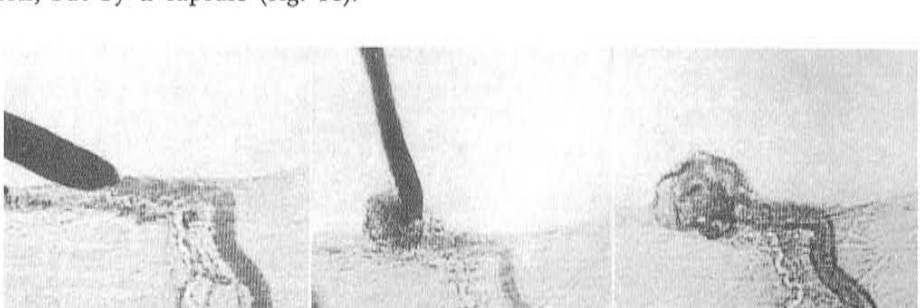
The Part Played by Platelets in the Formation of an Efficient Hemostatic Plug

Department of Internal Medicine, University of Liège, Belgium

J. Roskam, J. Hugues, Y. Bounameaux and J. Salmon

The mechanism by which bleeding from a small wound is arrested differs according to the nature of the damaged vessels (1, 38). Capillary hemorrhages, unless they are prevented by adherence of endothelial cells just when the vessel is nicked, are stopped by blood coagulation, similar to that occurring in a test tube.

On the other hand, the arrest of bleeding from a small artery or vein, much more important than that of capillary oozing, results from the formation of a "hemostatic plug": the blood streaming out of the open vessel immediately yields platelets which adhere to the lip of the wound and to each other as well. Thus a white thrombus is formed outside the vessel; it grows quickly downstream, and at last seals the wound in the manner of a bottle closed, not by a cork, but by a capsule (fig. 1c).



a b c

Fig. 1: a) Blood streaming out of a transected mesenteric artery while the wound is washed with a solution containing 10% sodium citrate (Snapshot taken 10 min. after the injury). — b) Blood streaming out of the mesenteric artery through a pervious platelet thrombus formed while the wound is washed with a solution containing 1% sodium citrate (Snapshot taken 6 min. after this solution has been substituted for the preceding one). — c) Efficient hemostatic plug closing the vascular wound (Snapshot taken 2 min. after the normal Zweifach solution has been substituted for the 1% citrated one).

At the beginning, parts of the platelet plug are often carried away by the blood stream, but it rebuilds itself immediately. At that time, blood escaping from the vessel runs for a while also through channels inside the thrombus. But usually, in two or three minutes, the hemostatic plug stops bleeding for good (1, 16, 17, 38, 40).

Two questions arise then:

— What causes the platelets to stick to the damaged vessel walls and to each other in these conditions?

— How does the platelet plug cause effective hemostasis?

I. Formation of platelet plug

Platelets adhere to many foreign surfaces, but their sticking to such surfaces varies according to the nature of the surface (11, 39).

A. Platelet adherence to organic particles

Erythrocytes from animals of another species, yeasts and many microbes, when introduced into the blood stream or into moderately citrated mammalian blood, are rapidly and intensely covered by agglutinated platelets (38). Such a platelet loading does not occur if the anticoagulant concentration is high, for instance 2% sodium citrate. Nevertheless, when such foreign particles have been previously treated with fresh serum or moderately citrated plasma deprived of platelets and afterwards washed with saline, they adhere immediately and intensely to blood platelets even in 2% citrated plasma (26, 27).

The prerequisite for such a platelet loading is identical to the modification of the surfaces of many microbes, leading to their uptake in phagocytosis. It depends upon the presence in the plasma (or the serum) of proteins which are destroyed at 56° C and have been called "natural opsonins". That is why we also termed "opsonization" the modification of a surface leading to its platelet loading (26, 27).

As natural opsonization of a surface is prevented by every factor which prevents blood clotting, it has been supposed that this opsonization might have something to do with blood coagulation (26, 27, 28, 29). But there is no close relationship between these two phenomena, because neither a mixture of recognised coagulation factors clottable by addition of calcium chloride, nor each of these factors acting separately, "opsonize" foreign organic particles (11).

Recent experiments have shown that treating yeasts with serum at low temperatures (10 to 16° C) opsonizes them without any deviation of comple-

ment. Such a treatment also prevents a further opsonization of fresh yeasts with this serum as well as an eventual deviation of its complement except, probably, if "properdin" be added. This suggests that the opsonizing factor might be this new immunological factor called "properdin" (11, 39).

B. Platelet adherence to glass surfaces

Figure 2 (curves 1 and 2) shows the rate at which platelets disappear from heparinized blood, because they adhere to the glass of the tubes when rotated in the Helen Payling Wright rotator. After 60 to 80 minutes, platelet sticking stops. If we then decant the rotated blood into fresh tubes, the remaining plate-

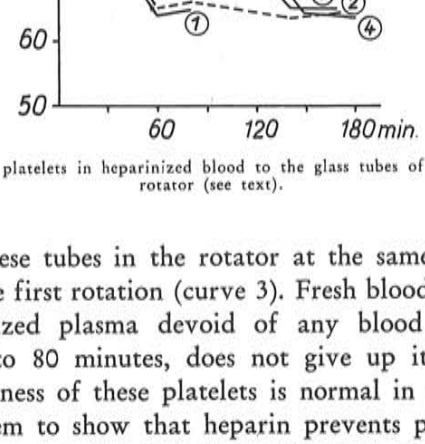


Fig. 2: Adhesiveness of platelets in heparinized blood to the glass tubes of the Helen Payling Wright rotator (see text).

lets will adhere to these tubes in the rotator at the same rate as the platelets which stuck during the first rotation (curve 3). Fresh blood rotated in pretreated tubes where heparinized plasma devoid of any blood corpuscles has been rotated also for 60 to 80 minutes, does not give up its platelets (curve 5), although the adhesiveness of these platelets is normal in fresh tubes (curve 4). These experiments seem to show that heparin prevents platelet sticking to the tubes after a while by means of a heparin modification of the glass surface. This modification is easily suppressed by washing the tubes with saline (6, 38, 11). Nevertheless — quite unlike yeasts — when the tubes of the rotator cause blood platelets to adhere, these tubes are not opsonized, for a previous rotation of serum in the tubes does not favour platelet adhesiveness.

The situation is entirely different if moderately citrated blood is used. If we rotate moderately citrated blood in the rotator, we again notice that platelet

sticking to the tubes will stop after about one hour (fig. 3, curves 1 and 2), But the citrated blood, after such a rotation, when decanted into fresh tubes, will not give up its platelets to these tubes when rotated anew (curve 3). On the other hand, fresh blood rotated in the tubes where citrated blood has been rotated for an hour, will lose its platelets (curve 4) as in fresh tubes (curve 5). The remaining platelets deprived by such a rotation of their adhesiveness will recover it if they are separated from their plasma, washed twice in saline, kept in saline, to which calcium chloride has been added, for 24 hours at 4° C and

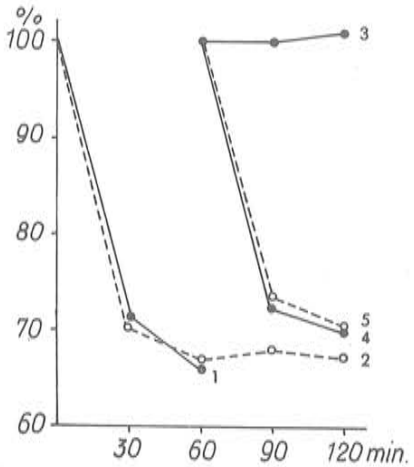


Fig. 3: Adhesiveness of platelets in citrated blood to the glass tubes of the Helen Payling Wright rotator (see text).

then resuspended in citrated plasma. This, along with other experiments, shows that platelets lose their adhesiveness during rotation in citrated plasma, because in this process they give up calcium ions so far retained around them (3, 38, 11), in what has been called their "plasmatic atmosphere" (24, 25, 26, 27).

Adhesiveness of platelets also requires the presence of prothrombin in their "plasmatic atmosphere" (4, 5, 38, 11). The latter has recently been shown to contain, besides albumin, α, β and γ globulins (30), fibrinogen (31, 36, 33, 39), prothrombin (4, 10), calcium (3,23), proaccelerin, proconvertin, antihemophilic globulin, Christmas factor, Stuart-Prower factor (10, 22, 15, 35, 39), and perhaps also antifibrinolysin (37). Thus it is possible that the slow adhesion of platelets to the tubes of a rotator has something to do with some clotting process in their plasmatic atmosphere.

To conclude: adhesion of platelets to glass surfaces seems to depend on two factors: a) probably on a modification of the glass surface. (This factor should

explain why adhesiveness is inhibited by heparin); b) certainly on the presence of a "plasmatic atmosphere" around the platelets, containing calcium ions and various coagulation factors.

This should be kept in mind when tests on platelet adhesiveness are used in clinical work.

C. Platelet adherence to some inorganic particles

A sudden platelet loading — which appears to be purely mechanical — occurs if platelet-rich plasma is mixed with a suspension of some inorganic particles such as $BaSO_4$ or alumina, even if the final concentration of sodium citrate is 2% (11, 39).

D. Platelet adherence to damaged vessel wall

Platelet clumping at the wound of a damaged vessel has not yet been sufficiently analysed (14). Nevertheless, the speed at which such clumping occurs (18) and the possibility of impeding the process by washing the vessel wound with sodium citrate or with heparin at high concentration (16, 17), suggest that it is similar, up to a certain point, to the platelet loading of organic particles, for instance yeasts.

II. Viscous metamorphosis of the platelets and firmness of hemostatic plugs

The adherence of platelets to foreign surfaces and their agglutination to each other are usually followed by their viscous metamorphosis, the latter resulting in their amalgamation (fig. 4). Such a fusion seems extremely important in determining the firmness of the hemostatic plug.

Indeed, nearly all antihistaminic drugs are able to prevent viscous metamorphosis of the platelets which they make spherical (fig. 5) though they increase their adhesiveness, at least in the rotator of H. P. Wright (8). If a wound is washed with a solution of one of these antihistaminics, for instance thephorine, platelets adhere as usual to the damaged vessel, but the hemostatic plug remains pervious to the blood stream (19). The firmness of the plug is also decreased by washing the wound with anticoagulants at sufficiently high doses (16; fig. 1a and 1b).

As adhesiveness and viscous metamorphosis are separate processes, we have now to consider the mechanism of viscous metamorphosis. The study of clot retraction has been used as a method to throw some light on this problem, for

a clot retracts only if the platelets of the blood are physiologically intact when coagulation starts and if they undergo viscous metamorphosis during clotting. This method has shown that a clot proceeding from fibrinogen, well washed platelets and thrombin, does not contract unless a dialysable factor has been added to the initial mixture (20, 39). This dialysable factor must contain calcium,

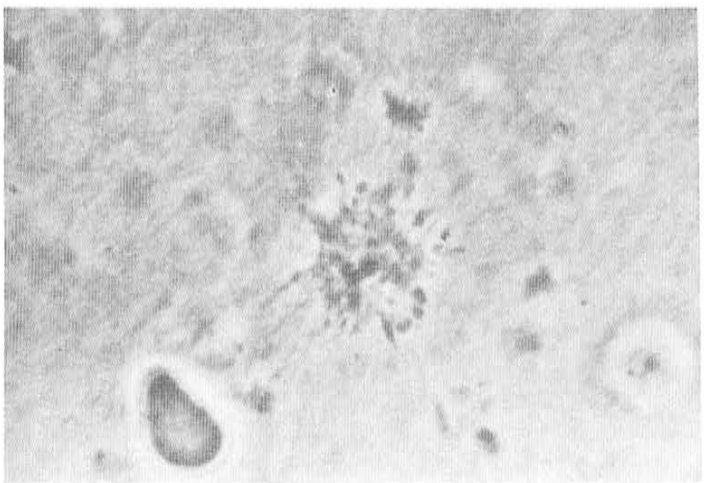


Fig. 4: Platelets having undergone viscous metamorphosis during blood coagulation.

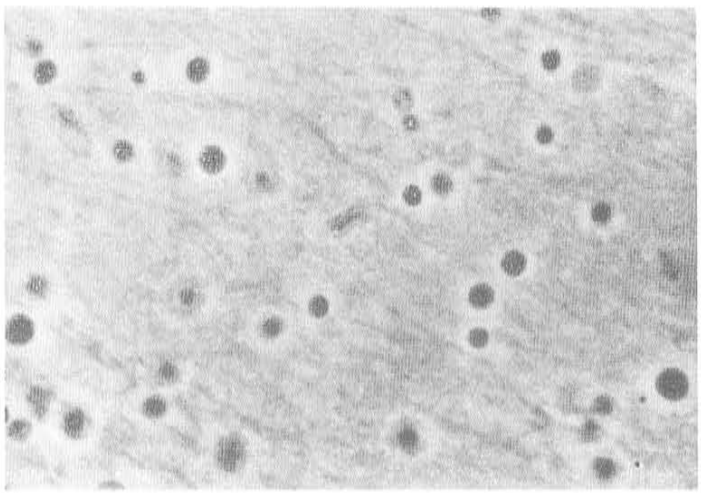


Fig. 5: Platelets made spherical by thephorine and thus prevented from undergoing viscous metamorphosis during blood coagulation.

glucose (7, 9, 21, 39) and adjuvant substances, i.e. substances with the radical COOH, phosphate, cysteine, glutathione, etc. (9, 39).

On the other hand, viscous metamorphosis was studied directly under the microscope. At least in man, ox and sheep, it seems to need thrombin, calcium and adjuvant substances, but not glucose. However, the latter is necessary for viscous metamorphosis to occur in the rat and in the rabbit (13).

As mentioned above, fibrinogen is present around the platelets, in their "plasmatic atmosphere". Immunological techniques, including immuno-electrophoresis, have also shown the presence of much larger quantities of fibrinogen inside the platelets themselves (32, 33). Further experiments are needed in order to specify the relationship between this platelet fibrinogen and the "clottable factor" of W a r e and S e e g e r s, or "protein S" of L ü s c h e r (39).

Anyway, the question arises whether this fibrinogen contributes to make hemostatic plugs effective. Up to now, histological methods have failed to prove the presence of fibrin inside the "magma" resulting from platelet viscous metamorphosis (38). Does this discard the hypothesis that a fibrin precursor might determine the firmness of hemostatic plugs? To answer this question, viscous metamorphosis and hemostasis should be studied in afibrinogenemia, where platelets appear to be deprived of fibrinogen (34).

Scattered fibrin bands have been described in some hemostatic plugs, but their formation does not seem to be an early process, nor a necessary one, to stop bleeding (38). It is logical to think of these bands as strengthening hemostatic plugs in the way iron bars do for ferro-cement, and thus preventing renewed bleeding. It is also highly probable that platelets play a prominent part in the clotting process, giving birth to these fibrin bands as they do in the intrinsic coagulation system (39). But direct observation of spontaneous hemostasis furnishes evidence that the fundamental part taken by platelets in the arrest of bleeding, is that of a building material: the dam which stops the blood flowing from a small artery or vein, is made of stones of peculiar type, namely the platelets, which are quickly converted into cement by their viscous metamorphosis.

Summary

The arrest of bleeding from small arteries or veins depends on the formation of a hemostatic plug made of platelets. In spite of recent information on the conditions required for platelet adhesion to various foreign surfaces, little is known about the precise mechanism of platelet adhesion to the lips of the vessel wound. The effectiveness of the hemostatic plug is due to the viscous metamorphosis of the agglutinated platelets.

Résumé

L'arrêt des saignements au niveau des artérioles et des veinules dépend essentiellement de la formation d'un clou hémostatique fait de plaquettes sanguines.

En dépit de récentes découvertes sur les conditions de l'accolement des plaquettes à diverses surfaces étrangères, le mécanisme précis de leur adhésion à une plaie vasculaire n'est guère élucidé.

L'efficacité des clous hémostatiques est due à la métamorphose visqueuse des plaquettes agglutinées.

Zusammenfassung

Die Stillung von Blutungen aus kleinen Arterien und Venen hängt von der Bildung eines hämostatischen Pfropfes aus den Plättchen ab. Trotz der neuen Informationen über die Bedingungen unter denen die Thrombozyten an verschiedenen fremden Oberflächen haften, ist wenig über den genauen Mechanismus des Haftens von Thrombozyten an den Rändern von Gefäßwunden bekannt. Die Wirksamkeit des hämostatischen Pfropfes beruht auf der viskösen Metamorphose der agglutinierten Thrombozyten.

References

(1) Apitz, K.: Die Bedeutung der Gerinnung und Thrombose für die Blutstillung. Virchows Arch. Path. Anat. 308: 540 (1942).
(2) Bounameaux, Y.: Cinétique de l'adhérence des plaquettes in vitro, C. R. Soc. Biol. (Paris) 149: 603 (1955).
(3) Bounameaux, Y.: Du rôle du calcium dans l'adhésivité des plaquettes in vitro C. R. Soc. Biol. (Paris) 149: 817 (1955).
(4) Bounameaux, Y.: Adhésivité des plaquettes in vitro. Du rôle de divers éléments plasmatiques dont la prothrombine C. R. Soc. Biol. (Paris) 149: 1059 (1955).
(5) Bounameaux, Y.: Action de la thromboplastine sur l'adhésivité des plaquettes in vitro C. R. Soc. Biol. (Paris) 149: 1285 (1955).
(6) Bounameaux, Y.: Cinétique de l'adhésion des plaquettes en sang hépariné. Arch. int. Physiol. et Bioch. 63: 241 (1955).
(7) Bounameaux, Y.: Recherches sur le mécanisme de la rétraction du caillot et de la métamorphose visqueuse des plaquettes. Experientia (Basel) 12: 355 (1956).
(8) Bounameaux, Y.: Action des antihistaminiques de synthèse sur la morphologie plaquettaire et sur les propriétés d'adhésivité des plaquettes in vitro. Rev. Hémat. 11: 17 (1956).
(9) Bounameaux, Y.: Recherches sur le mécanisme de la rétraction et de la métamorphose visqueuse des plaquettes. Rev. Hémat. 12: 16 (1957).

(10) Bounameaux, Y.: Dosage des facteurs de coagulation contenus dans l'atmosphère plasmatique des plaquettes humaines. Rev. Franç. Etudes Clin. et Biol. 2: 52 (1957).
(11) Bounameaux, Y.: Recherches sur l'emplaquettement des surfaces étrangères et sur la coagulation sanguine. Mém. Acad. Royale de Médecine de Belgique 3, fasc. 4, 1—73 (1958).
(12) Bounameaux, Y.: Action des antihistaminiques de synthèse sur la métamorphose visqueuse des plaquettes. Arch. int. Pharmacodyn. 116: 252 (1958).
(13) Bounameaux, Y.: Action de la thrombine et de la Reptilase sur la métamorphose visqueuse des plaquettes de diverses espèces animales. Rev. Franç. Etudes Clin. et Biol. 4: 54 (1959).
(14) Bounameaux, Y.: L'accolement des plaquettes aux fibres sous-endothéliales. Soc. belge Biologie, 30 mai 1959 in C. R. Soc. Biol. (Paris) in press.
(15) Hjort, P., Rapaport, I. and Owren, P. A.: Evidence that platelet accelerator (Platelet factor I) is adsorbed plasma accelerin. Blood 10: 1139 (1955).
(16) Hugues, J.: Contribution à l'étude des facteurs vasculaires et sanguins dans l'hémostase spontanée. Arch. int. Physiol. 61: 565 (1953).
(17) Hugues, J.: Thrombose et hémostase spontanée. Sem. Hôp. Paris, Suppl. 1957, 6th March, n° 14.
(18) Hugues, J.: Agglutination précoce des plaquettes au cours de la formation du clou hémostatique. Thromb. Diath. haem. 3: 177 (1959).
(19) Hugues, J.: Métamorphose visqueuse des plaquettes et formation du clou hémostatique. Thromb. Diath. haem. 3: 34 (1959).
(20) Lüscher, E. F.: Viscous metamorphosis and clot retraction. Vox Sanguinis 1: 133 (1956).
(21) Lüscher, E. F.: Glukose als Cofaktor bei der Retraktion der Blutgerinnsel. Experientia (Basel) 12: 294 (1956).
(22) Mann, F. D.: Reactivity of hemophilic plasma to platelet thromboplastin. J. Lab. clin. Med. 48: 51 (1956).
(23) Odell, T. T. Jr. and Upton, A. G.: Distribution of Calcium^{45} in platelets and bone marrow of rats. Acta haemat. (Basel) 14: 291 (1955).
(24) Roskam, J.: Fonction antixénique, plasma et globulins (plaquettes) C. R. Soc. Biol. (Paris) 85: 733 (1921).
(25) Roskam, J.: Le rôle du plasma dans l'agglutination des globulins (plaquettes) C. R. Soc. Biol. (Paris) 86: 733 (1922).
(26) Roskam, J.: Contribution à l'étude de la physiologie normale et pathologique du globulin (plaquette de Bizzozero). Arch. int. Physiol. 20: 241 (1923).
(27) Roskam, J.: Physiologie normale et pathologique du globulin (plaquette de Bizzozero). Presses Univ., France, publish. 151 (1927).
(28) Roskam, J.: Sur la nature de l'opsonisation C. R. 1er Congrès international Microbiol., Paris 2: 218 (1930).
(29) Roskam, J.: Mécanisme de la prévention et du traitement des thromboses par l'héparine. Arch. Int. Pharmacodyn. 68: 66 (1942).
(30) Salmon, J.: Etude immunoélectrophorétique des àntigènes plaquettaires humains. Schweiz. med. Wschr. 88: 1047 (1958).
(31) Salmon, J. and Bounameaux, Y.: Recherches sur l'antigénicité des plaquettes et du fibrinogène bovins C. R. Soc. Biol. (Paris) 150: 2278 (1956).
(32) Salmon, J. and Bounameaux, Y.: Situation du fibrinogène décelé dans les thrombocytes bovins isolés et lavés. Arch. int. Physiol. and Bioch. 65: 502 (1957).
(33) Salmon, J. and Bounameaux, Y.: Etude des antigènes plaquettaires et en particulier du fibrinogène. Thromb. Diath. haem. 2: 93 (1958).
(34) Salmon, J., Verstraete, M. and Bounameaux, Y.: Fibrinogène plaquettaire et afibrinogénémie. Arch. int. Physiol. et Bioch. 65: 632 (1957).

(35) Seibert, R. H., Margolius, A. and Ratnof, O. D.: Observations on hemophilia, parahemophilia and coexistent hemophilia and parahemophilia. J. Lab. clin. Med. 52: 449 (1958).
(36) Seligmann, M., Goudemand, B., Janin, A., Bernard, J. and Grabar, P.: Etudes immunochimiques sur la présence de fibrinogène dans des extraits de plaquettes humaines lavées et dans certains extraits leucocytaires. Rev. Hémat. 12: 302 (1957).
(37) Stefanini, M. and Murphy, I. S.: Studies on platelets. XIV. Human platelets as source of antifibrinolysin. J. clin. Invest. 35: 355 (1956).
(38) 1er Symposium de la Fondation Valentino Baldacci: Hémostase spontanée, plaquettes sanguines et parois vasculaires. Madrid, 27—30 mai 1955. Omnia.med. (Pisa) publish., 190 pp.
(39) 2me Symposium de la Fondation Valentino Baldacci: Hémostase spontanée, thrombose et coagulation sanguine. Seteais-Lisbonne, 7—11 juin 1957. Omnia med. (Pisa) publish., 214 pp.
(40) Zucker, M. B.: Platelet agglutination and vasoconstriction as factors in spontaneous hemostasis in normal, thrombocytopenic, heparinized and hypoprothrombinemic rats. Amer. J. Physiol. 148: 275 (1959).