A REVIEW

The Platelet as a Sponge: A Review

By Edward Adelson, Jack J. Rheingold and William H. Crosby

Many functions have been ascribed to platelets. Johnson and Seegers list eleven so-called platelet factors related to clotting. The general belief is that these factors are contained in the platelet structure as derived from the megakaryocyte. However, since Roskam's classic description of the "atmosphere plasmaticque" of the platelet, a large number of articles in the coagulation literature give evidence that many of the platelet activities are derived from materials adsorbed onto the platelet surface from the plasma. The purpose of this paper is to summarize this evidence. Our viewpoint is admittedly a biased one in favor of the concept that the ability of the platelet to act as a sponge is a basic function. We believe that the platelet adsorbs and concentrates coagulation and other factors and carries them through the circulation. When a vessel wall is ruptured, the platelet is attracted to the site of injury. Once there, the adsorbed proteins and calcium are somehow triggered to produce platelet clumping followed by viscous metamorphosis. In the small vessels this process is sufficient to stop the flow of blood. In the larger vessels hemostasis does not occur until the process continues to fibrin formation. The platelet surface thus serves as the link relating platelet agglutination and small vessel hemostasis to coagulation. This theory is in contrast to the generally accepted concept that hemostasis in arterioles and venules is a thing apart from blood coagulation.

The concept of the platelet as a sponge had its beginnings with Roskam's work in 1922. He has reiterated his concept through the years and has pointed out the close relationship between platelet adhesiveness, clumping and viscous metamorphosis. These processes are all dependent on the preservation of the plasma coating on the platelet surface. Bounameaux has demonstrated that without this coating, or by modifying this coating, platelet adhesiveness and clumping are lost. He has also pointed out that those factors on the platelet surface which are necessary for platelet adhesiveness are the same factors which are necessary for blood clotting. Lüscher, like Bounameaux, pictures viscous metamorphosis as occurring when thrombin is generated on the platelet surface. In Lüscher's scheme, this thrombin unites with a plasma cofactor and calcium to initiate viscous metamorphosis. A protein which he calls "protein-S" is then extruded from the platelet. This protein, like fibrinogen, clots under the influence of thrombin. The protein-S clot then spontaneously retracts and is responsible for the firmness of the platelet aggregates and for the subsequent clot retraction. The entire process is quite similar to coagulation, except...
that protein-S is substituted for fibrinogen. Unlike fibrinogen, protein-S requires adenosine triphosphate (ATP) in order to clot and retract. This may explain the decrease in platelet ATP which occurs with clotting.

At one time or another, nearly every one of the plasma clotting factors has been found in or on the platelet:

**Calcium**

Bounameaux has shown that washed platelets clump when exposed to thromboplastin. This clumping tendency is lost when the platelets are incubated in citrated or oxalated plasma. A similar loss of clumping ability occurs when platelets are incubated in saline. However, if calcium is added to the saline the clumping ability is regained, but the addition of citrated or oxalated plasma does not restore the clumping ability. Using radioactive calcium, Odell was able to show that the level of platelet calcium radioactivity rose and fell in parallel with the changing level of plasma calcium radioactivity.

**Prothrombin**

Bounameaux incubated washed platelets in plasma that had been deprothrombinized by adsorption with barium sulfate. These platelets lost their ability to clump on exposure to thromboplastin. Addition of fresh oxalated plasma restored this ability. Addition of normal serum did not. This he interpreted as demonstrating that prothrombin coats the platelet. Bounameaux was able to quantify the amount of prothrombin on the platelet surface. After two washings with saline he found that although the platelets occupy only 1 per cent of the total plasma volume, they hold on their surface 5 per cent of the total plasma prothrombin activity.

**Factor VIII (Antihemophilic globulin)**

In 1910 Sahl demonstrated that normal platelets partially correct the defect of hemophilic plasma and he suggested that platelets carry a substance responsible for this correction. Feissly confirmed this finding in 1924. More recently Duckert, Seibert et al. and Mann have found the adsorption of antihemophilic globulin on the platelet. Bounameaux was able to demonstrate this adsorbed antihemophilic globulin on normal platelets by the thromboplastin generation test. Platelets from hemophiliacs lacked the globulin. Repeated washings of normal platelets removed a small portion of the adsorbed globulin. If small amounts of antihemophilic globulin were added to the washing fluid, the amount of adsorbed globulin did not diminish with successive washings.

**Factors IX and X (PTC and Stuart factors)**

Duckert pointed out that PTC and Stuart factor deficiencies are better demonstrated by tissue thromboplastin than by platelet thromboplastin and suggested that this might be due to material adsorbed on the platelet surface. Bounameaux was able to demonstrate the presence of adsorbed PTC and Stuart factor on the platelet surface by the thromboplastin generation test. These proteins can be washed off more readily than antihemophilic globulin.
Platelet factor 3

Perry and Craddock showed that when platelets are incubated in saline for 10 minutes at 37°C, the substance necessary for the generation of thromboplastin is eluted. This substance can be demonstrated in the supernatant saline by the thromboplastin generation test, and it can be readorsed from the saline by the platelets from which it had been eluted. This substance is probably related to Seegers' platelet factor 3. Others, however, believe that platelet factor 3 is a part of platelet structure and is not an adsorbed factor.

Factor V (Proaccelerin)

Platelet factor 1 of Seegers is similar to proaccelerin. In 1947, Mann et al. demonstrated that proaccelerin activity is adsorbed onto platelets. In 1955, Hjort, Rappaport and Owren confirmed this finding. They showed that about 6 per cent of all the proaccelerin activity is adsorbed on the platelet and cannot be removed by washing. This globulin is activated tenfold by thrombin and it is therefore more like proaccelerin than accelerin. Platelets from patients with parahemophilia (Factor V deficiency) lack this factor. They are, however, able to adsorb it during incubation in normal plasma. When normal platelets are incubated with trypsin, which destroys plasma proaccelerin activity, the platelet proaccelerin activity is also destroyed, yet the platelet morphology is unchanged. This suggests that the proaccelerin is on the platelet surface rather than inside the platelet. Bounameaux found 37 per cent of plasma proaccelerin activity on the platelet surface after two washings and confirmed the fact that it was not readily removed by further washings.

Factor VII (Stable factor)

Bounameaux found that 2.1 per cent of plasma Stable Factor activity was present on the platelet surface from which it could not be removed by two washings.

Fibrinogen

Many authors have pointed out that the addition of pure thrombin to washed platelets leads to platelet clumping and viscous metamorphosis followed by the formation of a coagulum which is very similar to fibrin. Whether this coagulum is actually fibrin is uncertain. Evidence against the two being identical is the occurrence of this coagulum when thrombin is added to platelets from patients with a fibrinogenemia. However, it is possible that such patients form minute amounts of fibrinogen, all of which the platelets may adsorb. This can occur if the platelet surface has a higher affinity for clotting factors than the plasma.

Seligmann et al. and Salmon et al. have demonstrated the presence of fibrinogen in platelet extracts by means of immunoelectrophoresis and agar diffusion cells using fibrinogen antibody. The addition of thrombin to platelet extracts caused the formation of the coagulum described by Ware et al. and simultaneously caused the disappearance of identifiable fibrinogen. Seligmann
et al. theorized that fibrinogen is on the platelet surface rather than inside the platelet because platelet antibodies have anti-fibrinogen activity. Salmon et al.\textsuperscript{26} felt that the fibrinogen is inside the platelet since fibrinogen antibodies have no action against whole platelets but are active against platelet extract.

Lüsch\textsuperscript{45,46,47} attributed the coagulum which occurs when thrombin is added to platelets to a substance that he calls Protein-S and that he believes is similar to actinomyosin in its ability to retract. He believes that fibrinogen becomes involved in the coagulum only secondarily by being trapped in the interstices of the Protein-S strands.

**Antifibrinolysin**

Johnson et al.\textsuperscript{25} found that the coagulum formed by the addition of thrombin to washed platelets was resistant to the action of fibrinolysin. They were able to show that this resistance was not due to absence of fibrin, but to high levels of antifibrinolysin in or on the platelets. In fact, a large percentage of all the antifibrinolysin in whole blood is carried by the platelet. Stefanini et al.\textsuperscript{27} confirmed these findings and also showed that some of this antifibrinolytic activity could be washed off the platelet and that some was given off by the platelet during clotting. This favored the theory that antifibrinolysin was adsorbed onto the platelet.

There is no evidence of the adsorption of fibrinolysin or of antithrombin,\textsuperscript{11} which suggests that the platelet adsorbs those factors which help and avoids those factors which interfere with the hemostatic and coagulation mechanism. The sponge-like activity of the platelet may therefore not be a passive process, but rather an active and highly selective one.

The studies of Izak et al.\textsuperscript{23} on the adsorption of serotonin by platelets confirm the fact that platelet adsorption is an active process. The ability to adsorb serotonin persists only so long as the platelet is viable. These authors use this ability to adsorb serotonin as a measure of viability.

The selectivity of platelet adsorption is further demonstrated by the evidence of epinephrine and norepinephrine adsorption.\textsuperscript{20} Seventy to eighty percent of the plasma epinephrine activity is carried in or on the platelet. These hormones, along with serotonin, may play a role in vasoconstrictor phenomena at the site of platelet clumping. However, the contribution of serotonin to local vasoconstrictor phenomena at the site of vessel injury is very much in doubt since loss of platelet serotonin during reserpine therapy produces no change in hemostasis.\textsuperscript{30}

That much of platelet adsorption may be an active process requiring the expenditure of energy is suggested by the finding of adenosine triphosphate (ATP) on the platelet.\textsuperscript{34} Born et al.\textsuperscript{47,48} have shown that the ability of the platelet to adsorb epinephrine, norepinephrine, and serotonin is directly related to the concentration of platelet ATP. Incidentally, ATP may have the additional function of supplying energy for the process of coagulation since the platelet ATP breaks down during coagulation.\textsuperscript{49}

Many enzymes are present on the platelet surface from which they are readily removed by washing. For this reason, Waller et al.\textsuperscript{50} carry out their
PLATELET AS A SPONGE

enzyme determinations on unwashed platelets. The concentration of these enzymes on the platelet is so much higher than that in the plasma that the amount of contaminating plasma in the unwashed platelets cannot account for the platelet levels of the enzymes. Indeed, three of the enzymes which are readily washed off from the platelets—pyruvate kinase, 3-phosphoglycerate-1-kinase, and L-a-glycerophosphate dehydrogenase—are not even present in the plasma. This may argue against the plasma as the source of platelet enzymes, or it may indicate the ability of the platelet to clear these enzymes from the plasma by a process of adsorption and concentration.

In spite of the selectivity of platelet adsorption, there are some substances adsorbed on the platelet surface which appear to serve no useful purpose. There is evidence of A and B blood group substances, Rho(D) antigen, and histamine, the role of which is not clear.

In addition to the specific substances which coat the platelet, there is evidence of nonspecific coating of platelets by the plasma proteins in which they are suspended. There is also evidence that platelets adsorb particulate material onto their surface and may thus help to clear the blood of foreign matter such as bacteria and viruses. Salvidio and Crosby have recently described the activity of platelets in clearing blood of injected India ink.

The infusion of dextran into normal subjects causes a prolonged bleeding time, a positive tourniquet test, and impaired prothrombin consumption. By means of carbon-14 tagged dextran, we demonstrated adsorption of dextran onto the platelet surface. This has been confirmed by Ponder who found that dextran alters the rate of movement of platelets as measured by micro-electrophoresis, and by Ross and Ebert who found that dextran alters the isoelectric point of platelets. We believe that the bleeding tendency caused by dextran is due to the entanglement of the macromolecule on the platelet surface with a resultant interference with the normal function of the platelet. This may be another example of the platelet's attempting to clear the blood of foreign material.

This same phenomenon probably occurs in macroglobulinemia and may account, at least in some measure, for the purpura of that disease. By means of immunochemical and fluorescent antibody techniques, and by electron microscopy, Pachter et al. have detected macroglobulins coating the platelet in this disease. It may well be that a similar phenomenon explains the purpura of multiple myeloma and of other hyperglobulinemias. In the bleeding tendency of uremia, certain abnormalities of silicone clotting time and prothrombin consumption have been found. Geiger et al. have noted an abnormal plasma phosphatid in uremic blood. This same phosphatid is found by chromatography of platelet extracts. When normal platelets are incubated in uremic plasma, they adsorb the abnormal phosphatid. These authors believe that this absorbed phosphatid is the cause of uremic bleeding. Indeed, in any disease in which the
plasma contains an abnormal constituent the platelets may adsorb the abnormal material. Part of the hemostatic defect in cirrhosis\textsuperscript{44} may be due to such adsorption.

**Summary**

This paper summarizes the evidence in favor of the theory that many of the platelet's factors are adsorbed onto its surface from the plasma. It is suggested that this ability of the platelet is one of its basic functions. The platelet adsorbs coagulation and perhaps vascular factors on its surface and carries them through the circulation to the area where they are needed most—at the site of vessel wall injury. This adsorption by the platelet may be an active rather than a passive process, since it requires the expenditure of energy and since it continues only so long as the platelet is viable. In those diseases in which there is marked abnormality of the plasma proteins, there is evidence to suggest that associated coagulation and hemostatic defects may be due to interference with this "atmosphère plasmatique."

**SUMMARIO IN INTERLINGUA**

Iste articulo sumniarisa he evidentia in favor del theoria que multes del factores del plachetta es adsorbite ad su superficie ab le plasma. Es suggerite que iste capacitale del plachetta es un de su functiones basic. Le plachetta adsorbe factores de coagulation e possibilemente factores vascular ad su superficie e porta los per le circulation al region ubi illos es requirite le plus—al sito del injuriate pariete vascular. Iste adsorption per le plachetta pote esser un processo active plus tosto que passive, proque illo require un expender de energia e proque illo continua solmente durante que le plachetta es viabile. In ille maladias in que il ha anormalitate marcate del proteinas del plasma, il ha evidentia que suggere que defectos associate coagulational e hemostatic pote esser causate per interferentia in iste "atmosphère plasmatique."

**REFERENCES**

PLATELET AS A SPONGE

1285–6, 1955.

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