METABOLISM AND FUNCTION OF NORMAL PLATELETS

E. F. Lüscher

Theodor Kocher Institute, University of Berne

The history of platelet metabolism and platelet function is rather unusual: At the end of the last century most workers agreed that platelets were of the greatest importance for hemostasis, as well as for thrombus formation (cf. 12,38,50,39). In 1905 Morawitz (83) published what is sometimes called the classical scheme of blood coagulation with platelets playing a cardinal role in prothrombin activation and in 1912 Warburg (120), soon followed by others (73,94) showed that platelets display respiratory activity. Many years characterized by general disinterest in platelets followed, and it was only two decades ago that their importance under physiological and pathological conditions was rediscovered. This more recent work has shown beyond doubt that platelets are amazingly complex structures with a remarkable spectrum of energy-dependent activities.

W. L. Holmes (ed.), *Blood Cells as a Tissue*

METABOLISM OF RESTING PLATELETS

A comprehensive study on the energy metabolism of normal human platelets was published in 1959 by Waller et al. (119). This work confirmed and considerably extended earlier reports (cf. 20,21) on the presence of the complete enzyme systems of glycolysis, of the citric acid cycle, and of the respiratory chain. Furthermore the presence of the pentose phosphate shunt was established, a finding which soon was confirmed by others (2,128). More recent studies seem to show that the importance of this pathway for platelet activity is limitted (52).

Even under aerobic conditions, glycolysis was found to be the major pathway of carbohydrate degradation, lactate and pyruvate accounting for 50 % of the added substrate, as compared to only 20 % which were metabolized to CO_2 and water. Similar conclusions were also reached by Chernyak (23). It should be noted though, that in view of the much higher yield of adenosine triphosphate (ATP), respiration nevertheless appears as an important source of energy for the resting platelet.

Platelets contain considerable amounts of glycogen, which is easily detectable by electron microscopy (for reviews see 101,77,79). The glycogen level of more than 2 % of the dry weight, or 28 $\pm$ 10 µmoles expressed as glucose per gram of viable platelets (48, cf. also 64), approximately equals that found in skeletal muscle. Both glycogen synthesizing and degrading enzymes have been described (115,102).

Adenine nucleotides play a particularly important part in platelet activities. ATP is found in remarkably high amounts; values up to 23.6 µmoles/gram wet weight

have been reported (100). Also present are adenosine diphosphate (ADP) and adenosine-5-monophosphate (AMP); reported ratios for ATP/ADP vary greatly, from 3 (119) to about 2 (64,81), and 1 (116). It should be noted that these values lie far below those reported for other cells.

Platelets are unable to take up nucleotides unless they are first dephosphorylated to adenosine which is then converted mainly into ATP and ADP. Experiments with lysed platelets suggest that adenosine kinase catalyzes the formation of AMP which is rapidly converted to ATP and ADP by adenylate kinase (54). Platelets contain an adenine phosphoribosyl transferase and therefore are able to synthesize adenyl-nucleotides from adenine; a de novo synthesis of nucleotides is not observed (55). The transferase, together with pyruvate kinase is solubilized on disruption of the platelets, whereas most of the adenylate kinase remains attached to cell organelles or fragments (56). Other enzymes involved in nucleotide metabolism and identified as platelet constituents are adenosine deaminase, 5'-AMP-deaminase, purine nucleoside phosphorylase (54), adenyl cyclase (129,127), probably several ATPases, and an ADP-splitting enzyme (117,118,22). As will be shown later, all of these enzymes are of functional significance for platelet activity.

Many other enzymes have been described in blood platelets. There can be no doubt that they also are of importance for platelet function; however, in most cases direct proof is as yet missing. Exceptions are perhaps glutathionreductase (84), missing in thrombasthenic platelets, and fibrinstabilizing factor (factor XIII) (74,37,66,70), which is found in platelets

in amounts much higher than in plasma. It should be kept in mind that there exists the possibility that at least some of the observed activities in platelets may be left-overs from the megakaryocyte. This is certainly true for protein synthesis (13,14), which is much more pronounced in younger than in older platelets (111).

For other metabolic activities and the corresponding enzymes the reader is referred to several more recent reviews (77,79,28,80).

PLATELET FUNCTION

Blood Coagulation

The procoagulant activity of platelets is required for the formation of the prothrombin-converting enzyme complex by the intrinsic pathway. The essential component has been termed platelet factor 3 (PF. 3) and consists of phospholipid, probably present as a phospholipoprotein-complex and mainly localized in membraneous material. Cephalins, i.e. phosphatidyl-ethanolamine and phosphatidyl serine, and therefore erythrocyte stroma or other cell membranes can substitute for platelets in this reaction (cf. 77,79). What makes the platelet unique is the ease with which this procoagulant, which is masked in the normal, circulating cell, becomes available. More recent work (51,4) shows that in intrinsic prothrombin activation two enzyme-phospholipid complexes are formed: one of them comprising factors VIII (antihemophilic globulin), factor IX (PTC, Christmas-factor) and calcium, the other factors V (proaccelerin, Ac-globulin), X (Stuart-Prower factor), and calcium. The latter complex converts prothrombin into thrombin. All these important reactions therefore must take place on the platelet surface, where PF 3 becomes available, thus making the platelet the

center of thrombin formation by the intrinsic pathway.

Other factors which participate in blood coagulation have also been identified in platelets (see reviews 75,77,79). Some of them appear to be adsorbed or taken up from plasma; others are autonomous platelet constituents. To the latter belong platelet-fibrinogen, present in up to 13 % in platelet cytoplasm (cf. 30), platelet factor 2, an accelerator of fibrinformation under the influence of thrombin (40), and platelet factor 4, first described as a heparin-neutralizing polypeptide (40,19,36). PF 4 furthermore has the ability to combine with partially degraded fibrinogen, thus leading to the formation of complexes with pronounced cofactor-activity in platelet aggregation (93).

The Role of Platelets in Hemostasis

The spontaneous arrest of hemorrhage is a complex phenomenon in which vascular, plasmatic, and cellular factors play equally important roles. Examination of this process under the microscope shows that platelets adhere almost instantaneously to the site of a vascular injury and that these adhering platelets become the center for the deposition of new circulating platelets which in turn become adhesive. In this way a rapidly growing aggregate is formed which first remains fragile and permeable before it spontaneously consolidates to a functional, impermeable "hemostatic plug". Only then does fibrin formation begin and contribute secondarily to hemostasis.

It is a most intriguing problem why the inert circulating platelet under the stimulus of an endothelial injury is transformed into a sticky form enabling it to aggregate.

Platelet "viscous metamorphosis": A comparison of normal and tightly aggregated platelets, particularly by electron microscopy, shows that aggregation consists by no means in the assembly of otherwise unaltered cells. Normal platelets are disk-shaped; they show a complex internal structure with different types of organelles, particularly a considerable number of strongly osmiophilic granules (α-granules; cf. 101). In a hemostatic plug or an equivalent aggregate produced in vitro, platelets are tightly fused together; they have lost their disk shape and appear as irregular, swollen elements with interlinking protrusions. α-granules and certain other organelles have disappeared.

Platelet organelles have been partly separated and characterized (90,104,103,78). By sucrose density gradient centrifugation a top fraction consisting of membraneous material is followed by a heterogenous vesicular zone containing lysosomes, followed by still heavier mitochondriae fraction and a bottom fraction of socalled dense bodies, including α-granules. Recent work has shown beyond doubt that the dense granules are storage organs (35,34,106,122,95). Platelets are known to contain several biogenic amines, in particular serotonin, adrenaline, noradrenaline, and, in some species, histamine, which can be taken up by active transport or by diffusion (123,99,26). The disappearance of the storage granules is the consequence of the release of their contents into the surrounding medium. As will be shown, this release reaction is of the greatest importance for platelet function; besides biogenic amines, adenine nucleotides, platelet factor 4 (41), and calcium ions (88) are released in a remarkably fast reaction, which is energy-dependent. Other components

released are certain enzymes, proteins and carbohydrate (58,33,53). The involvement of the energy metabolism in aggregation and release is evidenced by a transient activation, followed by a progressive decline of metabolic activity. Finally, platelet aggregates formed in vitro show spontaneous contraction, thus relating platelet alterations in the course of irreversible aggregation to the well-known phenomenon of clot retraction (see reviews 18,77,75). Contractile activity of platelets is due to an actomyosin-like protein, termed thrombosthenin (9,46) in concentrations of up to 15 % of total platelet protein.

The described sequence of reactions is observed whenever platelets are exposed to thrombin, provided Ca^{++}ions are present. The total of the changes thus induced has been termed the "viscous metamorphosis" (VM) of the platelets (97), an expression proposed for the morphological phenomenon alone in 1886 by Eberth and Schimmelbusch (39).

Platelets and Vascular Integrity

As exemplified by the increased vascular fragility in severe thrombocytopenia, platelets bear a relationship to the vascular wall. As mentioned above, they adhere immediately to the site of vascular injury and further work has shown that it is connective tissue and in particular collagen with which they have a specific affinity (61,130,109,59,60). The nature of this reaction is still obscure. It has been shown that collagen devoid of telopeptides retains its full activity (126); however, blocking of free amino groups destroys its effect on platelets (124).

There is some reason to believe that basically the same mechanism is responsible for the endothelium-

platelet relationship. Platelets were shown to adhere to gaps between endothelial cells (114); and it is suggestive that this represents a physiological and continuing repair function.

BIOCHEMISTRY OF PLATELET ACTIVATION

Induction of Activated State

It has been mentioned above that thrombin induces far-reaching alterations culminating in irreversible aggregation (VM) in platelets. Certain other proteases with trypsin-like specificities can replace thrombin in this reaction (32,31); there is however no parallelism between the fibrinogen-clotting and VM-inducing properties of such enzymes (31). This makes it unlikely that fibrinogen is the substrate for thrombin on the platelet surface. Platelets contain several other thrombin-labile proteins (29,44,43,30), to mention only factor XIII (66), and a recently characterized β-globulin (43). Most remarkable is the finding that the myosin-like moiety of thrombosthenin (thrombosthenin M) is split by thrombin (24). The possible role of thrombosthenin in platelet function will be discussed later.

All these findings make it appear that activation of platelets consists in a proteolytic step. More remarkable then are findings that a variety of agents devoid of enzymatic properties are capable of causing similar effects. Thus, collagen in the presence of Ca^{++}ions not only is adhesive for platelets, but also leads to alterations not unlike VM, i.e. to a release reaction, degranulation, and to metabolic changes (96, 72). The same is true for immune complexes (86,85) and for certain opsonized particles such as latex or carbon (45,87). Finally, adrenaline, noradrenaline, (82,16,113)

and, under certain circumstances, serotonin (5,17) will also induce platelet aggregation.

The similarity of the platelet's response to this variety of external stimuli led to the postulate that a common product, perhaps resulting from a variety of primary effects, entered into play. Such a material was indeed found in the form of ADP. Gaarder et al. (42) were first to demonstrate that ADP in minute amounts aggregated platelets in plasma. Shortly afterwards, Käser-Glanzmann and Lüscher (65) were able to show that ADP is released from platelets in the course of VM in sufficient amounts to cause their mutual aggregation. Haslam (49) finally demonstrated that the removal of ADP from the incubation mixture prevents platelet aggregation by thrombin.

Release Reaction

The earlier work had shown already that the ratio of adenosine nucleotides released during VM was different from that in intact platelets (65). Recent studies (57) have given a most plausible explanation for this by the discovery of two different nucleotide pools: a metabolically active pool, present in the cytoplasmic compartment, capable of incorporating adenosine, adenine or phosphate and a metabolically inert pool localized in dense granules. The ATP/ADP ratios in both pools were different. In the organelles it lay from 0.6 - 1.1. Release experiments performed shortly after labeling the nucleotides showed that only this inert unlabeled pool was discharged and that it amounted to 60 % of the total sum of ATP + ADP of the platelet. About 30 % of the platelets nucleotides, with an ATP/ADP-ratio of about 10, remain unchanged during release; they are in equilibrium with perhaps

a third pool, consisting of ATP only, which is converted to IMP in the course of the release reaction.

Biogenic amines are always released together with the adenosine nucleotides, platelet factor 4, K^+ and small amounts of Zn^{++} (112,57), suggesting that all these substances are stored in the same organelles. This agrees with observations made as early as 1959 on ATP and adrenaline (1). The release reaction is a fast and energy-dependent process (89). The induction of platelet aggregation and release is linked to a transient stimulation of the platelets energy metabolism (7,121,64,25), but whether this is directly linked to the energy requirement of the release reaction is not yet clear. Another possibility, plausible because of its considerable speed, would be a linkage to the ATP-IMP-transformation, as suggested by Holmsen, Day and Storm (57).

Platelet Aggregation by ADP

Small amounts of ADP will aggregate platelets reversibly. This effect is dependent on the presence of external fibrinogen (69,27,108); complexes formed from partially degraded fibrinogen being even better cofactors (107,71,3). It should be noted, however, that smaller fibrinogenolytic degradation products interfere with platelet aggregation (125,62,68,67). As first shown by Macmillan (76), larger amounts of ADP induce irreversible aggregation, characterized by degranulation and release reactions (131,132,92,105). Thus, ADP ranks together with thrombin and collagen among the inducers of irreversible platelet aggregation. In this reaction it is potentiated by adrenaline and serotonin (16,113,5).

Several authors have speculated about the mechanism whereby ADP aggregates platelets. Most of them assume an inhibitory effect on a membrane-located ATPase. This "ecto-ATPase" could be thrombosthenin (98,15, cf. also 63,91), an assumption which is supported by the finding that specific antithrombosthenin-antibodies interfere with clot retraction (24,133). For others it is more related to the ATP-splitting enzyme in the membrane of sarcoplasmic reticulum, functioning as a Ca^{++}-pump (110). This latter hypothesis relates platelet alterations to the activation of the contractile system by interference with a "relaxing factor" (cf. 47).

The contractile system of platelets

It seems plausible that thrombosthenin plays an essential part in platelet activity. Release could be mediated by it, and its participation in morphological changes, not to speak of contractile manifestations, is obvious. Thrombosthenin is closely related to muscle actomyosin, and even more so to comparable proteins in other non-muscular cells (cf. 9,46,11,10,6,8). There can be no doubt that thrombosthenin is located mainly within the cells, as shown by its superprecipitation in glycerol-extracted cells (fig 1, 2a and b). Whether it is in fact accessible from the outside, must be subject to further studies.

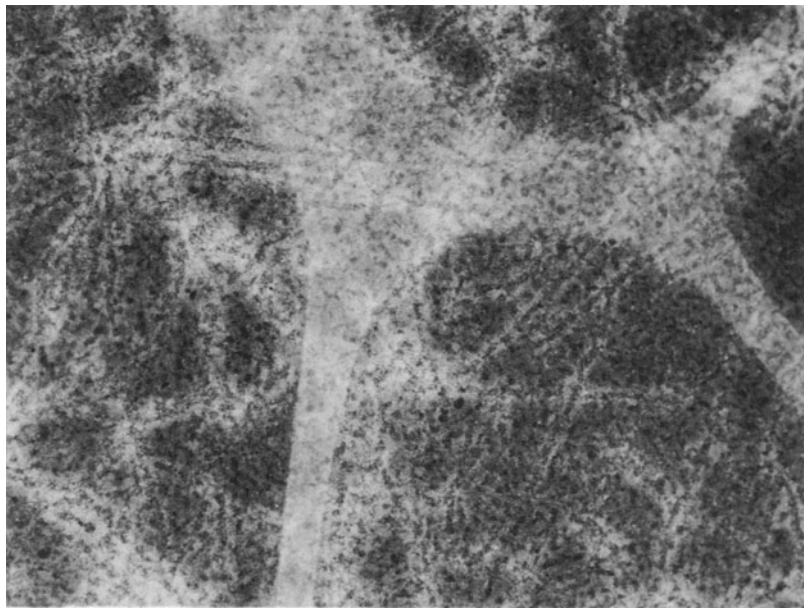

Fig. 1. Electron-micrograph of isolated actin-moiety of thrombosthenin (thrombosthenin A; F-form) in negative contrast staining. Long flexible threads show nodular substructure. Light background is carrier material for preparation. 1 : 200 000 (Courtesy of Drs. Bettex-Galland and Weibel.)

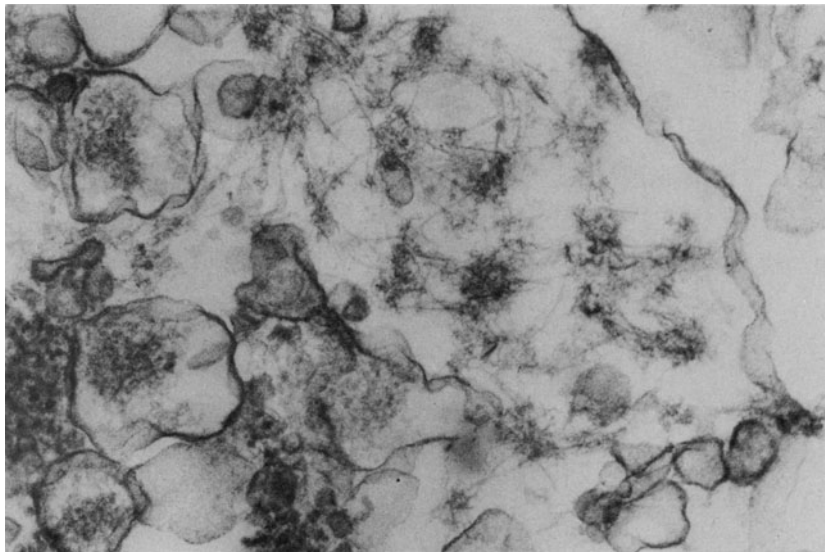

Fig. 2a. Glycerol extracted platelets showing precipitated thrombosthenin in cytoplasm; long threads of thrombosthenin A prevail.

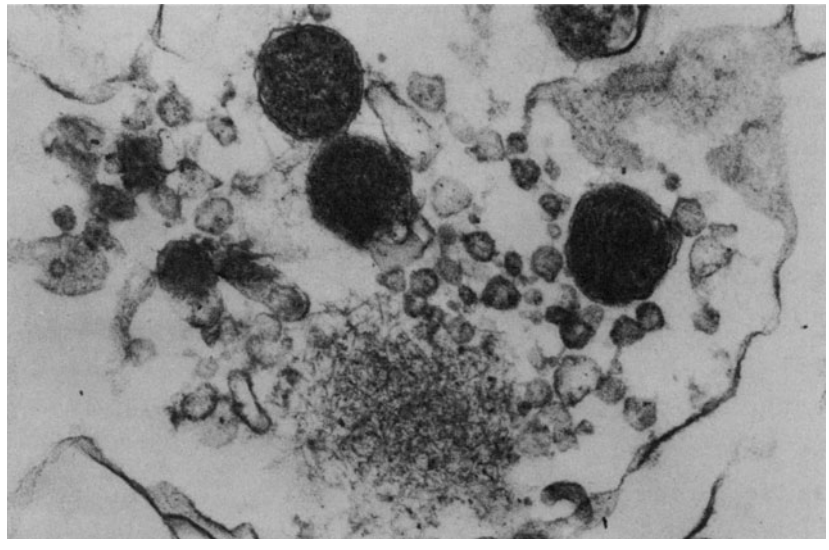

Fig. 2b. Superprecipitated thrombosthenin after addition of ATP to glycerol-extracted platelets. Contracted protein has accumulated in one site. Thrombosthenin A has largely disappeared in masses of stiff thrombosthenin M - polymers. Both pictures 1 : 62 000. (Courtesy of Drs. Bettex-Galland and Weibel.)

CONCLUSION

Platelets are metabolically very active cells and susceptible to a variety of external stimuli which convert them from an inert, circulating form to a transient, activated form which shows metabolic and morphological alterations, release reaction and which tends first to reversible and then to irreversible aggregation.

The formation of the hemostatic plug, the most prominent manifestation of the normal platelet, consists in the superposition of many aspects of platelet activity:

- the adherence to connective tissue
- the transformation into an activated form under the influence of collagen (plus Ca^{++}), and extrinsic thrombin
- the release reaction with liberation of adenosine nucleotides, biogenic amines, Ca^{++}, K^{+}, and PF 4
- the unmasking of PF 3, followed by the formation of intrinsic thrombin
- the superposition of the effects of ADP, biogenic amines, and thrombin on new platelets, leading to the growth of an aggregate
- the activation of the contractile system, resulting in morphological changes, and in the consolidation of the aggregate
- excessive thrombin formation, leading to plasma fibrin deposition.

Several, although not all, aspects of this complex scheme of reactivity have been dealt with. They should be sufficient to show that the smallest blood cell is by no means the simplest.

REFERENCES

1 Baker, R.V., H. Blaschko, G.V.R. Born: The isolation from blood platelets of particles containing 5-hydroxytryptamine and adenosine-triphosphate. J. Physiol. (Lond.) 149, 55 (1959)

2 Balogh, K.,jr.,R.B. Cohen: Histochemical demonstration of diaphorases and dehydrogenases in normal human leukocytes and platelets. Blood 17, 491 (1961)

3 Barnhart, M.I., D.C. Cress, R.C. Henry, J.M. Riddle: Influence of fibrinogen split products on platelets. Thrombos.Diathes.haemorrh. 17, 78 (1967)

4 Barton, P.G.: Sequence theories of blood coagulation re-evaluated with reference to lipid-protein interactions. Nature, Lond. 215, 1508 (1967)

5 Baumgartner, H.R., G.V.R. Born: Effects of 5-hydroxytryptamine on platelet aggregation. Nature 218, 137 (1968)

6 Bettex-Galland, M., E.F. Lüscher: Extraction of an actomyosin-like protein from human thrombocytes. Nature (Lond.) 184, 276 (1959)

7 Bettex-Galland, M., E.F. Lüscher: Studies on the metabolism of human blood platelets in relation to clot retraction. Thrombos.Diathes.haemorrh. 4, 178 (1960)

8 Bettex-Galland, M., E.F. Lüscher: Thrombosthenin - a contractile protein from thrombocytes. Its extraction from human blood platelets and some of its properties. Biochim.Biophys. Acta 49, 536 (1961)

9 Bettex-Galland, M., E.F. Lüscher: Thrombosthenin, the contractile protein from blood platelets, and its relation to other contractile proteins. Advanc. Protein Chem. 20, 1 (1965)

10 Bettex-Galland, M., H. Portzehl, E.F. Lüscher: Dissociation of thrombosthenin into two components comparable with actin and myosin. Nature (Lond.) 193, 777 (1962)

11 Bettex-Galland, M., H. Portzehl, E.F. Lüscher: Dissoziation des Thrombosthenins in seine zwei Komponenten. Untersuchung ihrer Adenosintriphosphatase-Aktivität. Helv.Chim.Acta 46, 1595 (1963)

12 Bizzozero, V.: Ueber einen neuen Formbestandteil des Blutes und dessen Rolle bei der Thrombose und Blutgerinnung. Virchows Arch. path. Anat. 90, 261 (1882)

13 Booyse, F., M.E. Rafelson,jr.: In vitro incorporation of amino acids into the contractile protein of human blood platelets. Nature (Lond.) 215, 383 (1967)

14 Booyse, F.M., M.E. Rafelson: Stable messenger RNA in the synthesis of contractile protein in human platelets. Biochim.Biophys. Acta 30, 1 (1967)

15 Booyse, F.M., M.E. Rafelson,jr.: Hypothesis: Studies on human platelets III: A contractile protein model for platelet aggregation. Blood 33, 100 (1969)

16 Born, G.V.R.: The influence of adrenaline on platelet aggregation. in: Platelets in Hemostasis; Exper. Biol. Med. 3, 71 (1968) Karger, Basel, N.Y.

17 O'Brien, J.R.: Comparison of platelet aggregation produced by seven compounds and comparison of their inhibitors. J.clin.Path. 17, 275 (1965)

18 Budtz-Olsen, O.E.: Clot retraction. Blackwell, Oxford 1951

19 Burstein, M., A. Guinand: Plaquettes et héparine. Arch. Int. Pharmacodyn. 111, 155 (1957)

20 Campbell, E.W., W.J. Small, W. Dameshek: Metabolic activity of human blood platelets. J. Lab. & Clin. Med. 47, 835 (1956)

21 Campbell, E.W., W.J. Small, E. LoPilato, W. Dameshek: Glycolytic intermediates of human platelets: Their separation and identification. Proc. Soc. Exp. Biol. & Med. 94, 505 (1957)

22 Chambers, D.A., E.W. Salzman, L.L. Neri: Characterization of Ecto-ATPase of human blood platelets. Arch. Biochem. 119, 173 (1967)

23 Chernyak, N.B.: Processes of formation and utilisation of energy in human blood platelets. Clin.chim. Acta 12, 244 (1965)

24 Cohen, I., Z. Bohak, A. de Vries, E. Katchalski: Thrombosthenin M. Purification and interaction with thrombin. European J. Biochem. 10, 388(1969)

25 Corn, M.: Effect of thrombin, ADP, connective tissue and endotoxin on platelet glycolysis. Nature Lond. 212, 508 (1966)

26 Crawford, N., M. Sutton, G.I. Horsfield: Platelets in the carcinoid syndrome: a chemical and ultrastructural investigation. Brit.J.Haemat. 13, 181 (1967)

27 Cross, M.J.: Effect of fibrinogen on the aggregation of platelets by adenosine diphosphate. Thrombos. Diathes. haemorrh. 12, 524 (1964)

28 Dastugue, G., P. Bastide, P.M. Plat: Les enzymes plaquettaires. Nouv.Rev.franç.Hemat. 6, 265 (1966)

29 Davey, M.G., E.F. Lüscher: The action of thrombin on platelet proteins, in "Diffuse Intravascular Clotting". Thrombos. Diathes. haemorrh. suppl. 20, 283 (1966)

30 Davey, M.G., E.F. Lüscher: Platelet proteins. Biochemistry of Blood Platelets, 9-22; E. Kowalski, S. Niewiarowski, Ed., Academic Press, London/N.Y. 1967

31 Davey, M.G., E.F. Lüscher: Actions of thrombin and other coagulant and proteolytic enzymes on blood platelets. Nature, Lond. 216, 857 (1967)

32 Davey, M.G., E.F. Lüscher: Effects of some coagulant snake venoms upon human platelets. Proc. 10th Congr. europ. Soc. Haemat. 2, 1118 (1967)

33 Davey, M.G. E.F. Lüscher: Release reactions of human platelets induced by thrombin and other agents. Biochim.Biophys.Acta 165, 490 (1968)

34 Davis, R.B., J.G. White: Localization of 5-hydroxytryptamine in blood platelets: on autoradiographix and ultrastructural study. Brit. J. Haemat. 15, 93 (1968)

35 Day, H.J., H. Holmsen, T. Hovig: Subcellular particles of human platelets - A biochemical and electronmicroscopic study with particular reference to the influence of fractionation techniques. Scand. J. Haemat., Suppl. 7, 1969

36 Deutsch, E.: Eine einfache Methode zur Bestimmung von Thrombocytenfaktor 4. Thrombos. Diathes. haemorrh. 4, 93 (1959)

37 McDonagh, J., R.P. McDonagh, jr., J.M. Delâge, R.H. Wagner: Factor XIII in human plasma and platelets. J. clin. Invest. 48, 940 (1969)

38 Eberth, J.C., C. Schimmelbusch: Die Blutplättchen und die Blutgerinnung. Virchows Arch. path. Anat. 101, 201 (1885)

39 Eberth, J.C., C. Schimmelbusch: Experimentelle Untersuchungen über Thrombose. Virchows Arch. path. Anat. 103, 39 (1886)

40 Farbiszewski, R., S. Niewiaroski, A. Poplawski: Purification and separation of platelet factor 2 (fibrinogen activating factor) and of platelet factor 4 (antiheparin factor). Biochim. Biophys. Acta 115, 397 (1966)

41 Farbiszewski, R., S. Niewiarowski, K. Worowski, B. Lipinski: Release of platelet factor 4 in vivo durin intravascular coagulation and in thrombotic states. Thrombos. Diathes. haemorrh. 19, 578 (1968)

42 Gaarder, A., J. Jonsen, S. Laland, A. Hellem, P.A. Owren: Adenosine diphosphate in red cells as a factor in the adhesiveness of human blood platelets. Nature (Lond.) 192, 531 (1961)

43 Ganguly, P.: Studies on platelet proteins. II. Effect of thrombin. Blood 33, 590 (1969)

44 Ganguly, P., R. Moore: Studies on human platelet proteins. Clin. chim. Acta. 17, 153 (1967)

45 Glynn, M.F., R. Herren, J.F. Mustard: Adherence of latex particles to platelets. Nature, Lond. 212, 79 (1966)

46 Grette, K.: Studies on the mechanism of thrombin-catalyzed hemostatic reactions in blood platelets. Norwegian Monographs on Med. Science, 1962

47 Grette, K.: Relaxing factor in extracts of blood platelets and its function in the cells. Nature (Lond.) 198, 488 (1963)

48 Gross, R.: Metabolic aspects of normal and pathological platelets. in: Blood platelets; S.A. Johnson, R.W. Monto, R.W. Rebuck, R.C. Horn,jr. eds., Little, Brown & Co., Boston, 1961 p. 407.

49 Haslam, R.J.: Role of adenosine diphosphate in the aggregation of human blood platelets by thrombin and by fatty acids. Nature (Lond.) 202, 765 (1964)

50 Hayem, G.: Du sang et des ses altérations anatomiques. G. Masson, Paris 1889

51 Hemker, H.C., M.J.P. Kahn: Reaction sequence of blood coagulation. Nature, Lond. 215, 1201 (1967)

52 Holmsen, H.: Incorporation in vitro of P^{32} into blood platelet acid-soluble organophosphates and their chromatographic identification. Scand. J. clin. & Lab. Invest. 17, 230 (1965)

53 Holmsen, H., H.J. Day: Thrombin-induced platelet release reaction and platelet lysosomes. Nature (Lond.) 219, 760 (1968)

54 Holmsen, H., M.C. Rozenberg: Adenine nucleotide metabolism of blood platelets. I. Adenosine kinase and nucleotide formation from exogenous adenosine and AMP. Biochim.Biophys. Acta 155, 326 (1968)

55 Holmsen, H., M.C. Rozenberg: Adenine nucleotide metabolism of blood platelets. III Adenine phosphoribo syl transferase and nucleotide formation from exogenous adenine. Biochim.Biophys. Acta 157, 266 (1968)

56 Holmsen, H., H.J. Day, M.A. Pimentel: Adenine nucleotide metabolism of blood platelets. V. Subcellular localization and kinetics of some related enzymes. Universitetsforlaget, Oslo, 1969

57 Holmsen, H., H.J. Day, E. Storm: Adenine nucleotide metabolism of blood platelets. VI Subcellular localization of nucleotide pools with different functions in the platelet release reaction. Universitetsforlaget, Oslo, 1969

58 Holmsen, H., H.J. Day, H. Stormorken: The blood platelet release reaction. Scand.J. Haemat., Suppl. 8m 1969

59 Hovig, T.: Aggregation of rabbit blood platelets produced in vitro by saline "extract" of tendous. Thrombos.Diathes.haemorrh.(Stuttg.) 9, 248 (1963)

60 Hugues, J.: Accolement des plaquettes au collagène C.R. Soc. Biol. (Paris) 154, 866 (1960)

61 Hugues, J., J. Roskam: The clumping of platelets with collagen. C.R. Soc. biol. (Paris) 154, 866 1960

62 Jerushalmy, Z., M.B. Zucker: Some effects of fibrinogen degradation products (FDP) on blood platelets. Thrombos.Diathes.haemorrh. 15, 413 (1966)

63 Jones, B.M.: A unifying hypothesis of cell adhesion Nature (Lond.) 212, 362 (1966)

64 Karpatkin, S., R.M. Langer: Biochemical energetics of simulated platelet plug formation. Effect of thrombin, adenosine diphosphate and epinephrine on intra- and extracellular adenine nucleotide kinetics. J. clin. Invest. 47, 2158 (1968)

65 Käser-Glanzmann, R., E.F. Lüscher: The mechanism of platelet aggregation in relation to hemostasis. Thrombos.Diathes.haemorrh. 7, 480 (1962)

66 Kiesselbach, T.H., R.H. Wagner: Fibrin-stabilising factor: Thrombin-labile platelet protein. Amer. J. Physiol. 211, 1472 (1966)

67 Kopec, M., A. Budzynski, J. Stachurska, Z. Wegrzynowicz, E. Kowalski: Studies on the mechanism of interference by fibrinogen degradation products (FDP) with the platelet function. Role of fibrinogen in the platelet atmosphere. Thrombos. Diathes.haemorrh. 15, 476 (1966)

68 Larrieu, M.J., S. Inceman, V. Marder: Action des produits de dégradation du fibrinogène sur les fonctions plaquettaires. Nouv. Rev. franç. Hémat. 7, 691 (1967)

69 McLean, J.R., R.E. Maxwell, D. Hertler: Fibrinogen and adenosine diphosphate-induced aggregation of platelet. Nature Lond. 202, 605 (1964)

70 Lewis, J.H., W.L. Bayer, J.H. Wilson, I.L.F. Szeto: Factor XIII in platelets. Abstr.3rd. Conf.on Platelets, Oak Ridge; Blood 30, 543 (1967)

71 Lipinski, B., Z. Wegrzynowicz, A.Z. Budzynski, M. Kopec, Z.S. Latallo, E. Kowalski: Soluble unclottable complexes formed in the presence of fibrinogen degradation products (FDP) during the fibrinogen-fibrin conversion and their potential significance in pathology. Thrombos.Diathes. haemorrh. 17, 65 (1967)

72 Loder, P.B., J. Hirsh, G.C. de Gruchy: The effect of collagen on platelet glycolysis and nucleotide metabolism. Brit.J.Haemat. 14, 563 (1968)

73 Loeber, J.: Zur Physiologie der Blutplättchen. Pflügers Arch. ges. Physiol. 140, 281 (1911)

74 Lüscher, E.F.: Ein fibrinstabilisierender Faktor aus Thrombozyten. Schweiz.Med.Wschr. 87, 1220 (1957)

75 Lüscher, E.F.: Thrombocytenfaktoren. Ergebn. Physiol. 50, 1 (1959)

76 Macmillan, D.C.: Secondary clumping effect in human citrated platelet-rich plasma produced by adenosine diphosphate and adrenaline. Nature, Lond. 211, 140 (1966)

77 Marcus, A.J., M.B. Zucker: The physiology of blood platelets. Grune & Stratton, New York, London, 1965

78 Marcus, A.J., D. Zucker-Franklin, L.B. Safier, H.L. Ullman: Studies on human platelet granules and membranes. J. clin. Invest 45, 14 (1966)

79 Maupin, B.: Blood platelets in man and animals (Vol. 1 and 2) Pergamon, Oxford, 1969

80 Maupin, B., J. Saint-Blancard: Introduction à l'étude des enzymes plaquettaires. Nouv. Rev. franç. Hémat. 6, 255 (1966)

81 Mills, D.C.B., D.P. Thomas: Blood platelet nucleotides in man and other species. Nature, Lond. 222, 991 (1969)

82 Mills, D.C.B., I.A. Robb, G.C.K. Roberts: The release of nucleotides, 5-hydroxytryptamine and enzymes from human blood platelets during aggregation. J. Physiol. (Lond.) 195, 715 (1968)

83 Morawitz, P.: Die Chemie der Blutgerinnung. Ergebn. Physiol. 4, 307 (1905)

84 Moser, K., K. Lechner, H. Vinazzer: A hitherto not described enzyme defect in thrombasthenia: glutathionreductase deficiency. Thrombos.Diathes. haemorrh. 19, 46 (1968)

85 Movat, H.Z., J.F. Mustard, N.S. Taichmann, T. Urinhara: Platelet aggregation and release of ADP, serotonin and histamine associated with phagocytosis of antigen-antibody complexes. Proc. Soc. exper. Biol. & Med. 120, 232 (1965)

86 Mueller-Eckhardt, Ch., E.F. Lüscher: Immune reactions of human blood platelets. I. A comparative study on the effects of heterologous antiplatelet antiserum, of antigen-antibody complexes, of aggregated gamma-globulin and of thrombin on platelets. Thrombos.Diathes.haemorrh. 20, 155 (1968)

87 Mueller-Eckhard, Ch., E.F. Lüscher: Immune reactions of human blood platelets. II. The effect of latex particles coated with gamma globulin in relation to complement activation. Thrombos. Diathes. haemorrh. 20, 168 (1968)

88 Mürer, E.H.: Release of calcium from blood platelets induced by thrombin. (unpublished)

89 Mürer, E., A.J. Hellem, M.C. Rozenberg: Energy metabolism and platelet function. Scand.J. clin. Lab. Invest. 19, 280 (1967)

90 Nachman, R.L., A.J. Marcus: Immunological studies of proteins associated with the subcellular fractions of thrombasthenic and afibringenaemic platelets. Brit.J. Haemat. 15, 181 (1968)

91 Nachman, R.L., A.J. Marcus, L.B. Safier: Platelet thrombosthenin: Subcellular localization and function. J. clin. Invest. 46, 1380 (1967)

92 Niewiarowski, S., D.P. Thomas: Platelet aggregation by ADP and thrombin. Nature 212, 1544 (1966)

93 Niewiarowski, S., A. Poplawski, B. Lipinski, R. Farbiszewski: The release of platelet clotting factors during aggregation and viscous metamorphosis. Exper.Biol.Med. 3, 121, Karger, Basel/ New York 1968

94 Onaka, M.: Ueber Oxydationen im Blut. Hoppe-Seylers Z. physiol. Chem. 71, 193 (1911)

95 da Prada, M., A. Pletscher, J.P. Tranzer, H. Knuchel: Subcellular localization of 5-hydroxytryptamine and histamine in blood platelets. Nature, Lond. 216, 1315 (1967)

96 Puszkin, E., Z. Jerushalmy: Effect of connective tissue and collagen on platelet lactate. Proc. Soc. exper. Biol. N.Y. 129, 346 (1968)

97 Roskam, J.: Proposals for certain definitions in haemostasis and thrombosis, in "Diffuse Intravascular Coagulation". Thrombos. Diathes. haemorrh., suppl. 20, 317 (1966)

98 Salzman, E.W., D.A. Chambers, L.L. Neri: Possible mechanism of aggregation of blood platelets by adenosine diphosphate. Nature (Lond.) 210, 167 (1966)

99 Sano, I., Y. Kakimoto, K. Tanizuchi, M. Takesada: Active transport of epinephrine into blood platelets. Amer.J. Physiol. 197, 81 (1959)

100 Schmitz, H., T. Schleipen, R. Gross: Freie Nucleotide in normalen menschlichen Blutplättchen. Klin. Wschr. 40, 13 (1962)

101 Schulz, H.: Thrombocyten und Thrombose im elektronenoptischen Bild. Springer, Berlin, Heidelberg, New York, 1968

102 Scott, R.B.: Activation of glycogen phosphorylase in blood platelets. Blood 30, 321 (1967)

103 Siegel, A.: Die Isolierung und Charakterisierung der subzellulären Strukturen menschlicher Thrombocyten. Diss., Ph.D., University of Berne, 1966

104 Siegel, A., E.F. Lüscher: Non-identity of the α-granules of human blood platelets with typical lysosomes. Nature (Lond.), 215, 745 (1967)

105 Sixma, J.J., J.J. Geuze: Degranulation of human platelets during ADP-induced aggregation. Vox Sang. 15, 309 (1968)

106 Solatunturi, E., M.K. Paasonen: Intracellular Distribution of monoamine oxidase, 5-hydroxytryptamine and histamine in blood platelets of rabbits. Ann. Med. exp. Fenn. 44, 427 (1966)

107 Solum, N.O.: Platelet aggregation during fibrin polymerization: Scandinav. J.clin. & Lab. Invest. 18, 577 (1966)

108 Solum, O., H. Stormorken: Influence of fibrinogen on the aggregation of washed human blood platelets induced by adenosine diphosphate, thrombin, collagen and adrenaline. Scand. J. clin. Lab. Invest. 17, Suppl. 84 170 (1965)

109 Spaet, T.H., J. Cintron, M. Spivak: Some properties of the platelets-connective tissue mixed agglutination reaction. Proc. Soc. exper. Biol. N.Y., III, 292 (1962)

110 Statland, B.E., B.M. Heagan, J.G. White: Uptake of calcium by platelet relaxing factor. Nature, Lond. 223, 521 (1969)

111 Steiner, M., M. Baldini: Protein synthesis in aging blood platelets. Blood 33, 628 (1969)

112 Stormorken, H.: The release reaction of secretion. A general basic phenomenon related to phagocytosis/pinocytosis. Scand.J.Haemat. Suppl. 9, 1969

113 Thomas, D.P.: Effect of catecholamines on platelet aggregation caused by thrombin. Nature, Lond. 215, 298 (1967

114 Tranzer, J.P., H.R. Baumgartner: Filling gaps in the vascular endothelium with blood platelets. Nature, 216, 1126 (1967)

115 Vainer, H., R. Wattiaux: Glycogen synthetase activity in blood platelets. Nature, Lond. 217, 951 (1968)

116 Vainer, H., D. Depernet, J. Caen: Etude des nucleotides, des ATP-ases et des adenylatekinases dans les plaquettes humaines, fraîches, congelées et lyophilisées. Coagulation 1, 211 (1968)

117 Vainer, H., D. Depernet, C. Cousin, J. Caen: Activités phosphohydrolytiques des plaquettes humaines congélées. Nouv.Rev.franç.Hemat. 6, 291 (1966)

118 Vethamany, V.G., S.S. Lazarus: Ultrastructural localization of Mg^{2+} dependent Dinitrophenol-stimulated adenosine triphosphatase in human blood platelets. J. Histochem. Cytochem. 15, 267 (1967)

119 Waller, H.D., G.W. Löhr, F. Grignani, R. Gross: Ueber den Energiestoffwechsel normaler menschlicher Thrombocyten. Thrombos. Diathes. haemorrh. 3, 520 (1959)

120 Warburg, O.: Untersuchungen über die Oxydations-Prozesse in Zellen. Münch. med. Wschr. 1911, 289

121 Warshaw, A.L., L. Laster, N.R. Shulman: The stimulation by thrombin of glucose oxication in human platelets. J. clin. Invest. 45, 1923 (1966)

122 Weber, E., H. Mondt: Verteilung und Beeinflussung von Metaboliten in fraktionierten Homogenaten aus Blutplättchen. Klin. Wschr. 45, 165 (1967)

123 Weissbach, H., D.F. Bogdansky, S. Udenfriend: Binding of serotonin and other amines by blood platelet. Arch.Biochem. 73, 492 (1958)

124 Wilner, G.D., H.G. Nossel, E.C. LeRoy: Aggregation of platelets by collagen. J. clin. Invest. 47, 2616 (1968)

125 Wilson, P.A., G.P. McNicol, A.S. Douglas: Effect of fibrinogen degradation products on platelet aggregation. J. clin. Pathol. 21, 147 (1968)

126 Wojtecka-Lukasik, E., I. Sopata, J. Wize, M. Skonieczna, I. Niedzwiecka-Namyslowska, E. Kowalski: Adhesion of platelets to collagen devoid of telopeptides. Thrombos.Diathes. haemorrh. 18, 76 (1967)

127 Wolfe, S.M., N.R. Shulman: Adenyl cyclase activity in human platelets. Biochem. Biophys. Res. Comm. 35, 265 (1969)

128 Wurzel, H., T. McCreary, L. Baker, L. Gumeman: Glucose - 6 - Phosphate dehydrogenase activity in platelets. Blood 17, 314 (1961)

129 Zieve, P.D., W.B. Greenough: Adenyl cyclase in human platelets: Activity and responsiveness. Biochem.Biophys. Res. Comm. 35, 462 (1969)

130 Zucker, M.B., J. Borrelli: Platelet viscous metamorphosis produced by connective tissue particles. Fed. Proc. (Abstr.) 20/1, 61 (1961)

131 Zucker, M.B., J. Peterson: Serotonin, platelet factor 3 activity, and platelet aggregating agent released by adenosine diphosphate. Blood 30, 556 (1967)

132 Zucker, M.B., J. Peterson: Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet function by acetylsalicilic acid ingestion. Proc. Soc. exper. Biol. Med., N.Y. 127, 547 (1968)

133 Zucker-Franklin, D., R.L. Nachman, A.J. Marcus: Ultrastructure of thrombosthenin, the contractile protein of human blood platelets. Science 157, 945 (1967)