Recent Advances in
BLOOD COAGULATION

EDITED BY

L. POLLER

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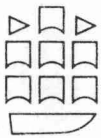

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Preface

The pace of research and development in blood coagulation has prompted a further volume in this series at a shorter time interval. The success of the previous volumes, necessitating reprinting, has been gratifying and appears to have firmly established the series in the blood coagulation literature.

The view of all the contributors has always been that the theme of topicality should prevail with emphasis throughout on recent developments. The subjects for review in this volume are those which were felt to be important and in which there have been major recent advances.

The editor is again extremely indebted to the individual chapter authors, not only for the high quality of their contributions but also for the great care with which they prepared their manuscripts and compiled their references. This again made editing a relatively light and pleasant duty.

Manchester, 1981

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Contents

1. Platelet structure, biochemistry and physiology

William L. Nichols Jonathan M. Gerrard Paul Didisheim

All this taken together makes it more apparent that blood coagulation is under the direct influence of the blood platelet. Thus, in the future in studying the function and changes of blood, this constant and well represented new constituent must always be taken into account.

*Giulio Bizzozero, 1882**

During the nearly 100 years since Bizzozero distinguished the blood platelets and their involvement in blood clotting, much has been learned about these cells and their functions. Qualitative platelet defects are now increasingly recognized in patients with bleeding or thrombotic problems. The study of such platelets has provided new understanding of platelet function and of the molecular mechanisms of hemostasis.

In this chapter we review major recent advances in platelet ultrastructure, biochemistry and physiology, and then in Chapter 2 we focus on the pathophysiology and diagnosis of the congenital and acquired qualitative platelet disorders, as these are understood at the beginning of the 1980s.

The burgeoning literature on platelets has become vast. In reviewing this literature, we have selectively synthesized that which we think contributes importantly toward a mechanistic understanding of processes important in platelet hemostatic function and dysfunction. Limitations of space and time preclude extensive bibliographic citations, and also preclude review of such topics as megakaryopoiesis and thrombopoiesis, platelet kinetics, blood rheology and platelet interactions with other formed elements of the blood, quantitative disorders of platelets (e.g. 'pure' thrombocytopenias unassociated with recognized qualitative abnormalities), platelet storage and transfusion, or platelet involvement in immune, inflammatory or neoplastic processes.

PLATELET STRUCTURE, BIOCHEMISTRY AND PHYSIOLOGY

General structure and function of blood platelets

The resting cell

Blood platelets in the quiescent state are small, anucleate discoid cells, much like miniature versions of discuses thrown by athletes (Fig. 1.1). In human blood, the platelets normally number 130 000 to 400 000 per microliter, have an average diameter of 2–3 microns and an average volume of about 8 cubic microns (Karpatkin, 1977; Stahl et al, 1978). The outside cell surface is generally smooth, with occasional pockmarked indentations where channels of the surface connected canalicular system (SCCS) exit. Inside the cell, organelles including dense granules, alpha granules,

* Quoted by Henry (1977)

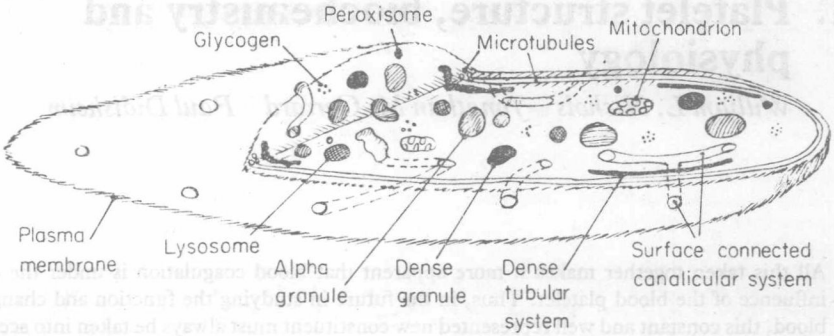


Fig. 1.1 Diagrammatic representation of an unactivated human platelet. In the resting cell, shown sectioned through the sagittal and equatorial planes, granules including dense granules, α-granules, lysosomes and peroxisomes are randomly distributed throughout the cell. Mitochondria and glycogen particles can also be seen. A microtubule band encircles the cell just beneath the plasma membrane. The surface-connected canalicular system and the dense tubular system are prominent in the region near the microtubule band.

lysosomes, peroxisomes, and mitochondria are randomly distributed. Also scattered throughout the cell cytoplasm are glycogen granules. In the plane of the largest diameter, a bundle of microtubules curves around just beneath the cell membrane, travelling the entire circumference of the cell. Two membrane systems weave throughout the cell interior. The SCCS, an invagination of the plasma membrane, contains channels which are continuous with the extracellular space. The dense tubular system (DTS), a smooth endoplasmic reticulum, occurs particularly in close association with the SCCS, and with the circumferential microtubule band. Contractile proteins exist in the cell cytoplasm, but these proteins are not easily seen in the resting cell, in part because of a dense intracellular matrix, and probably also because they exist to a considerable degree in a depolymerized, non-filamentous form. Resting or unactivated platelets are normally nonadherent to each other or to endothelialized vascular surfaces, but can be easily excited by a variety of stimuli to become sticky and activated.

Nomenclature of platelet activation phenomena

Platelet '*activation*' is a loosely defined term implying significant functional, biochemical and/or structural alteration of platelets, not found in the unstimulated and unexcited state. Such activation ordinarily requires metabolically intact platelets, and is usually defined operationally by one or more of the following terms. *Adhesion* refers to platelet attachment to the wall of a blood vessel or foreign surface. *Shape change* or viscous metamorphosis represents loss of the resting discoid shape with spherical transformation of the platelet followed by pseudopodial extension. *Aggregation* indicates non-immunologic cohesion of activated, sticky platelets to one another, while *agglutination* is a term reserved for immunologic clumping. *Release reaction* refers to the secretion by platelets of certain substances stored in intracellular organelles. This reaction usually follows one or more of the activation processes noted above, and has been subclassified by Holmsen (1975) into sequential stages (release I and II) in which with increasing stimulus strength, the contents of different storage

organelles (α-granules and dense granules, then lysosomes) are progressively extruded. Platelet *procoagulant activities* are mostly latent in resting platelets, but become manifest with platelet activation processes, serving ultimately to accelerate the generation of thrombin, and the conversion of fibrinogen to fibrin. *Primary hemostasis* encompasses the interaction of platelets with blood vessels and certain coagulation factors to form a *hemostatic plug* (reviewed in the chapter by Sixma), and is sometimes conceptually distinguished from 'secondary hemostasis' which mainly involves plasmatic coagulation and fibrin clot formation. *Platelet retention* reflects the progressive numerical diminution of the platelet content of whole blood exuding from a small wound or passing across a foreign surface. This phenomenon is thought to result from several platelet activation processes including adhesion, shape change, aggregation and release reactions.

Structural changes with cell activation

Major activation processes in platelets include: (1) adhesion of these cells to sites of vessel wall injury; (2) cohesion of platelets into cell aggregates (aggregation); (3) changes in cell shape with extension of pseudopods and centralization of granules; and (4) labilization of granule contents by fusion of granule and plasma membranes so as to connect the granule interior to the channels of the SCCS. These four processes can occur separately from one another, although usually one or more occur together. Thus, platelets initially adhering to sites of vessel wall injury are frequently discoid, but later shape change occurs and cell aggregates form (Baumgartner and Muggli, 1976). Platelet-platelet stickiness of discoid cells can occur during the first wave of epinephrine-induced aggregation. Usually shape change precedes aggregation as when ADP, thrombin, collagen or arachidonic acid is stirred with these cells to stimulate them.

With a change in cell shape and granule centralization (Fig. 1.2a), considerable polymerization of actin occurs, and microfilaments are visible principally in three locations in the cell: in the pseudopods, surrounding the centralized granules in close association with the microtubules, and as a central contractile gel in degranulated platelets (Gerrard & White, 1976). In cell aggregates (Fig. 1.2b), granules move to the center of the aggregate more often than to the center of the individual cells (Gerrard et al, 1979a). Thus a coordination exists between proteins involved in platelet stickiness and aggregation, and those involved in granule centralization. Granule secretion appears to involve both labilization of granules and granule centralization (Gerrard et al, 1977a).

The platelet plasma membrane

The plasma membrane represents the site of platelet interactions with the external environment, and is intimately involved in the control or generation of the many specialized functional properties of platelets. Unique morphologic features of the platelet plasma membrane, noted in the previous section, include numerous 'intracellular' invaginations through pores to form the SCCS, effectively providing for a relative excess of membrane surface, and for close apposition of internal organelles to the external surface. Techniques for isolating platelet plasma membranes have been the subject of reviews (Barber et al, 1971; Crawford & Taylor, 1977; Sixma & Lips, 1978), and it should be noted that most platelet plasma membrane preparations may

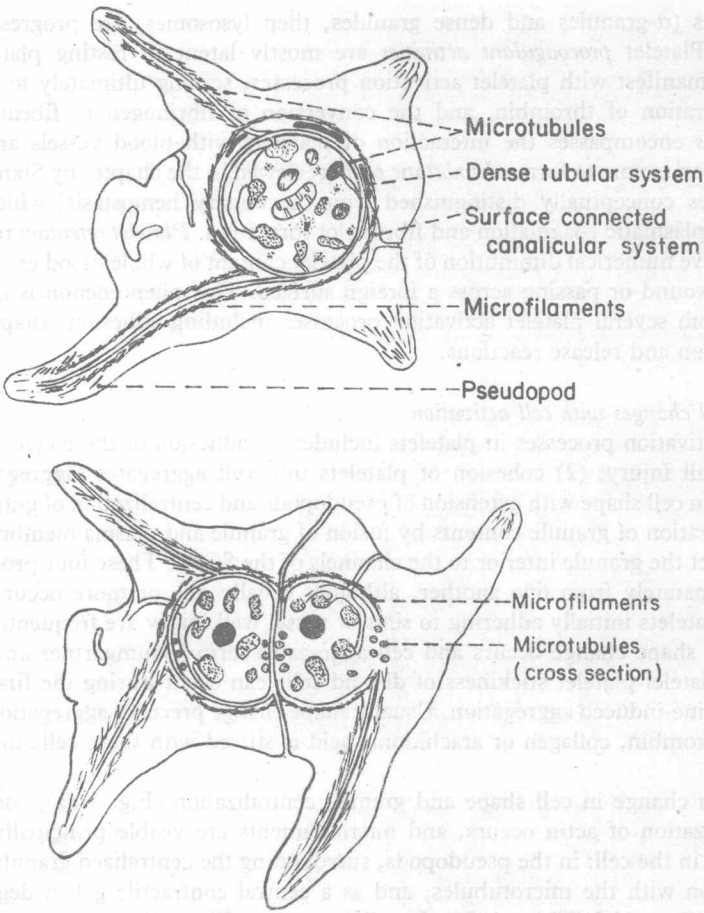


Fig. 1.2(a) A single activated platelet. In an activated cell which is not attached to other cells, pseudopods containing microfilaments are visible, and granules are centralized. Surrounding the centralized granules are microfilaments and microtubules. The surface connected canalicular system and dense tubular system continue a close association with the microtubules and centralizing granules.

(b) Two attached activated platelets. When activated cells are attached to one another, granule centralization now occurs toward the center of the small aggregate.

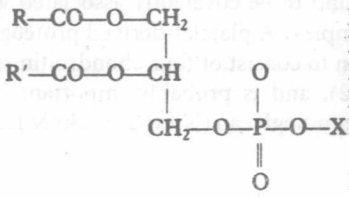
also contain some components from intraplatelet structures. Recent studies have discerned a number of unique molecular features of the platelet plasma membrane systems, and elucidate some biochemical mechanisms important for specific platelet functions.

Lipids

Human platelet membrane preparations contain about 35 per cent lipids, 8 per cent carbohydrates, and 57 per cent proteins by weight (Crawford & Taylor, 1977; Shattil & Cooper, 1978). The bulk of the membrane lipid bilayer is composed of phospho-

lipids (65 to 75 per cent by weight), while cholesterol comprises most of the neutral lipids (20 to 25 per cent), and glycolipids (2 to 5 per cent) account for almost all the remainder. On a molar basis membrane cholesterol is about half the phospholipid content. Cholesterol is not synthesized by human platelets, but can be readily incorporated into platelet membranes in vitro and probably in vivo from plasma lipoproteins (Colman, 1978; Shattil & Cooper, 1978; Stuart et al, 1980). Increased membrane cholesterol content renders the lipid bilayer more rigid (less fluid), and seems associated with the hyperreactivity of such platelets to various stimuli.

Membrane glycerophospholipids can be synthesized by platelets, using glycerol to form a three carbon backbone to which are attached (in most cases) one phosphate ester and two fatty acids:



Phosphatidylcholine: X = Choline
Phosphatidylserine: X = Serine
Phosphatidylinositol: X = Inositol
Phosphatidylethanolamine: X = Ethanolamine
Phosphatidic Acid: X = Hydrogen

R, R': = Hydrocarbon chain of fatty acid

Lysophospholipids lack one of the two fatty acid side chains.

The fatty acid moieties of membrane phospholipids can be saturated with hydrogen or unsaturated, the latter being mainly derived from essential fatty acids in the diet. The polyunsaturated fatty acid, arachidonic acid (20 carbons and 4 double bonds, 20:4), is one of the most abundant fatty acids in platelet membranes (Marcus, 1978). In non-activated platelets this fatty acid exists almost exclusively esterified into the phospholipids; phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidylcholine (PC) mainly attached to the middle carbon of the glycerol backbone (Marcus, 1978; Shattil & Cooper, 1978). Liberation of free arachidonic acid from platelet membrane phospholipids by specific lipases is believed to be a major control point of the platelet prostaglandin metabolic pathways, ultimately accelerating or limiting the generation of thromboxanes, potent mediators of platelet activation. (Prostaglandin metabolism is reviewed more fully in a subsequent section.)

There is reasonable evidence for asymmetric distribution of certain phospholipid species on the two sides of platelet membrane lipid bilayers (Shattil & Cooper, 1978; Zwaal, 1978; Schick, 1979). The preferential inside surface exposure of the acidic phospholipids PE, PS and PI in membrane systems of non-activated platelets is thought to be relevant to platelet prostaglandin metabolism. With platelet activation, PE and PS may become more externally exposed (Shattil & Cooper, 1978; Bevers et al, 1979; Schick, 1979) thus providing a catalytically important lipid surface (platelet procoagulant activity) for the generation of activated plasmatic coagulation factors.

Glycolipids contain about one-third of the platelet membrane carbohydrates (Glöckner et al, 1978; Schick, 1979), and probably are largely exposed on the external membrane surface, as are the glycoproteins. Because many glycolipids contain sialic acid, it is likely that they contribute significantly to the relatively large negative surface charge of the platelet. Other functions of platelet membrane glycolipids are unknown, but some components might serve as membrane antigens in a fashion

analogous to the erythrocyte ABH and Ii systems (Dejter-Juszynski et al, 1978; Watanabe et al, 1979).

Carbohydrates

The platelet glycocalyx or external plasma membrane coat is rich in carbohydrates which, in addition to the glycolipid contributions, are also provided by glycosaminoglycans or mucopolysaccharides, and by glycoproteins. The principal platelet glycosaminoglycan is chondroitin 4-sulfate (Hagen, 1972; Ward & Packham, 1979), a releasable surface component of both platelet plasma membranes and storage granule membranes. Functions of cell surface glycosaminoglycans are largely unknown, but they are thought to be important in cell-cell and cell-surface interactions (Lindahl & Höök, 1978). Most glycosaminoglycans are found to be covalently associated with a specific core protein to form a proteoglycan complex. A platelet-derived proteoglycan carrier of platelet factor 4 (PF4) has been shown to consist of four chondroitin sulfate chains and a core protein (Barber et al, 1972), and is probably important in the interaction of PF4 with heparin, another glycosaminoglycan (Niewiarowski & Levine, 1979; Lindahl & Höök, 1978).

Glycoproteins

The externally exposed plasma membrane proteins of platelets are largely, if not exclusively, glycoproteins, and generally are intrinsically bound in the lipid bilayer. Current analytical techniques have permitted identification of up to seven 'major' or relatively abundant externally exposed platelet membrane glycoproteins. Other 'minor' or less abundant surface glycoproteins are now recognized (Phillips & Agin, 1977b; Andersson & Gahmberg, 1978; McGregor et al, 1979; Nichols et al, 1979), so that in total, there are at least 15 discernible human platelet membrane glycoprotein species. These cell surface glycoproteins are believed to play important and diverse roles in platelet function, including mediation of cell-cell and cell-surface interactions, generation of surface specificity and antigenicity, activities as message receptors and transducers, and functions in membrane transport phenomena. In addition, loss of surface sialic acids or glycoproteins appears to be a principal mechanism of platelet senescence in vivo (Greenberg et al, 1979b).

Table 1.1 outlines some characteristics of the major platelet membrane glycoproteins. It should be noted that, as yet, there is no universally employed nomenclature for these glycoproteins. This reflects that relatively few platelet membrane glycoproteins have been isolated in sufficient quantity and homogeneity to permit comprehensive biochemical identification and characterization.

Among the components of the glycoprotein I (GP I) group, a readily solubilized or proteolytically liberated component, 'glycocalicin' or GP Is (Okumura & Jamieson, 1976; Nurden & Caen, 1978), has been isolated and partially characterized. It is rich in sialic acid, and seems implicated in platelet interaction with thrombin (Ganguly & Gould, 1979; Okumura et al, 1978), and in Willebrand factor-mediated adhesion of platelets to each other and to subendothelial surfaces (Okumura & Jamieson, 1976; Baumgartner et al, 1977). Glycocalicin shares some structural and functional characteristics with the integral membrane protein GP Ib, but recent studies suggest that these proteins are non-identical (Cooper et al, 1979; Moroi et al, 1979; Nachman et al, 1979; Solum et al, 1979). Both GP Is and GP Ib are quantitatively decreased in

Table 1.1 Major human platelet membrane glycoproteins.

Nomenclature[1]	Apparent Molecular Weight[2,3]	Comments
Ia	167 000	
Ib	143 000	Decreased in Bernard-Soulier disease
Is	148 000[4]	'Glycocalicin'; Decreased in Bernard-Soulier disease
Ic[3]	134 000	Minor component; ? Glycosylated
IIa	157 000	
IIb	132 000	Decreased in Glanzmann's thrombasthenia
IIc[5]	110 000[5]	
IIIa (III)[3,4]	114 000	α-actinin; Decreased in Glanzmann's thrombasthenia
IIIb (IV)[3,4]	97 000	

[1] Nurden & Caen, 1979.
[2] Electrophoretic estimate; reduced glycopeptide.
[3] Phillips & Agin, 1977a.
[4] Okumura & Jamieson, 1976
[5] George, 1978.

platelets of patients with Bernard-Soulier disease. These platelets display defects in adhesion to the subendothelium, do not aggregate in response to ristocetin and Willebrand factor, and show diminished responsiveness to thrombin (Weiss et al, 1974; Caen et al, 1976; Okumura et al, 1978; Jamieson & Okumura, 1978). Elucidation of the biochemical and functional relationships of GP Is and GP Ib awaits isolation and characterization of the membrane bound form.

Glycoproteins IIb and IIIa are quantitatively decreased in platelets of patients with Glanzmann's thrombasthenia (Phillips & Agin, 1977c; Nurden & Caen, 1978; Jamieson et al, 1979), and the platelet-specific alloantigen $P1^{A1}$ is also deficient (Kunicki & Aster, 1978). Glycoprotein IIIa has recently been tentatively identified as platelet alpha-actinin (Gerrard et al, 1979a) and as the structural carrier of $P1^{A1}$ activity (Kunicki & Aster, 1979). Gerrard et al (1979a) have suggested that membrane bound alpha-actinin (GP IIIa) may serve as an anchor point for attachment of actin filaments to the inside of the platelet membrane, thus linking the platelet contractile apparatus to the membrane in a fashion analogous to skeletal muscle Z-band attachment sites for actin. Phillips et al (1979) have provided evidence that GP IIb and GP IIIa are involved in the direct interaction of platelets with each other during aggregation.

Alloantibodies directed against specific surface antigens missing in Bernard-Soulier platelets (GP I) or in thrombasthenic platelets (GP II or III) confer the corresponding functional defects when incubated with normal platelets (Nurden & Caen, 1978). There is, therefore, abundant evidence that platelet membrane glycoproteins are critical for certain platelet functions.

Proteins

Analysis of detergent solubilized platelet membranes by high resolution electrophoretic techniques has permitted the recognition of a large number of different protein and glycoprotein components. Some represent membrane associated components of platelet contractile proteins (actin, myosin, tropomyosin). Functional identities of most of the remainder are currently unknown, but it is likely that a substantial portion of the different species are enzymes. Among the tentatively identified membrane

Table 1.2 Platelet receptors

Ligand	Sites/Platelet[a]	Affinity (M^{-1})[a,b]	Notes[c]	Reference
ADP	1×10^5	6×10^6	HM	Nachman & Ferris, 1974
ADP	1×10^5	5×10^5	HP	Born & Feinberg, 1975
ADP	- - - - - -	5×10^6	HM	Legrand & Caen, 1976
ADP	- - - - - -	3×10^6	HM	Adler & Handin, 1979
Epinephrine	1×10^5	- - - - - -	HP	McMillan et al, 1979
Dihydroergocryptine	2×10^2	1×10^9	HP	Newman et al, 1978
Dihydroergocryptine	5×10^2	2×10^8	HP	Kaywin et al, 1978
Clonidine	1×10^2	5×10^7	HP	Shattil et al, 1979b
Serotonin	7×10^3 (2×10^3)	4×10^7 (7×10^6)	RP	Drummond & Gordon, 1975
Serotonin	1×10^3 (7×10^3)	6×10^{10} (7×10^9)	HP	Boullin et al, 1977
Collagen α1 chain	2×10^5	2×10^6	HM	Chiang & Kang, 1976
PGD_2	2×10^2	2×10^7	HP	Cooper & Ahern, 1979
PGE_1	4×10^2 (- - - - - -)	2×10^8 (5×10^5)	HP	Schafer et al, 1979
PGI_2	1×10^2 (3×10^3)	8×10^7 (1×10^6)	HP	Siegel et al, 1979
Fibrinogen	5×10^3	1×10^7	H-HP	Marguerie et al, 1979
Fibrinogen	4×10^4	1×10^7	H-HP	Bennett & Vilaire, 1979
Thrombin	3×10^2 (3×10^3)	5×10^8 (8×10^6)	H-HP	Martin et al, 1976
Thrombin	7×10^2 (6×10^4)	3×10^8 (2×10^6)	H-HM	Tam & Detwiler, 1979
Thrombin	5×10^2 (4×10^4)	3×10^8 (2×10^6)	H-HP	Tam & Detwiler, 1979
Factor V	8×10^2	3×10^8	B-BP	Tracy et al, 1979
Factor Va	9×10^2 (4×10^3)	3×10^9 (3×10^8)	B-BP	Tracy et al, 1979
Factor Xa	2×10^2	3×10^{10}	H-HP	Miletich et al, 1978
Factor Xa	4×10^2	1×10^{10}	B-BP	Dahlbäck & Stenflo, 1978
Factor VIII R:Ag	3×10^5	2×10^9	H-HP	Kao et al, 1979a

[a] Estimated to one significant digit. Lower affinity sites in parentheses.
[b] Expressed as the association or affinity constant (Ka) of the equilibrium binding of ligand to receptor. Larger values imply greater affinity.
[c] Abbreviations: HM = human platelet membranes; HP = human platelets; RP = rat platelets; H-HP = human factor and human platelets; H-HM = human factor and human platelet membranes; B-BP = bovine factor and bovine platelets.

endoenzymes, those of particular interest relate to cyclic AMP metabolism (adenylate cyclase, cAMP dependent protein kinases: Smith & Damus, 1977; Steer & Wood, 1979), prostaglandin metabolism (diglyceride lipase, phospholipase A_2: Schoene, 1978; Bell et al, 1979), and membrane transport (calcium and magnesium dependent ATPases: Crawford & Taylor, 1977). Platelet collagen-glycosyl transferases are thought to be ectoenzymes, and may be of importance in the interaction of platelet surface glycoproteins with collagen (Michaeli & Orloff, 1976; Crawford & Taylor, 1977).

Receptors

A number of platelet 'receptors' for ligands of biologic or pharmacologic importance have been identified. Table 1.2 outlines properties of the more well defined platelet receptor activities. In most cases some, but not all of the usually accepted criteria for designation of membrane binding as a 'receptor' (Cuatrecasas & Hollenberg, 1976; Mills & MacFarlane, 1976; Ryan & Lee, 1976) have been met. These platelet receptor functions are probably largely mediated by membrane proteins, as evidenced by the marked reduction of binding sites observed in several studies following partial

proteolysis of the platelet surface. With few exceptions, the binding proteins are structurally unidentified.

Although fibrinogen is required for ADP-induced platelet aggregation (Mustard et al, 1978), ADP binding to the platelet membrane does not require fibrinogen. Adenine nucleotides are thought to be of major importance in the platelet activation process, and work is in progress to isolate receptor proteins from platelet membranes (Bennett et al, 1978; Adler & Handin, 1979).

Platelet membranes actively transport serotonin, and it has been suggested that the high affinity membrane binding of serotonin may represent a receptor site, while lower affinity sites may represent the transport component (Drummond & Gordon, 1975; Boullin et al, 1977). The physiological significance of alpha-adrenergic membrane receptors is unknown, despite the high membrane affinity and potent ability of such agonists to activate platelets in vitro.

Collagen fibrils, structural components of the vascular subendothelium and wall, are clearly important in primary hemostasis, but the complexity of these structures has compounded the difficulty of understanding the biochemical mechanisms of platelet-collagen interaction. Both the primary and the quaternary structures of collagen species seem important in platelet adhesion and subsequent aggregation (Chiang & Kang, 1976; Santoro & Cunningham, 1977; Fauvel et al, 1978; Fauvel et al, 1979; Brown et al, 1980).

Prostacyclin (PGI_2), produced by endothelial cells, exerts a profound inhibitory influence on platelets and is thought to represent a major mechanism for the non-thrombogenicity of intact, endothelialized vascular surfaces (Moncada & Vane, 1979). Characterization of platelet receptors for this endothelial 'hormone' will allow investigation of the possible role of platelet PGI_2 receptor abnormalities in patients with thrombotic diatheses (Siegel et al, 1979). Platelet PGI_2 receptors appear to be distinct from those for PGD_2, the major inhibitory prostaglandin produced by platelets (Cooper & Ahern, 1979).

Platelet receptors for immunoglobulin (Fc) and for complement components exist, but are biochemically less well defined. Cheng and Hawiger (1979) have reported isolation of a platelet Fc receptor which is a surface glycoprotein. Insulin receptors have also been demonstrated (Hajek et al, 1979), as have specific receptors for dipyridamole (Subbarao et al, 1977). Receptors for plasmatic coagulation factors are discussed in a subsequent section.

Antigens

Structural components of the platelet HLA antigen system have been solubilized and purified (Bernier et al, 1974; Gockerman & Jacob, 1979), and the protein structural components appear similar, but not identical to those isolated from lymphocytes (Springer et al, 1977). The probable glycolipid structure of platelet ABH antigens, and the glycoprotein structure of the $P1^{A1}$ antigen have been noted above. The structural basis for other platelet antigen systems such as $P1^{E}$ and Ko is unknown.

Internal membrane systems

Surface Connected Canalicular System (SCCS)

The SCCS, an invagination of the plasma membrane, serves as a conduit through