containing ethylenediaminetetraacetic acid as previously described (7,8,10). Subsequent to washing, the cells were stored at room temperature until used in the adhesion assay. Adhesion to either glass or carbon stabilized formvar surfaces was carried out at 37°C in a moist chamber. For these studies a 200 µl aliquot of washed platelets with a platelet concentration of 200,000 cells/µl was placed on a 13 mm glass coverslip which had 3 formvar coated grids uniformly positioned on the surface. Following the 40 minute adhesion period the nonadherent cells were removed by repeatedly dipping the grids in balance salts, and the adherent cells fixed by immersing the grids for 10 minutes in a cacodylate (0.1 M) buffered (pH 7.2) glutaraldehyde (2.5%).

The fixed cells were then washed in the cacodylate buffer, secondarily fixed for 3 minutes in buffered osmium tetroxide (1%), stained for 3-5 minutes in ethanolic uranyl acetate, dehydrated through a graded series of ethanol and dried from CO_2 by the critical point method. Dryness during the critical point process was assured through the use of molecular sieves both in the alcohols (Davison Molecular Sieves, alumina silicate, 10-16 mesh with 4 Å pore size) and in-line (Matheson Gas Purifier #451) from the tank of "bone-dry" CO_2. The dried cells were further prepared for scanning electron microscopy by sputter coating with gold-paladium prior to observation at 15 kV in a Philips SEM 501 microscope. Whole mount samples for transmission electron microscopy were coated in a rotary evaporator with carbon and then observed either at 125 kV in a Philips EM-400 or at 1000 kV using the JEM-1000 maintained as a National Institutes of Health resource in Boulder, Colorado. Stereo pair micrographs were taken of the cells in each of the microscopes at tilt angles as specified in the figure legends. In order to determine the identification of membranes in the adherent cells, select samples were reacted for peroxidase cytochemistry using the diaminobenzidine reaction as adapted for platelets by Breton-Gorius and Guichard (2) and Lewis et al. (9). Cells for these studies, subsequent to primary fixation, were washed extensively in the cacodylate buffer containing sucrose (1%). The fixed and washed cells were then maintained in the reaction medium (0.05 M Tris buffered to pH 7.4 and containing the DAB and hydrogen peroxide) at 4°C for 48-72 hours after which they were incubated at 37°C for 1 hour in fresh medium. Following the final incubation the cells were washed in the Tris buffer and then processed for whole mount microscopy by continuing with the osmium tetroxide step described above.

Results

Platelet contact with either glass or formvar surface resulted in rapid activation leading to a series of events which culminated in the spreading of cytoplasm from the central granulomere outward across the surface. The major stages in the adhesion process were similar for human and African green monkey platelets with the initial stage being a disk to sphere transformation. This was followed by the extension of numerous filopodia from the spherical granulomere region; and, ultimately, the spreading of a delicate hyalomere from the central region to interconnect adjacent filopodia completed the adhesion events (Figure 1). Under the conditions of these experiments most of the platelets were singly adherent, however, a small number (less than 1%) were in small aggregates. Within the aggregates cells at various stages of activation could be identified, and the stages of activation appeared to be similar to the singly adherent platelets.

The sequence of surface events in adhesion/activation coincided with major reorganization within the cytoplasm. When

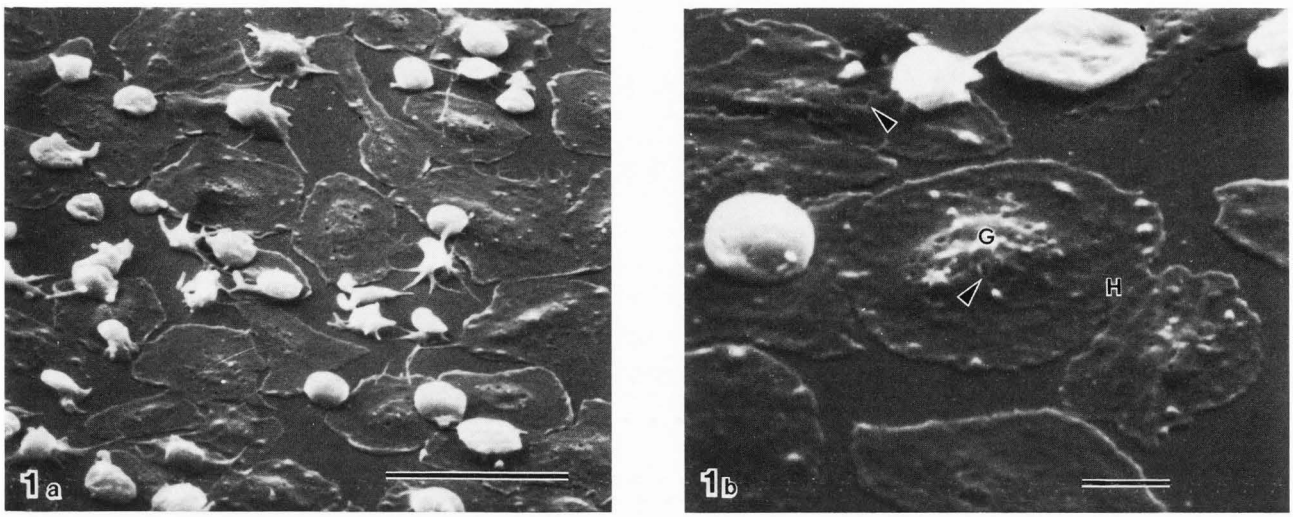


Figure 1. Scanning electron micrograph of adherent African green monkey platelets observed at 30° stage tilt. The majority of the basally located cells have spread fully during the 40 minute adhesion time and appear as flattened cytoplasmic sheets on the surface. Randomly dispersed on the surfaces of these basal cells are platelets in varying stages of activation (see a). As illustrated in the higher magnification micrograph (b), the spread cells are characterized by a flattened hyalomere (H) which often appears slightly raised from the surface at the cell margins. Plasma membranes over the centrally located granulomere (G) were typically pitted in appearance suggesting the location of elements of the open channel system (arrows). a, Bar=10 μm; b, Bar=2μm

observed by whole mount and stereo (3-D) microscopy (Figure 2) the initial stages were characterized by the transformation of the coarse, somewhat granular cytoplasm, which was typical of the unactivated platelet, to a delicate filamentous network. The network in the early stages of activation consisted of 40-160 Å diameter elements organized in a 3-dimensional lattice. Filament lengths within the lattice ranged from 100-2000 Å, with the longer filaments generally being of larger diameter (Figure 3). Within the main body of the platelet no consistent orientation of the lattice was apparent, as filaments meandered in a random fashion to form a maze of open cytoplasmic spaces defined by the filaments. Precise size of the filaments and the relative number of each could not be clearly established in the whole mount preparations of the early stages of activation; however, three diameters, 30-50 Å, 80-100 Å and 150-170 Å were consistently observed (Figure 3). The largest class of filaments was observed only in the area of the microtubule bundle in unactivated cells and remained outside the granulomere following activation (see Figure 4 for overview of organization). These 160 Å elements typically followed tortuous paths that were generally circumferential to the centrally located granulomere and defined the largest open spaces within the platelet. Frequently, the larger filaments had a helical appearance which seemed to repeat at intervals of 1500-2000 Å (not shown). The second size class of filaments, 80-100 Å, was most clearly observed in the open areas containing the largest filaments. As in the case of the 160 Å filaments the 80-100 Å elements appeared to contribute to the definition of the open spaces. When observed against the electron lucent background of the cytoplasmic open areas, the 80-100 Å filaments often seemed to be comprised of two smaller filaments which converged and diverged to form structures of lengths varying from 200-1500 Å. The shorter length 80-100 Å filaments comprised the bulk of the forming lattice during early activation where they existed in a honeycomb having an approximate center to center spacing of 200-250 Å. The delicate 30-50 Å filaments were throughout the matrix of the activating cells, but they were most evident as short structural elements which extended between and interconnected the larger filaments (see Figure 3). Within the lattice formed by the various filaments points of intersection, vertices, could be identified. Typically, these appeared as globular structures having diameters in the range 350-400 Å. The only major variation on this organizational pattern was in the filopodia where long slender filaments, having diameters of approximately 70 Å, extended in parallel tracks from the cell body out to the filopodial tips (Figure 5). These filopodial filaments were interconnected at intervals of 500 Å by the 30-50 filaments.

The cytoskeletal structure of cells at more advanced stages of adhesion consisted of three distinct zones. At the center of the platelet mass, in the granulomere region, were the alpha granules, dense bodies and the mitochondria interspersed with numerous membrane vesicles and channels (Figures 4 and 6). The cytoskeleton forming the backdrop for these organelles was intermediate between the granular appearance of

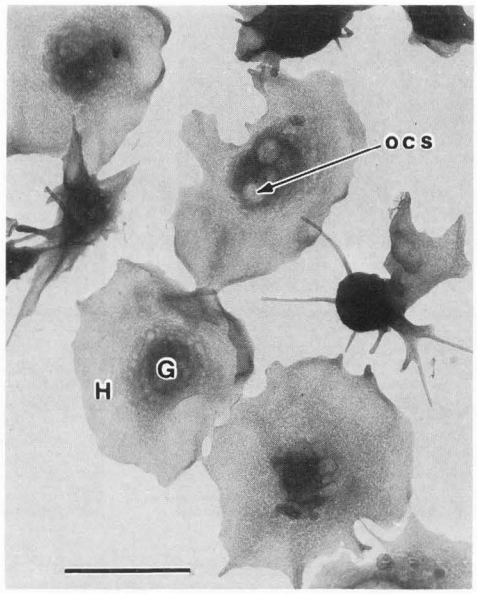
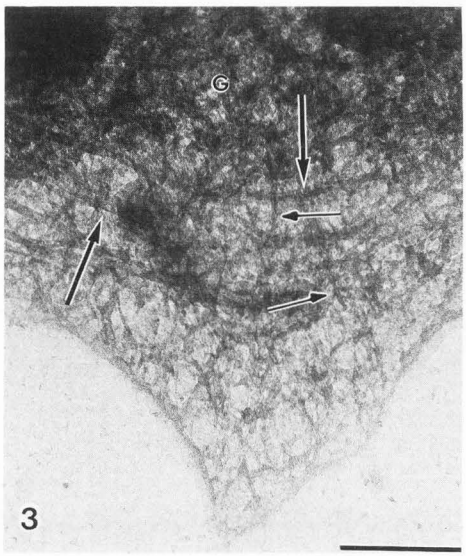


Figure 2. HVEM stereo-pair micrographs of African green monkey platelets similar to those shown in Figure 1. Note the delicately filamentous hyalomere (H), the electron dense granulomere (G) regions and the large distended cisternae of the open channel system (OCS). Bar=3 µm; Angle=36°



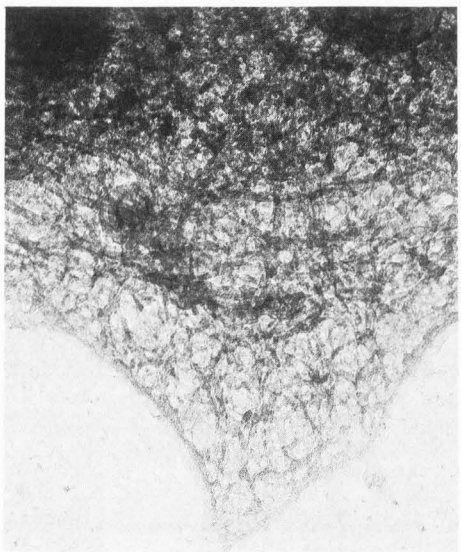
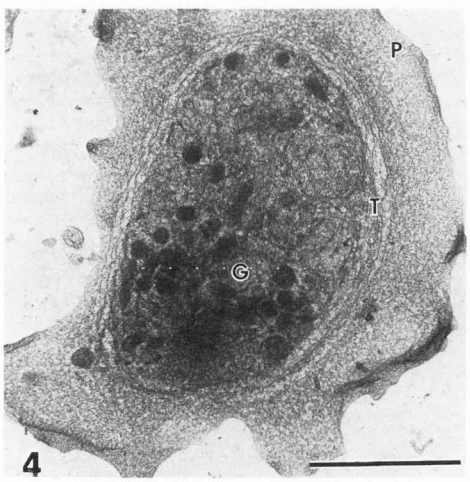
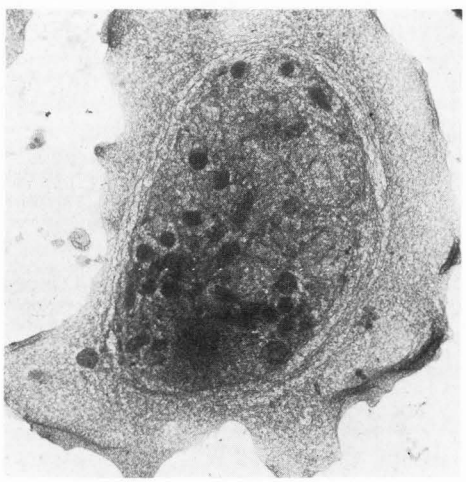


Figure 3. HVEM of select region from an African green monkey platelet in the early stages of activation. The electron dense granulomere (G) with its matrix of short filaments is flanked by the Trabecular Zone with its 3-D lattice of long (1000-2000 Å) filaments having diameters in the range 150-170 Å (see large arrow). Among the larger filaments are those having diameters in the range 80-100 Å (small arrow). Interconnecting the larger elements are delicate filaments, microtrabeculae, having diameters in the range 30-50 Å. Bar=0.2 µm





See facing page for the caption of Figure 4.

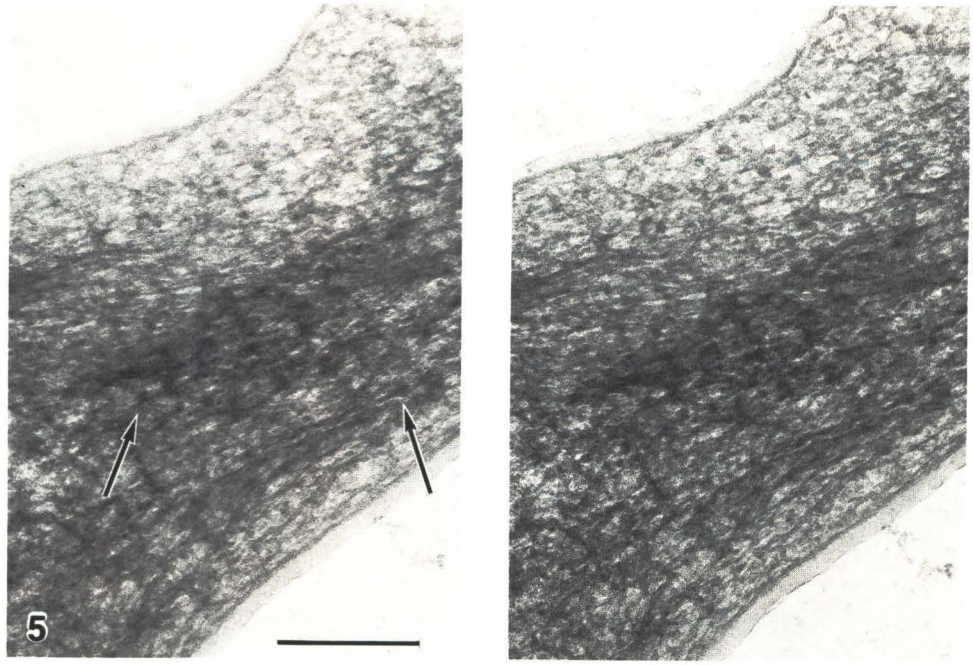


Figure 5. HVEM stereo pair micrographs of a filopodium from an early stage activated cell (African green monkey). Note the linear organization of 70 Å filaments which parallel the long axis of the filopodium. The 70 Å filaments are interlaced by a network of 30-50 Å elements. Globular vertices (arrows) can be identified at the intercepts of the 70 and 30 Å filaments. Bar= 0.2 μm; Angle=40°

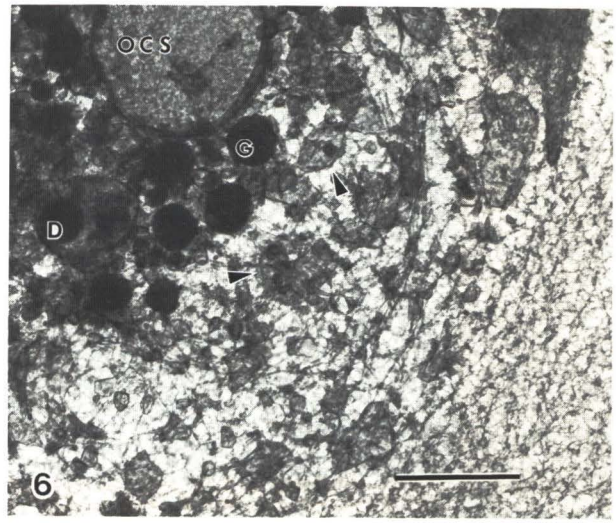
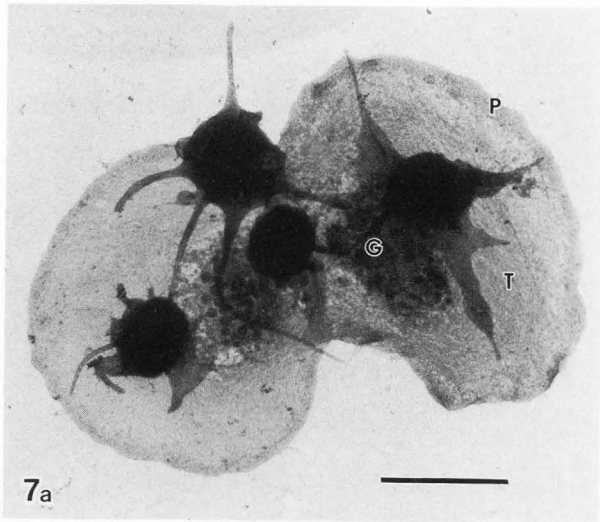


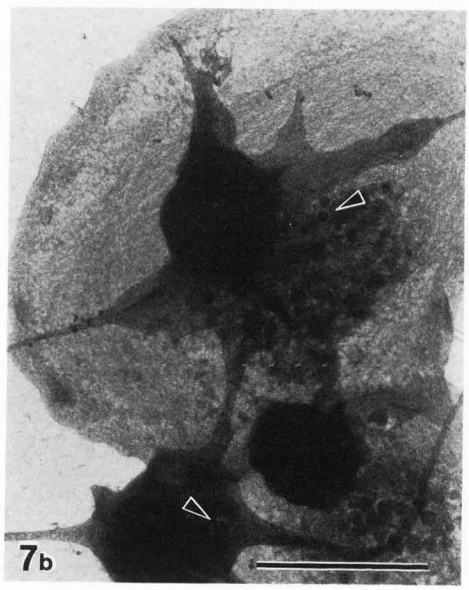
Figure 6. HVEM micrograph of a select region from a spread human platelet. The three zones described in Figure 4 can also be identified in the human cells. Contained within the granulomere are cisternae of the open channel system (OCS), alpha-granules (G), a dense body for serotonin storage (D), and elements of the dense tubular system (arrows). Typically dense bodies could be identified as an excentrically placed nucleoid within an electron lucent vacuole, whereas alpha granules lacked the electron lucent peripheral region. Although distinct zones of cytoplasmic filament organization are evident, it is clear that transitions from one zone to another takes place over a distance of several thousand angstroms. Bar=1 μm

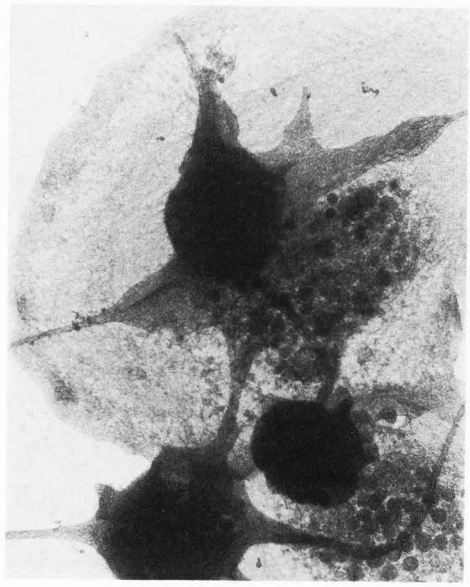
the unactivated cell and the delicate honeycomb lattice of 80-100 Å filaments observed in the early stages of activation. This Central Matrix was delineated by a more open region, the Trabecular Zone, which consisted of both the 80-100 Å and 160 Å filaments in an open network with cytoplasmic spaces often approaching 2000 Å in diameter. As in the early stages of activation, the larger filaments in the Trabecular Zone were interconnected by 30-50 Å filaments which extended laterally from the surfaces of the larger filaments to define the size of the intertrabecular spaces. Surrounding the Trabecular Zone and extending to the margins of the spreading hyalomere was an area, the Peripheral Web, in which the 70 Å filaments were interconnected in a delicate web-like network having center to center spacing in the range 150-300 Å. The width of the peripheral web was variable and was inversely proportionate to the Trabecular Zone. In advanced stages of spreading the Trabecular Zone often approached 1-2 microns in diameter and the Peripheral Web was restricted to a band of a few thousand angstroms wide at the hyalomere edge. Although the demarcation between these zones often appeared sharp at low

Figure 4. HVEM stereo pair micrographs of an African green monkey platelet in the advanced stages of spreading. Shown are three distinct zones of cytoplasmic filament organization. The Central Matrix (granulomere) (G) is circumscribed by a Trabecular Zone (T) containing large diameter filaments arranged in an open lattice. Extending from this Trabecular Zone to the cell margin is a more delicate lattice of 80-100 Å elements. The cytoplasm at the cell margins, the Peripheral Web (P), is a tight web-like mesh of 70 Å filaments. Note the ruffled cell margins corresponding to those described in Figure 1. Bar=2.0 μm; Angle=20°

Figure 7. HVEM micrographs of a small platelet aggregate (human) which is forming secondary to adhesion. The basally adherent cells have the three distinct organizational zones described for singly adherent cells. Note the positioning of the granulomere regions in the cytoplasm at the point of apposition between the basal platelets. Cells entering the aggregate at the top are in various stages of activation. Stereo pair micrographs in b illustrate the spatial arrangement within the aggregate. Well defined dense bodies are highlighted by the arrows in the granulomere of both basally located and the newly activated cells. Bars=2.0 μm; Angle=16°



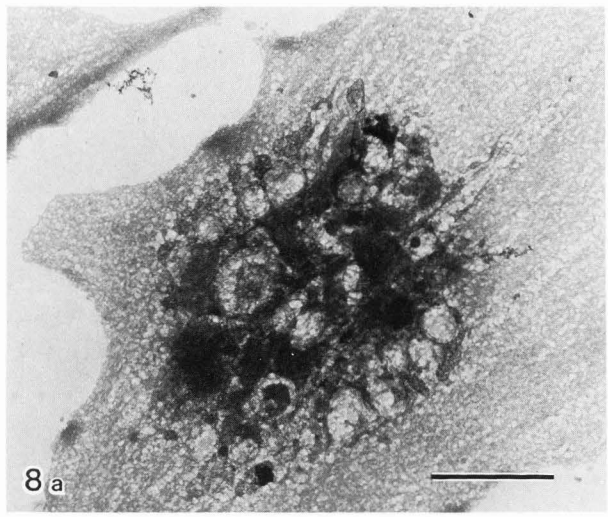


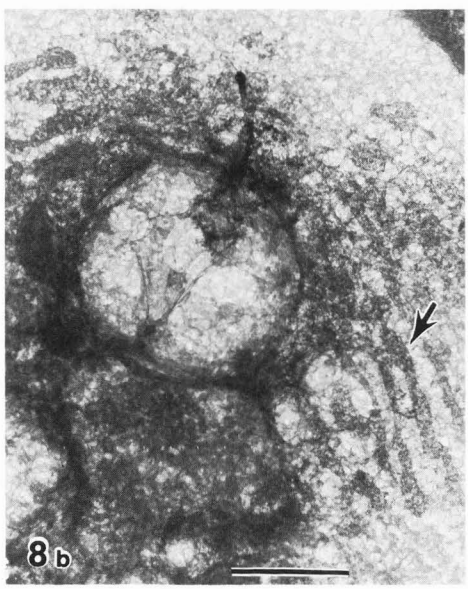


magnification, crisp delineation between zones was not the case when the cells were observed at high magnification. Typically when observed at higher magnification, as shown in Figure 6, a gradual transition was observed in the size of the filaments. The larger diameter and longer length filaments predominated in the trabecular region, and this more coarse configuration gradually gave way to the honeycomb lattice comprised of short length, 80-100 Å, filaments. The honeycomb lattice in turn gradually blended with the delicate web at the cell margins where 70 Å elements predominated.

The major zones of filament organization identified in singly adherent cells were also identified in platelets which had aggregated on the surface (Figure 7) Within the aggregates the basally located platelets (which were attached to the formvar) were specifically oriented with the Central Matrix and the granulomere at the point of platelet confluence near the center of the aggregate. The hyalomere with the Trabecular Zone in each of the constituent platelets extended radially from the aggregate center, and the Peripheral Web was clearly identified in each of the cells (see Figure 7).

Since the platelet dense tubular system (DTS) comprises a significant proportion of the cellular volume and has been proposed as the source of calcium and prostaglandins for regulation of contractile protein formation leading to granule release, the peroxidase reaction was used to more precisely identify membranes of the DTS in the fully adherent cells. As illustrated in Figure 8, the DAB reaction product in the fully adherent cells was found as an intensely electron opaque deposit in mitochondria and as a less dense granular precipitate in membranous elements





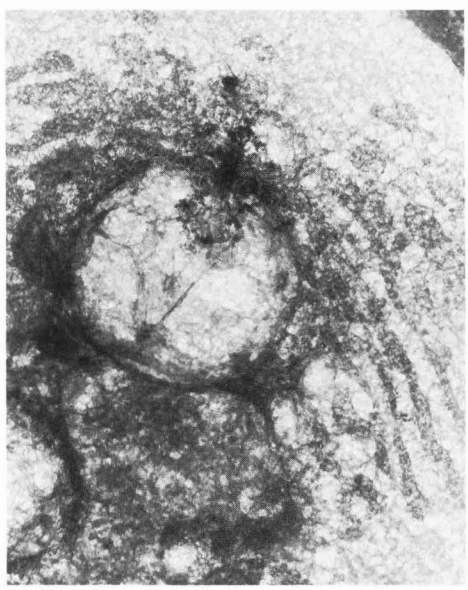


Figure 8. 120 kV electron micrographs of peroxidase reaction produced in adherent platelets. As illustrated in (a) the reaction product is localized to the granulomere region of the cells. Within this region (see 3-D in b) the delicate precipitate is contained in an anastomosing network of tubular, membrane-limited elements (arrow) similar to those described in Figure 6. Bar in a=1.0 μm, Bar in b=0.5 μm; Angle=6°

throughout the Central Matrix. The DAB-containing dense tubular system was similar in appearance to the membranes identified in the regular whole-mount cells (see Figure 6) and appeared as an anastomosing network of oval saccules having diameters in the range 600-1200 Å and lengths ranging from 1000-5000 Å. The saccules were interconnected both longitudinally and laterally by slender, 300-400 Å diameter, anastomoses which ranged in length from 500-2500 Å. In most cells the tubular system as defined by the peroxidase reaction was restricted to the Central Matrix region; but, occasional elements were observed in the Trabecular Zone. No reaction product was observed in the peripheral web zone of the platelets.

Discussion

Described in detail in the present study is the appearance of three distinct organizational zones within adherent platelets from both the African green monkey and normal human volunteers. These zones, which for convenience we have labeled the Central Matrix, the Trabecular Zone and the Peripheral Web, have in the past been noted by us in studies of the African green monkey platelets (10) and by others studying adherent human cells (1,12,14). In a recent study of whole mount and triton extracted platelets, Mattson and Zuiches (12) described a highly filamentous region in the hyalomere surrounding the densely packed granulomere. A similar zone, referred to as the "dense filamentous zone", has been reported by Albrecht and Lewis (1) and compares to the Trabecular Zone described in the present paper.

Consistent with these earlier studies, we found two major classes of filaments within this region. The first, which through morphometric analysis had an average diameter of 165 Å and lengths ranging to 2000 Å, comprised 27% of the filaments (data not shown) and compared favorably to the 150 Å filaments reported by Mattson and Zuiches (12). The second major class of filaments in the Trabecular Zone averaged 93 Å in diameter and accounted for 29% of the total number of filaments. These larger filaments (range 80-100 Å) were similar to the 60-120 Å filaments reported by Mattson and Zuiches (12) as being the major component of the peri-granulomere region. In addition to these two major classes of filaments the present paper describes a delicate lattice-work of 30-50 Å microtrabeculae (18) which extend between and interconnect the larger filaments. It is noteworthy that these small trabecular structures accounted for 15-20% of the filaments in both whole mounted cells and in triton residues of adherent African green monkey platelets (triton data not shown - see Lewis et al.) (8). The outer-most area of the platelet cytoplasm, the Peripheral Web, as described in the present study and in the previous work of Albrecht and Lewis (1) was similar to the condensed cortex reported both by Mattson and Zuiches (12) and Nachmias et al. (14), who studied negative stain preparations of platelets collected on polylysine-coated grids. In addition to confirming the presence of a distinct peripheral zone, the present observations extend the previous work by describing the 3-dimensional organization of this peripheral region as a web-like network consisting of 70 Å filaments of short length.

Although the biochemical nature of the various filaments was not established in our studies, it is noteworthy that the 70 Å filaments in the Peripheral Web were similar to the size reported for polymerized actin (4,11,16). The position of the 70 Å filaments in the submembraneous cortex corresponded to one of the major actin locations described by Debus et al. (3) through the use of immunofluorescence microscopy. Similarly, both Zucker-Franklin and Grusky (19) and Nachmias and Asch (13) used heavy meromyosin binding to document that filaments of similar size in platelets were actin. Several workers have studied the relationship of the acto-myosin complex to platelet function and through the use of immunofluorescence the localization of myosin has been reported in activated platelets. Most pertinent to the present study is the report by Debus et al. (3) who described in platelets a ring of myosin fluorescence in the area we have described as the Trabecular Zone. Interestingly, one of the major filaments we observed in this zone, the 165 Å filament, had a size comparable to that reported for myosin (16). The second major filament in this zone had a size similar to actin (although in our study the mean diameter 93 Å was slightly larger). Interestingly, the size range of the smaller filaments (80-100 Å in the present paper and 60-100 Å in the study of Mattson and Zuiches) was not unlike that reported for filaments in actin networks of other cells. In the present paper we indicated that these smaller filaments often consisted of two smaller entwined elements. This observation and the filament bundling described by Mattson and Zuiches (12) are in accordance with the recent reports of lateral associations between actin filaments polymerized in vitro (4,5).

The highly organized nature of the cytoplasmic lattice in the two outer zones of adherent platelets was in marked contrast to the less defined character of the Central Matrix zone. This contrast was interesting, since the Central Matrix was the region of the platelet which contained the alpha granules, dense bodies, elements of the dense tubular system (as identified by peroxidase cytochemistry in the present report), and cisternae of the open channel system. It was, therefore, the matrix in this central region which provided the contractile force needed to position the various organelles for the release reaction. Although less well defined than the outer zones, the central matrix had considerably more definition than the ground substance of the unactivated platelet (14,15). The globular nature of the unactivated platelet matrix gave rise to the central matrix in which 80-100 Å filaments of short length predominated. Although lacking precise superstructure, the organization of the central matrix region may have been of functional significance. Conceivably, the complex nature of cellular movements and molecular reorganizations required for release required a matrix that remained in a dynamic and somewhat fluid state. Seen in this light the highly organized configurations of the Trabecular Zone and to a lesser extent the Peripheral Web would have been less flexible and therefore less compatible with the release process. On the other hand, the more rigid configurations of these outer regions were ideally suited for providing structural support (Trabecular Zone) or controlled cytoplasmic spreading (Peripheral Web).

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Discussion with Reviewers

J.C. Mattson: You use washed platelets suspended in a balanced salts solution in your assay. Do you assume that plasma cofactors (i.e., von Willebrand factor, fibrinogen and cold insoluble globulin) play no role in adhesion to formvar or glass in your system?
Authors: The role of various plasma proteins in platelet adhesion under physiologic conditions has unequivocally been established; however, under the experimental conditions typically used in our studies the effects of plasma proteins are not in accordance with the in vivo role. The von Willebrand factor, for example, is required for normal platelet adhesion and aggregation on damaged vascular surfaces under normal conditions of flow. Under static conditions, however, the necessity for the von Willebrand factor is greatly diminished. In view of such disparities we made the decision several years ago to use a standardized washed platelet assay and thereby minimize variability; our present experiments are in keeping with this decision. Although our system is artificial in this aspect it is noteworthy that our observations on cytoskeletal organization parallel those reported by you and others who have conducted adhesion assays in the presence of plasma proteins.

J.C. Mattson: Please clarify the exact limits of the "Peripheral Web" as you define it. In Figure 4, the circumferential band of filaments is very narrow. Is all of the extensive spreading hyaloplasm peripheral to this also considered part of the Trabecular Zone and only the condensed fine filamentous network just beneath the platelet membrane considered the Peripheral Web?
R.M. Albrecht: The platelets in Figure 7a and b appear to have a less densely staining zone between the "Trabecular" Zone "T" and the Peripheral Web "P". Would the authors care to comment?
Authors: Both these questions address variability in the appearance of the platelet hyaloplasm in the region between Trabecular Zone and the Peripheral Web. My impression of cytoskeletal polymerization in the adhering platelet is that the process is dynamic with constant remodeling to accommodate hyalomere extension across the substrate. Due to this evolving nature the relationships between adjacent zones is constantly changing. The limits of the Trabecular Zone and the Peripheral Web cannot, therefore, be precisely defined. In the present manuscript we describe the region between the Trabecular Zone and the Peripheral Web as being one of transition with the coarse filaments of the Trabecular Zone giving way to the delicate lattice of the Peripheral Web. There are two points inherent to the concept of the transition: the first is the lack of precise demarcation between the zones and the second is that variability is to be expected in the region of transition.

R.M. Albrecht: It is difficult to delineate specific zones in the peroxidase stained platelets. Do the authors feel the dense

tubular system often extends partially into the "Trabecular" (inner filamentous) Zone or is it usually restricted to the granulomere? It is not entirely clear to me if the authors refer to the "Central Matrix" as including only the granulomere and associated cytoskeletal elements or if the "Central Matrix" includes a portion of the "Trabecular" Zone which contains elements of the dense tubular system.
Authors: It was not our intent to suggest exclusive Central Matrix localization for the DTS; but, rather, our observations support the Central Matrix as the primary location. The data obtained using peroxidase cytochemistry are consistent with routine whole mount 3-D observations of intact cells. Under our normal preparative conditions, most elements of smooth endoplasmic reticulum have been localized in the Central Matrix; however, a limited amount of smooth endoplasmic reticulum has been observed in the Trabecular Zone. It is our impression that the smooth endoplasmic reticulum observed in regular whole-mount preparations corresponds to the dense tubular system identified using the cytochemistry.

J.C. Mattson: You speculate that the Central Matrix is the likely center of contractile activity while the Trabecular Zone has a cytoskeletal organization more consistent with providing structural support. Yet Dubus et al. (text ref. 3) found myosin concentrated in the region surrounding the granulomere comparable to your Trabecular Zone. Is this position for myosin consistent with the Trabecular Zone having a structural rather than a contractile function?
J.J. Wolosewick: I feel that you have over-interpreted your data in the last paragraph. What are your reasons for suggesting that the configurations seen in your micrographs are flexible or rigid?
Authors: The last paragraph of our discussion is speculative, and we have extrapolated data from the static state to a dynamic process. Although speculative, our suggestion of a rigid superstructure in the Trabecular Zone is not without precedent, for it is known that both actin and myosin are in this region and the two proteins can exist in a non-contracting rigid state depending upon calcium levels, regulatory protein activity and local availability of ATP. More directly in support of our suggestion are the observations of Allen et al., who have reported the use of high resolution AVEC-DIC microscopy to study platelet adhesion under conditions similar to those described by us (Allen RD. 1983. Cell Motility, Videodisc Supplement 1). When observed using time lapse and video enhancement, the region which corresponds to our Trabecular Zone remained relatively stationary in the adhering platelets. The Peripheral Zone on the other hand was dynamically active with cytoplasmic pulsatile waves and membrane extension. Consistent with our observations, Allen et al. have reported the release of granules from a region corresponding with our Central Matrix.

J.C. Mattson: What happens to the microtubules during spreading?
Authors: We have previously reported the depolymerization of microtubules upon adhesion/activation (text ref. 10). Our present observations are consistent with the earlier studies.