American Journal of Pathology, Vol. 136, No. 2, February 1990

Surface-activated Bovine Platelets Do Not Spread, They Unfold

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The present study has examined the response of bovine platelets to surface activation and compared it to the reaction of human cells. Human platelets react to surfaces by losing their discoid shape, extending pseudopods, converting to dendritic forms, and finally, spreading into thin films resembling pancakes. Bovine platelets do not spread, they unfold. Surface activation causes them to transform from discs to irregular, flattened shapes resembling dendritic platelets, but they are unable to fill in spaces between pseudopods, a step required for spreading. Bovine platelets lack the surface-connected open canalicular system (OCS), which serves as a reservoir of membrane for human platelet spreading. Its absence may be the major factor in the failure of bovine platelet spreading, but there are other possible factors. Circumferential microtubules are more resistant to disassembly in surface-activated bovine than human cells, and their stability as rings or fractured bundles may limit spreading. Actin filament assembly is similar in human and bovine platelets, but the organization is different. Human platelets form a peripheral weave of actin that expands the membrane between pseudopods. A peripheral weave does not form in surface-activated bovine platelets. The absence of the OCS and differences in cytoskeletal organization in bovine platelets may also affect spreading of the surface membrane. Fibrinogen-gold (Fgn-Au) probes added to spread human platelet move from pseudopods and the cell margin toward the center and concentrate in the OCS. Fgn-Au particles bind to surface-activated bovine cells, but move very little, or not at all. All of these factors may contribute to the inability of bovine platelets to react to surfaces by spreading like human cells, but absence of the OCS appears to be the major cause. (Am J Pathol 1990, 136:399–408)

Human platelet physical transformation after exposure to foreign surfaces *in vivo* and *in vitro* has been well characterized.[1–6] Resting cells are normally discoid in form. After contact with a surface, platelets extend long, finger-like processes and develop a dendritic appearance. Dendritic platelets undergo conversion to spread forms as the central body sinks into the surface and cytoplasm fills spaces between extended pseudopods.[7] Recent studies in our laboratory using fibrinogen coupled to colloidal gold (fgn-Au) as a probe for glycoprotein IIb-IIIa (GPIIb-IIIa) receptors have shown that evagination of the surface-connected open canalicular system (OCS) is a major factor contributing to human platelet spreading on surfaces.[8]

Zucker-Franklin and coworkers[9] were first to demonstrate that bovine platelets are similar to human cells in most respects, but lack the OCS found in human cells and platelets from most other mammals. As a result, the bovine platelet secretes products of alpha granules by fusion of the organelles to the surface membrane before extrusion,[10] whereas secretory organelles in human platelets fuse with channels of the OCS and use this route for discharge of products.[11]

The absence of the OCS in bovine platelets suggested it might be a useful model for other studies. Because our recent investigation identified involvement of the human platelet OCS in spreading,[8] it seemed reasonable to examine interaction of bovine platelets with foreign surfaces and the binding of fgn-Au receptor probes after surface activation. In contrast to human cells, bovine platelets do not spread, they unfold. After surface activation, bovine cells bind fgn-Au particles but do not move them to cell centers or the OCS, as human platelets do.

Accepted for publication October 2, 1989.

The work submitted is part of the academic thesis (Ph.D.) of Lynette H. Grouse, B.S.

Supported by NIH grants HL-11880 and GM-22167 and a grant from the March of Dimes (1-886).

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Materials and Methods

General

Blood for the present study was obtained from healthy adult cattle housed under direct supervision of the School of Veterinary Sciences at the University of Minnesota. Samples aspirated from the internal jugular vein were mixed immediately with citrate-citric acid-dextrose (CCD) (93 mmol/l trisodium citrate, 70 mmol/l citric acid, 140 mmol/l dextrose, pH 6.5) in a ratio of nine parts blood to one part anticoagulant.[4,12] Blood was sedimented at room temperature under a force of 200*g* for 20 minutes. Platelet-rich plasma (C-PRP) above the buffy coat was aspirated and transferred to separate tubes.

Human blood was obtained after informed consent from young adults who had volunteered for our studies on many previous occasions. None was taking drugs of any kind at the time or for at least 2 weeks before the study. Blood aspirated from an antecubital vein was mixed immediately with CCD anticoagulant in the same ratio as for bovine blood, and C-PRP was prepared as mentioned for bovine samples.

Light Microscopy

Drops of human and bovine C-PRP were placed on polylysine-coated glass slides and thin cover slips were placed on them. The interaction of platelets with polylysine-coated glass was viewed under oil through 63X and 100X oil-immersion objectives adapted for phase contrast and interference phase contrast observation. Interactions between platelets and glass were followed at intervals for 15 to 60 minutes. Between observations the slides were maintained in a 37 C oven.

Immunofluorescence

Samples of bovine and human C-PRP were mixed with an equal volume of the citrate anticoagulant and centrifuged to pellets. The platelets were resuspended in phosphate-buffered saline (PBS; NaCl, 8 g; KCl 0.2 g; KH_2PO_4, 0.2 g; Na_2HPO_4, 1.15 g/l adjusted to pH 7.2 with 1.0 mol/l NaOH). Twenty microliters of washed platelets were placed on each of a number of polylysine-coated glass slides, which were inserted in a moist chamber and placed in an oven maintained at 37 C to settle. At intervals of 15, 30, and 60 minutes, slides were fixed in methanol at −10 C for 10 minutes followed by brief immersions (three times for three minutes each) in acetone at −10 C. A stock monoclonal antibody to beta-tubulin (Amersham, Arlington Heights, IL) was diluted to a concentration of 0.01 mg/ml in PBS with 1% bovine serum albumin (BSA).[13] Slides were washed once with PBS, and the platelet spots covered with 20 to 40 ul of the antitubulin antibody. After 30 minutes, the slides were washed with PBS and the spots covered with a fluorescein-conjugated, anti-immunoglobulin antibody (Cappel, Cochranville, PA). After incubation for another 30 minutes, the slides were washed well with PBS and mounted under coverslips in a solution of p-phenylene- diamine-PBS-glycerin using the method of Johnson et al.[14] The slides were studied under phase contrast and phase fluorescence in a Zeiss photomicroscope equipped with an ultraviolet power source and appropriate excitation and barrier filters. Platelets were observed and photographed through epifluorescence optics and 60X and 100X planapo objectives.

Detergent-extracted Cytoskeletons

Samples of detergent-extracted platelets were prepared after incubation at 37 C for 15, 30, or 60 minutes for ultrastructural study after negative staining according to the method of Small[15] as modified from Hoglund et al.[16] The Triton X-100 glutaraldehyde mixture consisted of 0.5% Triton X-100 and 0.25% glutaraldehyde.[13] 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 mmol/l; KCl, 5 mmol/l; Na_2HPO_4, 1.1 mmol/l; KH_2PO_4, 0.4 mmol/l; $NaHCO_3$, 4 mmol/l; glucose, 5.5 mmol/l; $MgCl_2$, 2 mmol/l; EGTA, 2 mmol/l; PIPES, 5 mmol/l; pH 6.0–6.1). After washing in the cytoskeleton 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 twice in distilled water and transferred sequentially through four 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 four drops of 3% sodium silicotungstate, drained of excess stain, and allowed to air dry.

Preparation of Fibrinogen Gold

Kabi grade L and Sigma F4883 fibrinogen preparations were obtained from commercial sources.[17] They were dia-

lized against deionized distilled water for at least six changes and 3 hours. Dialized fibrinogen solutions were then divided into aliquots, snap frozen, stored at −70 C, and thawed before adsorption. Colloidal gold particles having a diameter of 16 to 18 nm were prepared as described by Loftus and Albrecht.[18] One half milliliter of 4% tetrachloroacuric ($HAuCl_4$) acid solution was added to 200 ml of deionized distilled water and brought to a boil. Then 5 ml of 1% trisodium citrate was rapidly mixed into the boiling solution and the mixture was refluxed for 30 minutes. The fresh colloid was cooled and pH was adjusted to 6.5 with 0.2 N K_2CO_3 before use.

The minimum amount of protein necessary to stabilize the colloidal gold particles was determined by adsorption isotherms.[19,20] A series of fibrinogen solutions of increasing concentration was made and 0.5 ml of Au (pH 6.5) was added. After 1 minute, 1 ml of 10% NaCl was combined and rapidly mixed. The minimum concentration of fibrinogen plus 10% of that minimum amount that prevented flocculation by NaCl was added to 10 ml of gold. Based on the adsorption isotherm, the average concentration of fibrinogen was 6 μg per ml of gold colloid in each experiment. After 5 minutes, 0.5 ml of freshly made and prefiltered (0.45 μm) 1% polyethylene glycol (molecular weight, 20,000) was added to prevent flocculation of the fibrinogen-labeled gold (fgn-Au).[18,21,22] The fgn-Au was concentrated by centrifugation in polycarbonate tubes at an average speed of 10,000*g* for 30 minutes at 4 C. The concentrated fgn-Au was separated and resuspended to 1 ml with prefiltered (0.2 um) Tyrode's buffer (pH 7.4) with calcium and magnesium, but without albumin.

Staining with Fibrinogen-Gold (Fng-Au)

Drops of washed platelets were placed on carbon-stabilized, formvar-coated grids and allowed to interact with the surface for 20 minutes at 37 C.[17] In some experiments, the incubation time was changed to 15, 30, or 60 minutes. Nonadherent platelets were gently rinsed off the surface with HBSS before 10 μl of fgn-Au was applied. After incubation with the adherent platelets for 5 minutes at 37 C, unattached fgn-Au particles were removed by rinsing with HBSS. The grids were then fixed in 1% glutaraldehyde in cacodylate buffer and air dried for study in the electron microscope.

Results

Phase Contrast and Nomarski Interference Phase Contrast Microscopy of Bovine Platelets During Surface Activation

Examination of bovine platelets after interaction with glass slides for intervals of 15 to 60 minutes by light microscopic methods revealed a significant difference from human cells. Platelets from human subjects may attach to a foreign surface as discoid forms and remain in that state for only a few seconds up to a minute. The discoid cells then extend spikelike pseudopods radiating from a more spherical, central body and become dendritic forms. Slowly the central body sinks into the surface and cytoplasm fills in spaces between pseudopods, transforming dendritic platelets into spread forms.[3,5,6]

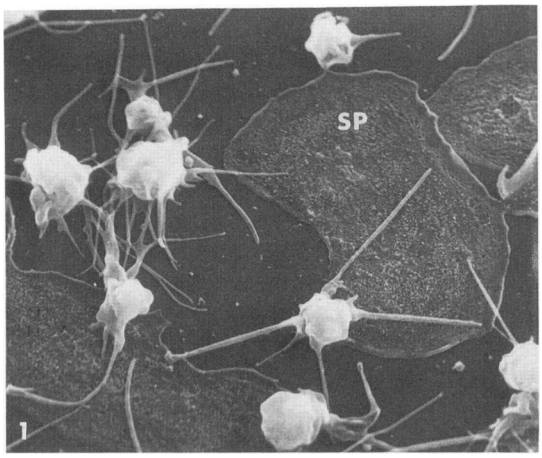

Figure 1. *Human platelets allowed to interact with a carbon-coated cover slip for 15 minutes, then fixed and critical-point dried for study in the electron microscope. Most of the platelets have undergone transformation from resting, discoid cells to dendritic shapes with long, spiky pseudopods and spread forms (SP). Magnification × 5000.*

Bovine platelets during intervals of up to 60 minutes did not spread on glass surfaces. The cells often remained discs for the entire 30-to-60-minute period of observation. Cells that did change shape underwent limited conversion, often appearing irregular or crescent shaped, but never spread. Some resembled dendritic human platelets, but they did not transform into fully spread cells like their human counterparts.

Scanning Electron Microscopy

Evaluation of human and bovine platelets by scanning electron microscopy confirmed the observations made by light microscopic methods.[7,23] Human platelets underwent the entire sequence of transformation from discoid platelets to dendritic forms and then into the thin films typical of spread cells (Figure 1). Bovine platelets also responded to surface activation by changing shape. However, the shape change was limited compared to human cells. Bovine platelets extended long, filiform processes

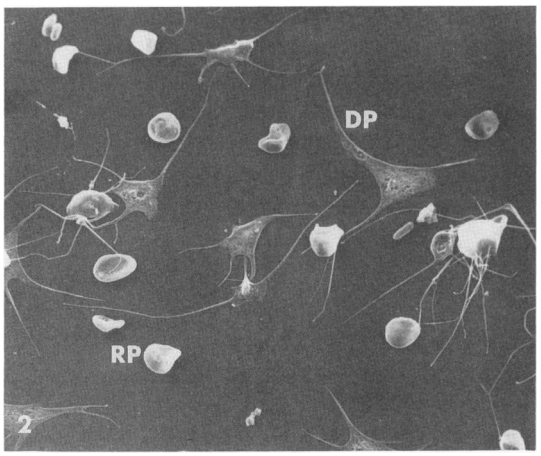

Figure 2. *Bovine platelets prepared in the same manner as the cells in Figure 1, but allowed to interact with the coverslip for 1 hour before fixation. Despite the long exposure, many platelets have retained a resting, discoid appearance (RP). Others have transformed into dendritic platelets (DP), but no spread forms are apparent. Magnification × 5000.*

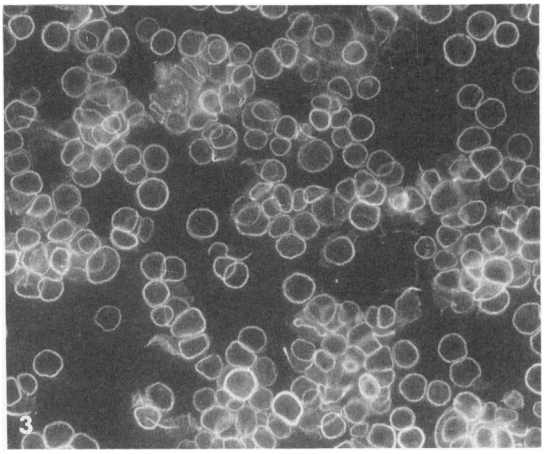

Figure 3. *Human platelets allowed to interact with a glass slide for 15 minutes, then fixed and exposed to anti-tubulin antibody followed by fluorescein conjugated anti-immunoglobulin. Most of the microtubule rings appear intact. Magnification × 630.*

after contact with the grid surfaces (Figure 2). Their bodies flattened to some extent, but never filled the spaces between surface extensions. Thus bovine platelets can change shape and extend filaform processes, but do not develop the spikelike pseudopods typical of human dendritic forms or fill spaces between them to become spread platelets.

Immunofluorescence

The unusual response of bovine platelets to glass slides was reflected by changes in the circumferential microtubule. Examination of human cells stained with an antibody to tubulin and then with a fluorescein-coupled antibody to the first immunoglobulin revealed uniform fluorescent rings at 15 and 30 minutes of glass exposure (Figure 3). Bright fluorescent rings were also visible at 60 minutes, but in many platelets they developed a disorganized appearance (Figure 4).

Circumferential microtubules in most bovine platelets were uniform fluorescent circles at 15 and 30 minutes. One hour after exposure to glass most of the rings were still intact (Figure 5), but some had fractured, leaving behind curved, spiral, or straight fluorescent bundles (Figure 6). The disorganized patterns observed in human cells were absent in bovine preparations. On the other hand, the spiral, curved, and straight fluorescent bundles in bovine preparations were uncommon in human platelet samples.

Detergent-extracted Cytoskeletons

The failure of bovine platelets to spread was clearly apparent in cytoskeletal preparations examined in the electron microscope. The thin, pancakelike forms typical of the human platelet response to surface activation were absent in bovine preparations (Figures 7 to 11). Discoid cells with coils of the circumferential microtubule forming the platelet margin were common (Figures 7 and 8). Crescent- and spindle-shaped platelets with fractured microtubule coils were also frequent (Figure 9). Other bovine platelets were jagged with broken microtubules or loosened coils extending into surface irregularities (Figures 10 and 11).

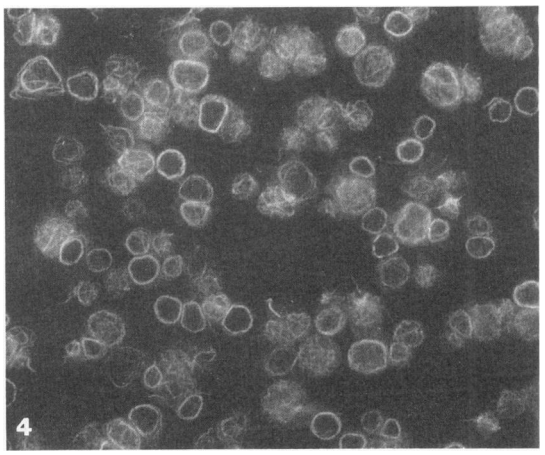

Figure 4. *Human platelets fixed 1 hour after exposure to a glass surface and the subunit protein of the microtubule, tubulin, stained by indirect immunofluorescence. Some microtubule rings retain a circumferential appearance, while most reveal various degrees of disorganization. Magnification × 630.*

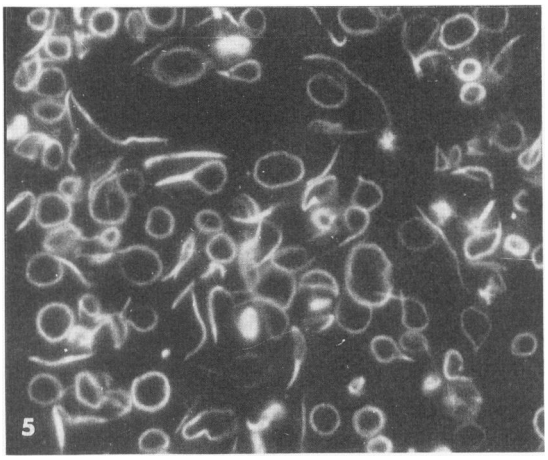

Figure 5. *Bovine platelets exposed to glass for 60 minutes before fixation and stained for tubulin. The majority of rings remain intact 1 hour after glass activation. Other marginal microtubules appear to have fractured. Coils have straightened into linear or curved bundles. Magnification × 1000.*

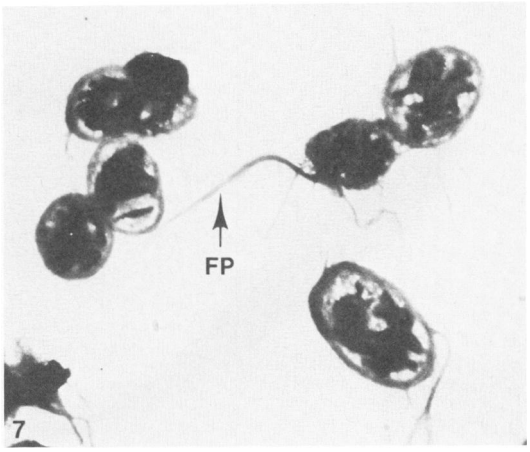

Figure 7. *Whole mount of bovine platelets fixed and simultaneously detergent extracted 30 minutes after exposure to the carbon-stabilized, formvar-coated grid. Most of the cells have retained a discoid configuration. Only a few have extended filiform processes (FP) in response to surface activation. Magnification × 4000.*

Some resembled kites and other irregular forms, but the radiating dendritic shapes and spread forms with a peripheral weave of actin typical of surface-activated human platelets (Figure 12) were not present in bovine preparations.

Interaction of Surface-activated Bovine Platelets with Fng-Au Probes

The distribution of fgn-Au probes on surface-activated bovine platelets differed strikingly from localization on human cells.[17,18] Neither bovine nor human discoid forms bound fgn-Au particles. However, dendritic forms in human and bovine preparations were covered with fgn-Au particles that extended onto pseudopods (Figures 13 to 16). As human dendritic platelets transformed into spread forms, fgn–Au-receptor complexes moved from peripheral margins toward cell centers and became concentrated in channels of the OCS[17] (Figure 17).

Fgn-Au particles did not appear to undergo reorganization on maximally altered bovine platelets in the manner observed on human platelets. The only apparent movement of ligand receptor complexes was on pseudopods

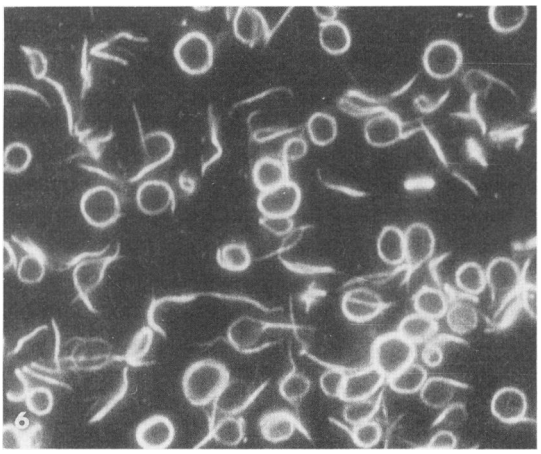

Figure 6. *Bovine platelets fixed 90 minutes after exposure to glass and stained for tubulin. Many rings are intact, but a significant proportion have fractured and straightened into linear or curved bundles. There is little difference between the changes at 60 and 90 minutes. Magnification × 1000.*

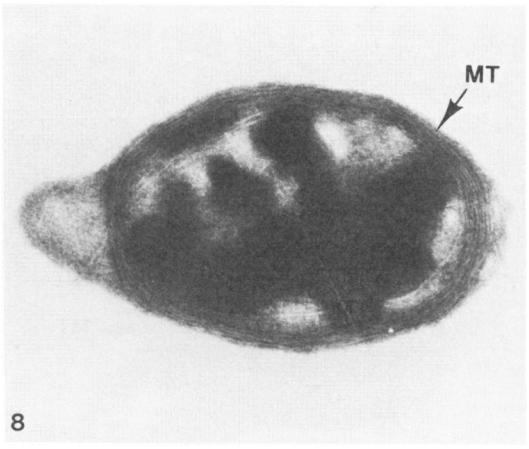

Figure 8. *Bovine platelet prepared in the same manner as cells in the previous illustration 60 minutes after exposure to the grid. All of the cell membranes have been removed, leaving only detergent-resistant elements of the cytoskeleton and residues of alpha granules. A circumferential microtubule (MT) forms the cell border. The only sign of activation is the small protrusion filled with actin filaments on the left side of the cell. Magnification × 17,000.*

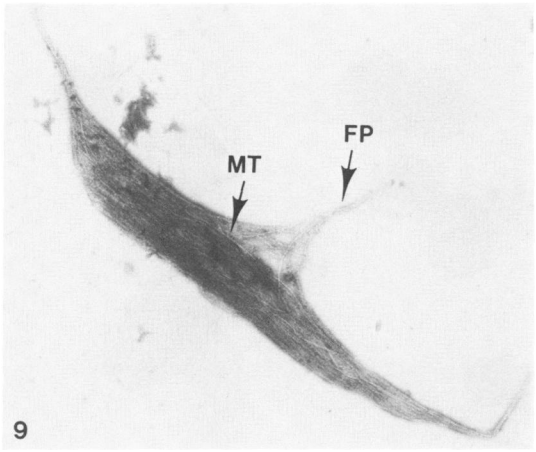

Figure 9. *Simultaneously fixed and detergent extracted bovine platelet prepared 60 minutes after exposure to the grid. The cell has assumed a form resembling a dendritic platelet but has extended only three filiform processes (FP). The circumferential microtubule coil has fractured and extends in the long axis of the cell. Microtubule (MT) fragments also extend into the central process. Magnification × 10,500.*

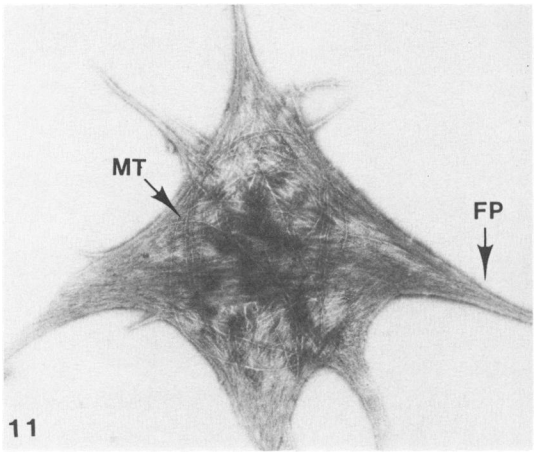

Figure 11. *Detergent resistant bovine platelet cytoskeleton prepared 90 minutes after exposure to the grid. The circumferential microtubule (MT) remains coiled in the cell, but elements have loosened and extend into filiform processes (FP). The cell has unfolded to the maximum extent achieved by bovine platelets. Magnification × 10,500.*

and at the edges of some platelet bodies (Figure 18). Fgn-Au particles moved from different locations around the pseudopod to form a linear pattern stretching the length of the pseudopod. The linear organization was occasionally observed at the inner or outer edge of dendritic bovine cells. However, the central movement of Fgn-Au observed routinely on human cells was not seen on bovine platelets.

Discussion

Bovine platelets, like their human counterparts, are critical components of the hemostatic mechanism, protecting the animal from hemorrhage after vascular injury.[2] Yet there are significant differences between human and bovine platelets, and clarifying them may improve our understanding of platelet function and pathology in humans. Cattle platelets are significantly smaller than human cells, have larger alpha granules, and circulate in larger numbers.[24] Zucker-Franklin and coworkers[9] demonstrated that cow platelets lack the surface-connected OCS present in similar cells in nearly all other mammalian species. As a result, activated bovine platelets fuse alpha granules to the surface membrane and discharge products directly to the exterior[10] rather than through channels of the OCS, as in stimulated human cells.[11]

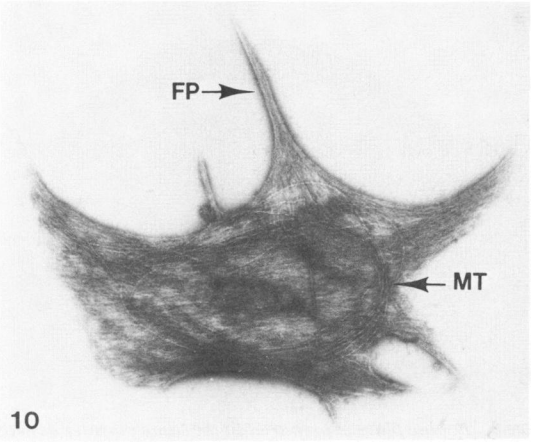

Figure 10. *Unfolded bovine platelet simultaneously fixed and extracted 90 minutes after exposure to the surface. The microtubule (MT) coils have loosened but remain circular for the most part. However, elements of the marginal microtubule extend into almost every filiform process (FP). Magnification × 10,500.*

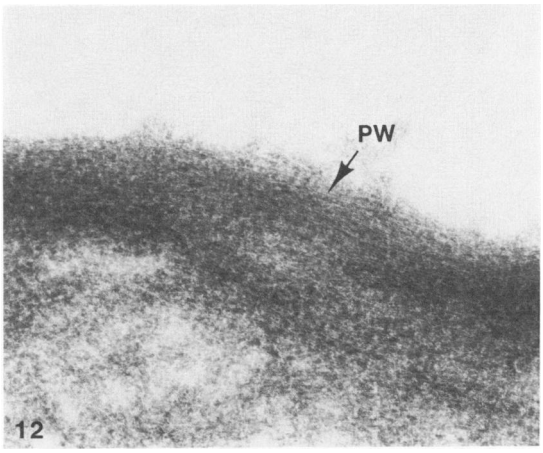

Figure 12. *Human platelet fixed and detergent extracted 30 minutes after exposure to the grid. The cell is fully spread. Actin filaments form a peripheral weave at the margin of the cell. Magnification × 40,000.*

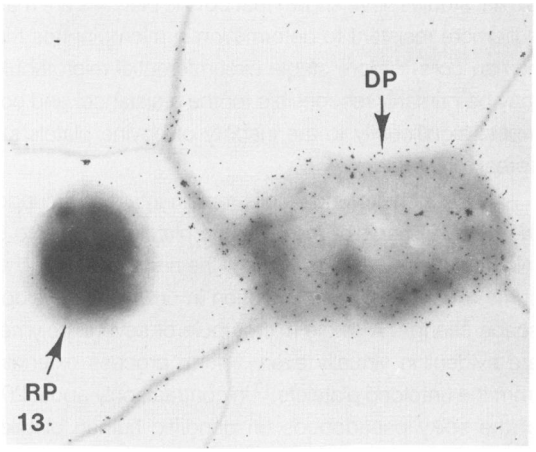

Figure 13. *Bovine platelets allowed to interact with a grid for 20 minutes and then exposed to fibrinogen-gold (Fgn-Au) for 5 minutes before fixation. A resting platelet (RP) has not bound Fgn-Au particles. An adjacent dendritic platelet (DP) has evenly distributed Fgn-Au over its body and filiform processes. Magnification × 10,500.*

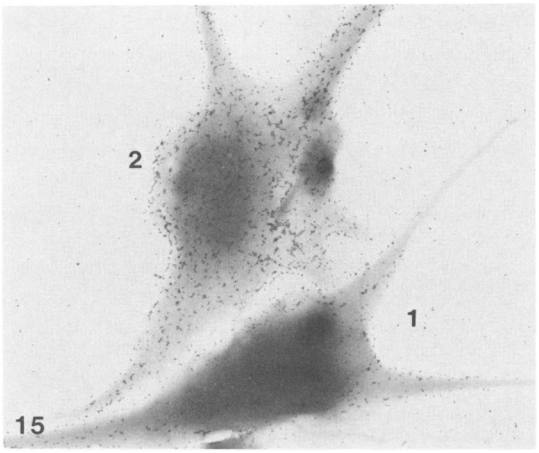

Figure 15. *Two bovine platelets combined with Fgn-Au for 5 minutes after exposure to the grid surface for 30 minutes. An early unfolded cell (1) has a light coating of Fgn-Au particles while the other (2) is heavily labeled. Magnification × 8000.*

The present study has shown an even more striking difference between human and bovine platelets. Surface activation is a powerful stimulus causing rapid changes in platelet morphology.[1–6] Human cells lose their resting discoid shape, become relatively spherical, and extend long, spiky pseudopods as they convert to dendritic forms. Continued interaction causes the spherical body to sink into the surface and cytoplasm to fill spaces between pseudopods, transforming dendritic platelets to spread forms.[7] If this process is inhibited significantly in humans, the bleeding time may be prolonged and hemorrhage may ensue.[25,26]

Bovine platelets also develop shape changes in response to surface activation. They adhere, lose discoid form, extend long, filiform processes, and sink into the surface. However, that is the extent of their reaction. They do not develop thicker, spikelike pseudopods or undergo conversion from dendritic to spread forms so typical of the human platelet response to surface stimulation.[3,5,6,21,22]

The variation in cow platelet reactivity[27] and spreading is not known to be associated with any hemorrhagic problem in the animals. Yet the differences in the response of human and bovine cells to surface activation may provide clues to the understanding of human hemostasis. An attractive rationale for the failure of bovine platelets to convert from unfolded or dendritic forms to fully spread cells like their human counterparts is the absence of the OCS.[9] Recent studies in our laboratory using fibrinogen coupled

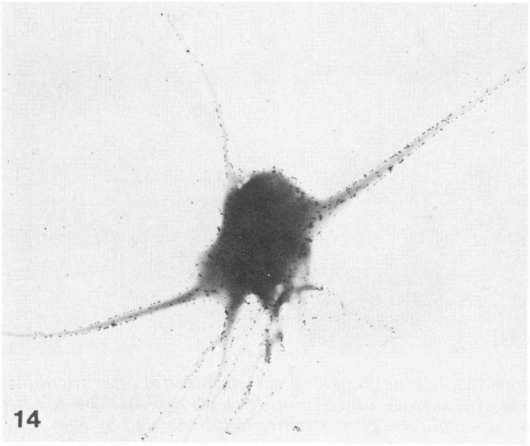

Figure 14. *Bovine platelet exposed to a grid for 20 minutes and treated with Fgn-Au for 5 minutes has many filiform processes. Fgn-Au particles are evenly distributed on the many extensions. Magnification × 6500.*

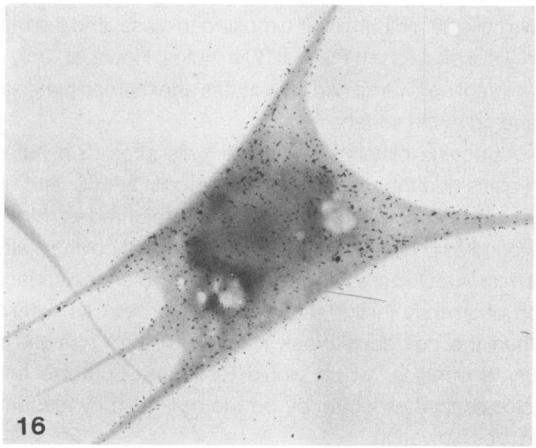

Figure 16. *Unfolded bovine platelet fixed after exposure to the grid for 30 minutes and Fgn-Au for 5 minutes. Fgn-Au particles are evenly distributed over the body and surface extensions. Magnification × 8000.*

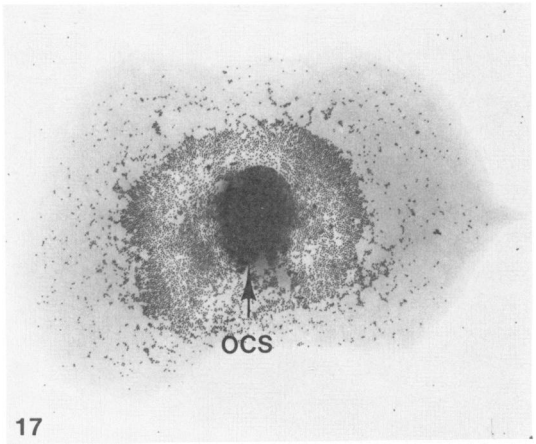

Figure 17. *Human platelet placed on a grid for 20 minutes and then treated with Fgn-Au for 5 minutes. The cell has not spread fully, but Fgn-Au particles have moved away from the cell margin toward the cell center. They are concentrated in an intermediate belt and in channels of the open canalicular system (OCS). Magnification × 8000.*

to colloidal gold as a probe for glycoprotein IIb-IIIa receptors have shown that the OCS of human platelets represents a significant membrane reservoir available for expansion of the cell-surface area after activation.[8] The absence of the OCS in bovine cells may limit the extent to which their membranes can expand after surface activation.

The fate of microtubule coils during surface activation of human and bovine cells offers another possible explanation for the observed differences.[13,26,28] Fluorescence microscopy of platelets stained with a monoclonal antibody to tubulin followed by fluorescein conjugated anti-immunoglobulin demonstrated that circumferential microtubules in bovine platelets are more resistant to disassembly after long-term surface activation than those in human cells. Nearly all human platelets contained intact microtubule coils 15 minutes after exposure to glass, and the majority still possessed them at 30 minutes. However, only a few intact coils remained 60 minutes after human platelets adhered to the surface.[13]

All bovine platelets contained coils after 15 minutes on glass and the majority contained coils 30, 60, and 90 minutes after activation. Coils that had fractured remained as bundles in bovine cells. The resistance of bovine platelet microtubule coils to disassembly on prolonged surface activation may inhibit shape change or limit its extent. When the coil does break it tends to form straight or curved bundles, which would foster the porpoise and sickle shapes assumed by bovine cells as they respond to glass activation.

Microtubule coils support the discoid shape of resting human platelets and contribute significantly to their resistance to deformation on aspiration into micropipettes.[29] Similar studies have shown that bovine platelets are markedly more resistant to deformation in micropipettes than human cells.[30] More stable circumferential microtubules may be primarily responsible for the resistance, and contribute significantly to the inability of bovine platelets to spread on surfaces.

Although microtubule coils remain intact and support the discoid shape of most bovine platelets for up to 90 minutes after surface activation, the rings loosen and assume a ball-of-yarn configuration in cells that develop a shape change. A single microtubule or several polymers are evident in virtually every filiform process extending from the unfolded platelets.[10] In contrast, only about 20% of the spiky pseudopods on dendritic human platelets contain microtubules.[12] Yet the filiform processes on unfolded bovine platelets do not develop the appearance of thicker, spiky pseudopods extending from dendritic human cells. Parallel actin filaments organized in bundles extend in the long axis of the filiform processes of bovine platelets, as they do in human platelet pseudopods. Why their polymerization and association in cattle platelets fails to produce spiky pseudopods remains obscure.

A similar failure may underlie the absence of spreading by bovine platelets. Polymerization of actin filaments and their association in a peripheral weave at the cell margin is a major factor in the conversion of dendritic human platelets into spread forms.[31-33] The margin of unfolded bovine platelets is filled with actin filaments, but they do not appear to assemble into the peripheral weave found in spread human cells.

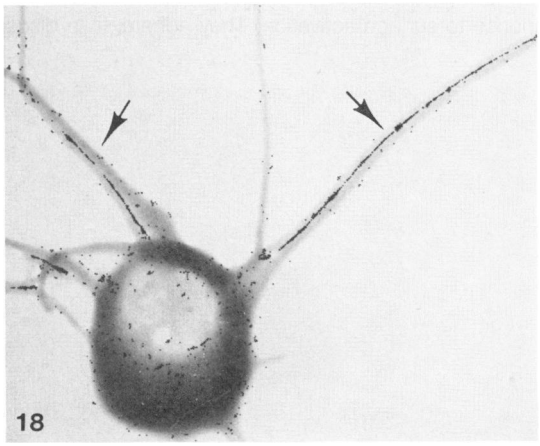

Figure 18. *Bovine platelet fixed 20 minutes after incubation on a grid surface and 5 minutes with Fgn-Au. The cell body and some filiform processes are lightly stained by Fgn-Au. On other surface extensions ( ) the Fgn-Au gold particles have formed a straight line in the long axis. The particles may lie in a groove between closely associated filiform processes on some cells, and on single surface extensions on the same or other platelets. Magnification × 16,000.*

The differences in cytoskeletal assembly and organization in surface-activated bovine platelets may extend to the surface membrane cytoskeleton. Fibrinogen coupled to colloidal gold (Fgn-Au) has been used to demonstrate the movement of the glycoprotein IIb-IIIa (GPIIb-IIIa) receptors on surface-activated human platelets.[18,21,22] The electron-dense probes cover the body and pseudopods of dendritic platelets. As the spiderlike cells convert to spread forms, the Fgn-Au particles move from pseudopods and peripheral margins move toward platelet centers and concentrate in channels of the OCS.[17]

Bovine platelets lack the OCS found in human and most other mammalian platelets.[9] Therefore, the directed movement of Fgn-Au, GPIIb-IIIa receptor complexes from peripheral margins to channels of the OCS on surface-activated human platelets might be expected to differ on bovine cells, and it does. Fgn-Au remained randomly dispersed on dendritic and unfolded bovine platelets. The only significant movement of receptor ligand complexes on bovine cells took place on filiform processes and cell margins. Fgn-Au particles diffusely covering pseudopods often formed linear configurations in the long axis of the surface extensions and occasionally developed similar lines of gold particles along internal or external margins. The movement of the Fgn-Au probes away from peripheral margins toward cell centers and the OCS of surface-activated human cells was not observed on the bovine platelets at any stage of transformation.

The lack of an OCS, however, should not block movement of Fgn-Au probes on bovine platelets.[34] Fully spread human platelets will still transport the ligand-receptor complexes toward cell centers, even though channels of the OCS have evaginated back onto the surface[8] or have closed as a result of the tension caused by spreading.[17] Preliminary studies in our laboratory have shown that the movement of GPIIb-IIIa receptor-ligand complexes is not due to assembly of the internal actin cytoskeleton, but to a cytochalasin B resistant cytoskeleton in the human platelet surface membrane.[35] Cytochalasin B, however, did not enhance movement of GPIIb-IIIa receptors on bovine platelets.

The present study has shown that bovine platelets do not spread like human cells after surface activation; instead they unfold. Absence of an OCS and the reservoir of membrane it represents in human cells for expansion of the surface area during spreading may explain why bovine platelets cannot go beyond an unfolded or dendritic phase during surface activation. The inherent resistance of their circumferential microtubules to disorganization or disassembly may be, in part, responsible for their inability to develop into the spread forms assumed by human platelets. While actin filaments polymerize after surface activation of bovine platelets, their organization may be ineffective in producing spikelike pseudopods and expanding the cytoplasm to fill spaces between them. The variation in internal cytoskeletal assembly may extend to the bovine cell surface. Exposure of bovine cells to fgn-Au probes during surface stimulation failed to result in the reorganization observed on similarly treated human cells. This may be due, in part, to absence of the OCS in bovine platelets. However, it may also be due to inability of GPIIb-IIIa receptors to be moved by a cytoskeleton within the membrane of the bovine cell. In that case a difference in the membrane cytoskeleton may also be a factor in the failure of the bovine platelet to form an OCS.

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