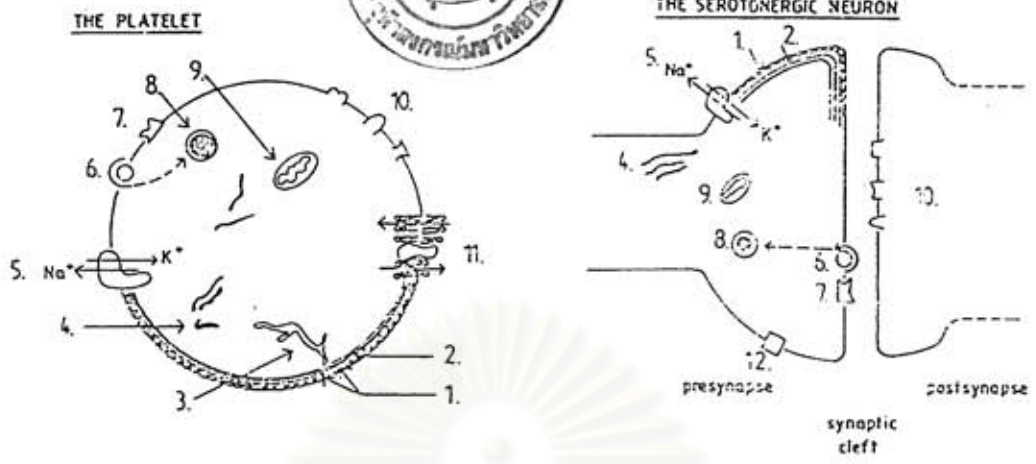
CHAPTER I

Introduction

The platelets are the smallest and anucleated cellular element in blood circulation, however, they possess a metabolic and functional complexity similar to that of the nucleated blood cells. They play a central role in maintaining haemostasis as well as contributing to the pathogenesis of thrombosis. Beyond their role in haemostasis, platelets also release a wide variety of substances that play important roles in many processes. These released products are extremely important, for example, in coordinating the activity of other cells in processes such as wound healing or inflammatory response. Table 1 summarizes the role of platelets in this and other normal and pathological processes (Crawford and Scrutton, 1994). Furthermore, there has also been an increasing interest in platelets as a faithful model for aminergic neurons (Rigmor and Lena, 1987). Both cells share many morphologic, biochemical, and pharmacologic characteristics (Figure 1). Several studies indicated the similarity of these two cells regrading accumulation and storage of serotonin, as well as the stimulus response-mediated release of biologically active substances such as serotonin, catecholamine and prostaglandins. Furthermore, their respective cell membranes contain similar receptors (α_2,β_2, 5-HT_2) and immipramine binding

Table I. Roles of blood platelets in physiological and pathological processes (Crawford and Scrutton, 1994)

Nature of process	Nature of platelet involvement
A. Physiological	
Haemostasis	Adherence to vessel wall injury sites, formation of platelet aggregates amplified by release of pro-aggregatory substances. Initial blood loss prevented. Initiation of coagulation through release of pro-coagulants and exposure of surface phospholipid for thrombin generation. Consolidation by binding and polymerization of fibrinogen. Retraction of fibrin-cell mass by force generated through platelet actomycin contraction.
Endothelial support function	Maintainance and/or restoration of vessel wall integrity by encouraging re-endothelialization processes at injury sites. Secretion of growth factors, e.g. platelet derived.growth factor, etc.
Detoxifying role	Uptake and transport of serotonin (5 -HT) from sites of synthesis and release to areas of function need or metabolic breakdown. Serotonin is the body's most potent vasoconstrictor and is involved in cardiovascular haemodynamics and regulation of peristasis.
Phagocytosis	Platelets capable of both phagocytosis and pinocytosis, but it is not known if such activities are operationally significant in the normal circulation.
Cytocidal	Platelets participate in cytocidal responses via a low affinity IgE receptor (CD23)
B. Pathological	
Inflammatory states and wound healing	Platelets release factors which not only increase vascular permeability directly (PGE2, HETE, cationic proteins, etc.) but also indirectly by generating mast cell degranulation and of histamine. Platelets also contain, and may release, leukocyte chemotactic factors and proteases and glycosidases capable of destroying integrity of connective tissue.
Transplant rejection	Aggregatory response to immune complexes causing vascular occlusion and damage leading to rejection of trasplanted organ.
Gout	Release of tissue-destroying proteases and other constiuents by monosodium water crystals.
Thrombosis and down-stream embolism	Over-activity of normal haemostatic processes after vascular injury or may occur in absence of damage by abnormal platlet-endothelial cell interactions.
Stenosis	Excessive recruitment of platelets to injury site (e.g. post-angioplasty) leading to local release of smooth muscle cell chemotactic or proliferative factors, e.g. PDGF
Cancer metastasis	Platelet interactions with circulating tumor cell facilitating arrest and extravasation. Platelets adhering to tumor cells may protect them from immune survillance. Release of platelet growth factors or angiogensis-promoting agents at both primary and secondary tumor sites.



1. Contractile microfilaments.
2. Microtubules.
3. Surface connecting system.
4. Dense tubules.
5. Ouabain sensitive Na^+/K^+ - ATP-ase.
6. 5-HT uptake system.
7. Imipramin binding site.
8. Dense body: storage of 5-HT, ATP, ADP, Ca^{2+}
9. Mitochondrion with membrane bound MAO.
10. 5-HT_2, α_2 - noradrenergic and $histamin_2$ - receptors.
11. Passiv diffusion of small molecules through the phospholipid bilayer.
12. 5-HT release-regulating receptor.

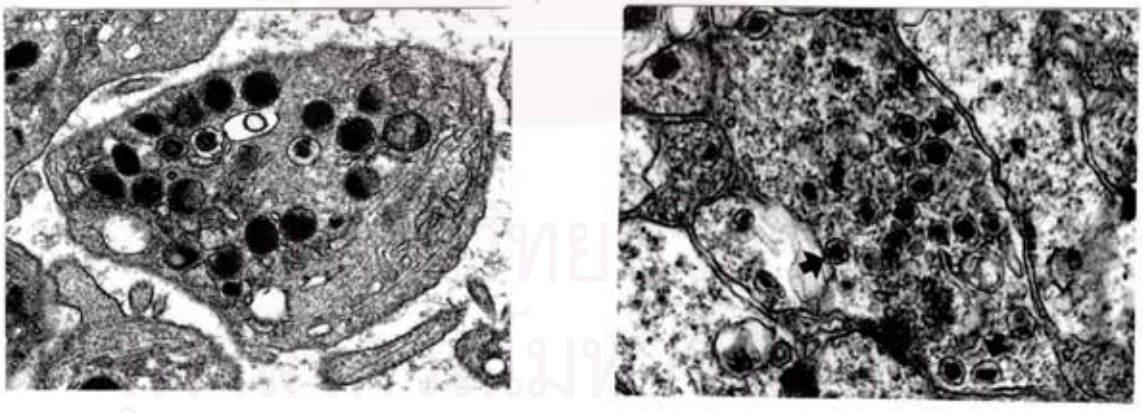


Figure 1. Above is a diagram showing platelet as a model for the aminergic neuron. (Malmgren and Hasselmark, 1989). Below showing the ultrastructural findings of platelets (left) and aminogenic neuron (right).

sites (Malmgren, 1988; Langer et al., 1988; Susanna and Noel, 1991). Both cell types make use of similar lipid metabolizing systems (Lapetina and Siess, 1985), and intracellular secretion involves Calcium (Ca^{2+}) and metabolites of the phosphatidylinositol phosphate cycle and the prostanoid pathway. Common enzymes, such as monoamine oxidase (MAO) and neuron-specific enolase (NSE) are found in both platelets and serotonergic neurons (White, 1979; Rigma, 1988). This evidence leads to the conclusion that the biogenic amine functions of platelets closely resemble those of aminergic neurons and strongly advocates platelets as a model for aminergic neurons.

Platelets are small fragments of megakaryocytes which shed their cytoplasm into platelet-sized fragments after following a unique pattern of differentiation and maturation (Pennington, 1981). The actual site of platelet formation is still controversial. Although the bone marrow has been widely accepted as the key site for platelet production, trapped pulmonary megakaryocytes may also act as a major source (Martin and Levin, 1991). Various properties of the platelet , e.g. size, cell volume, metabolic competence, receptor status etc., appear related to the ploidy class of the megakaryocyte from which they were derived (Pennington et al. 1974). The life span of the platelets in the circulation is 8-11 days (Abrahamsen, 1968; Crawford and Scruton, 1994).

Platelet morphology

On Wright stained blood smear, platelets appear as small bluish-gray oval to round bodies with several purple-red granules. The mean diameter of platelets varies (in different individuals) ranging from 1.5 to 2.5 micrometers. Under scanning electron microscopy platelets appear as disk-shaped cells with a long axis measuring 1.5 to 3.5 micrometers in length and 0.5 to 0.9 micrometer in width in the resting state (Figure 2). After activation, this discoid appearance disappears and the cell transforms its shape to an irregular sphere with long thin filopodia (Figure 3).

Ultrastructural studies under the transmission electron microscope reveal the complicated structural features of the platelets. These structures can be divided into four major regions: peripheral zone, sol gel zone, organelle zone and membrane system (White, 1994).

Peripheral zone

This zone consists of the membrane and closely associated structures providing the surface of the platelet connected with the open canaliculi system. The outermost of the peripheral zone is an exterior coat or glycocalyx which contains at least nine different glycoproteins , Ia, Ib, Ic, IIb, IIc, IIIa, IV,

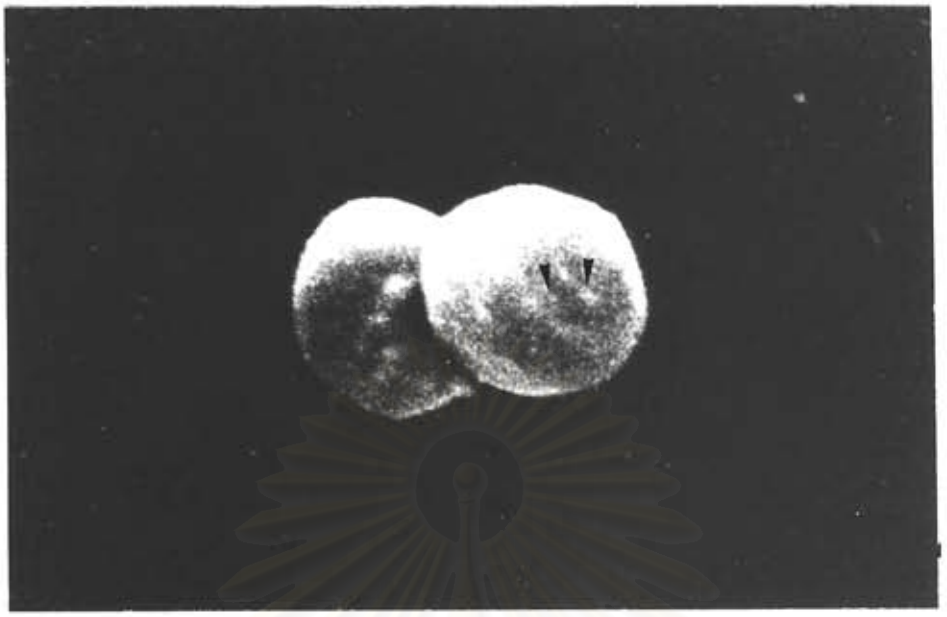


Figure 2. Scanning electron micrograph showing discoid appearance. The indentations were seen (arrows) indicating sites where channels of open canalicular system (OCS) communicate with the cell exterior (x15,000).

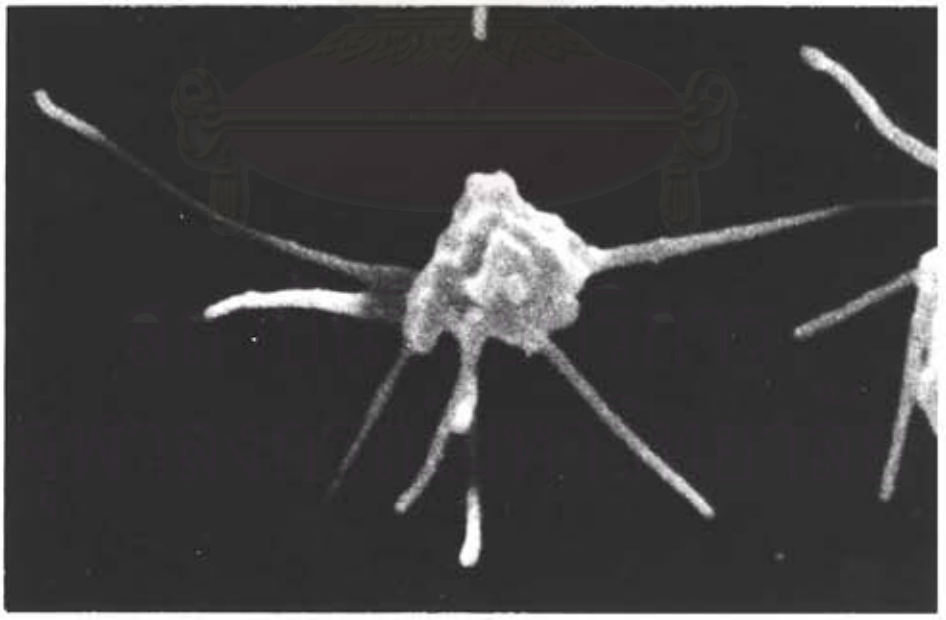


Figure 3. Platelets after activation, showing irregular sphere with long thin filopodia (x15,000)

V, and IX (Phillips and Poh-Agin, 1977; George, 1978). The glycoproteins (GP) are frequently associated in complexes serving as receptors for several aggregate ligands. Some of the known adhesion protein/receptor functions of platelet glycoproteins are listed in Table 2.

The platelet membrane has a trilaminar structure which is not different from that of other mammalian cells. The important components of the platelet membrane are Na^+/ K^+ ATPase and other anion and cation pumps which maintain the appropriate transmembrane ionic gradients (White, 1994).

Sol gel zone

The sol gel zone or cytoskeletal system is the matrix of the platelet cytoplasm. It contains three major fiber systems in various states of polymerization which support the discoid shape of the resting platelet and provide a contractile system involved in shape change, pseudopod extrusion, internal transformation, and secretion. These are submembrane filaments, microtubules and microfilaments (White, 1994).

The most prominent protein of the cytoskeletal system is the circumferential band of microtubules. Cross sections of platelets show microtubules as a group of 8-24 circular profiles, each approximately 25 nanometers in diameter, at the polar ends of the lentiform cell, whereas

Table 2. Receptor properties of some platelet membrane (Crawford and Scrutton, 1994)

Glycoprotein	Receptor role(s)
GP IIb/IIIa complex	Fibrinogen receptor
	Also binds vWf, fibronectin, vitronectin and thrombospondin
	Platelet activation required for expression of receptor function
GP Ib/IX complex	vWf receptor involve in platelet-subendothelium matrix
	binding, active in resting platelet
	Also binds thrombin
	Major surface sialoglycoprotein responsible for cell elctro-negativity
GP Ic/IIa	Fibronectin receptor active in resting platelet
	A closely related gene product binds laminin
GP Ia/IIa	Collagen receptor
	Identical to lymphocyte VLA-2 antigen
GP IV	Binds to collagen fibrils and to thrombospondin
GP V	Only major glycoprotein hydrolysed by thrombin

equatorial plane sections reveal the coil of microtubules immediately beneath the cell wall (Figure 4). Based on their location, microtubules are suggested to be the part of a cytoskeletal support system maintaining the discoid shape of resting platelets (Haydon and Taylor, 1965; White, 1983). Some studies revealed the constriction of the circumferential band of platelets after exposure to agents which stimulate a physiological response. However, White et al. demonstrated that removal of these microtubules did not alter

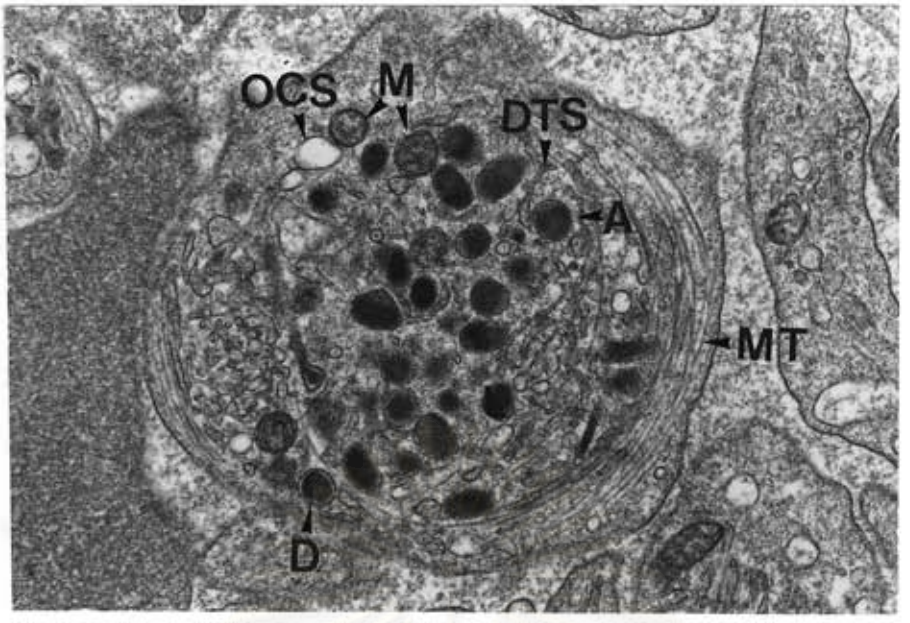


Figure 4. Ultrastructure of discoid platelet cut in equatorial plane showing circumferential microtubule (MT) beneath the cell membrane. Randomly dispersed organelles including mitochondria (M), dense granule (D), and alpha granule (A) are embedded in sol-gel-zone. The open canalicular system (OCS) and the dense tubular system (DTS) are also demonstrated. (x22,000)

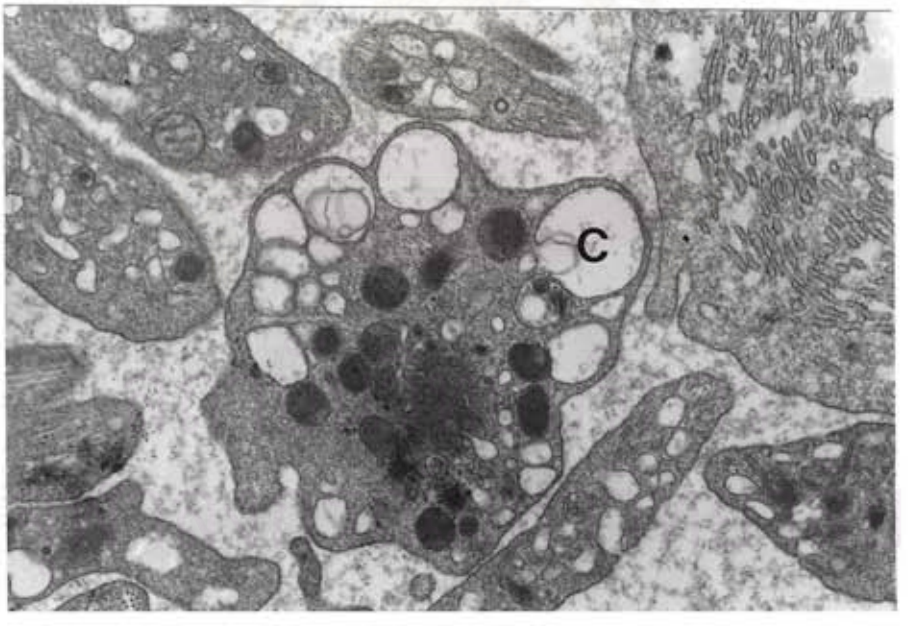


Figure 5. After activation, the platelet is irregular in shape. The organelles are concentrated in the central area. Note the dilatation of canalicular system resembling vacuoles of variable sitzes. (x22,000)

the platelet response to aggregatory agonists (White and Roe, 1983).

Microfilaments are bundles of polymerized actin, which represents 15-35% of the total platelet protein. The state of this filament in resting platelets is not clear but when platelets change shape, newly formed filopodia contain bundles of microfilaments made of actin and associated proteins (Ware and Coller, 1996).

Glycogen

Glycogen is an electron dense particle observed in platelet cytoplasm. Particle of glycogen occur single or in relatively large masses. Ordinary, they are not membrane bound or associated with specific organelles. However, in some platelets glycogen particles appear closely associated with circumferential microtubules (White, 1994).

Organelle zone

Study by transmission electron microscopy revealed several types of organelles in the cytoplasm of platelets (Figure 4).

Mitochondria

Mitochondria are easily differentiated from other organelles by the their internal membranes folding into cristae. Platelets contain approximately seven mitochondria of relatively small size. They are involved in oxidative energy metabolism since they contain the enzymes of the tricarboxylic acid cycle and of fatty acid oxidative phosphorylation (Holsen et al., 1982; Holsen, 1994; Crawford and Scrutton, 1994)

Lysosome

Platelets have lysosomal granules that contain several acid hydrolases such as β-glucuronidase, cathepsin, arylsulfatase, β-galactosidase etc. (Crawford and Scrumtun, 1994). When platelets undergo their secretory process, their lysosomal content is released more slowly and incompletely than the content of alpha and dense granules. Moreover, stronger inducers of activation are required to obtain any release of the lysosomal content (Holsen et al., 1982).

Alpha granules

These granules are 200 nanometers in diameter and demonstrate internal variation in electron density. They are the most abundant granules in

platelets (Figure 4). Ultrastructural immunocytochemistry employing monoclonal antibody has been performed by several groups. Their findings have revealed the presence of Von Willebrand factor, fibrinogen, plasma factor 4, growth factor and several proteins in the matrix of alpha granules. Some of the most important proteins present in alpha granules are listed in Table 3 (Steinberg et al., 1984; Harrison and Martin, 1993; Niewiarowski, 1994).

Dense granules

Dense granules are easily distinguishable from alpha granules and lysosomes in electron micrographs, because they are the most electron dense granules in platelets. These granules are 50 to 150 nm diameter in cross-section. Electron microscopy, ultrastructural autoradiography, analytical electron microscopy and biochemistry have demonstrated that these electron dense granules are the storage sites for the non metabolic pool of adenine nucleotides, serotonin and calcium and magnesium (Tranzer et al., 1966). Other constituents such as GTP and pyrophosphate are present at much lower concentration (Table 4). In human platelets ADP is the predominant nucleotide and calcium is the predominant divalent cation. However, this situation is not characteristic of all mammalian platelets since ATP and magnesium predominate in dense granules of rabbit and pig

Table 3. List of proteins present in alpha granule (Ware and Coller, 1994)

Platelet specific protein	
Platelet factor 4	
β Thromboglobulin family (platelet basic protein, low affinity platelet factor 4, β thromboglobulin and β thromboglobulin-F)	
Multimerin	
Adhesive glycoproteins	
Fibrinogen	Thrombospondin
von Willebrand factor	Vitronectin
Fibronectin	
Coaggulation factors	
Factor V	Factor XI
Factor S	
Mitogenic factor	
Platelet derived growth factor	Endothelial cell growth factor
Transforming growth factor-B	Epidermal growth factor
Fibrinolytic inhibitos:	
α2-Plasmin inhibitor	
Plasminogen activator inhibitor-1	
Albumin	
Immunoglobulin	
Membrane associated proteins	
P-selectin (CD62P)	GPIV (CD36)
GMP 33	Osteonectin
24-kD GTP-binding protein	

Table 4. List of contents of platelet dense granules (Ware and Coller, 1994)

ADP	653	mM
ATP	436	mM
Calcium	2181	mM
Serotonin	65	mM
Pyrophosphate	326	mM

platelets (Costa and Murphy, 1980). Other biogenic amines eg. noradrenalin or histamine may be present in dense granules, the latter being a major constituent in rabbit dense granules (Pletscher et al., 1974). Several studies on these granular membranes demonstrate a transport system (5-HT/H^+ symport) which is responsible for the selective uptake of 5-HT and possibly other biogenic amines from the cytosol (Da Prada et al., 1981; Crawford and Scrutton, 1994). Transportation of 5-HT into dense granules depends on the relative rate of (1) transfer of 5-HT from the cytosol into granules and (2) the cytosolic metabolism of 5-HT by enzymes such as monoamine oxidase and phenosulfotransferase. Normally, the balance favours granular uptake and only a small fraction is metabolized in the cytosol (Da Prada et al., 1981).

Membrane system

Platelets have two discrete membrane systems not found in other blood cells, the open canalicular system (OCS) and dense tubular system (DTS). The OCS is derived from the plasma membrane of megakaryocytes while DTS is the residual smooth endoplasmic reticulum of the parent cell (Menashi et al., 1981). The OCS and DTS are not completely isolated membrane systems since canaliculi of the OCS and DTS form intimate physical relationships in nearly every cell. The association of the two channel systems is usually restricted to one or two areas of the cytoplasm.

The OCS in such areas is gathered in clusters or groups, and small canaliculi of the DTS are scattered between the groups of OCS (White, 1994).

Open canalicular system (OCS)

The open canalicular system consists of invaginations of the cell wall tunnelling throughout the cytoplasm (Behnke, 1970; Behnke and White, 1994). The OCS may serve several functions. It provides a mechanism for entry of external elements into the interior of the platelet, as well as a potential route for the release of granule contents to the outside. This latter function is especially important because platelet granules appear to move to the center of the cell upon platelet activation rather than to the periphery (Figure 5). The granules then fused with the membrane of the OCS, releasing their contents into platelet's external enviorment. Although the opening of the canaliculi on the platelet surface may be narrowed or close in resting platelets, they are widened during secretion to permit the exterization of the granular contents (Stenberg et al.,1984). Additionally, OCS also represents an extensive internal membrane store. Both filopodia formation and platelet spreading after activation require a dramatic increase in surface plasma membrane compared to that of resting platelets. The cell cannot synthesize new membrane during the short time-course of these phenomena. Thus, the membranes of OCS most likely contribute to the increase in plasma membrane under these conditions.

Dense tubular system

Channels of the DTS can be distinguished from clear canaliculi of the OCS by the presence of amorphous material, similar in opacity to the surrounding cytoplasm (Figure 4). Several studies indicate DTS to be the calcium sequestenting site, as well as the site for prostaglandin synthesis in platelets (White, 1975; Menashi et al., 1982).

Apparently, each particular region in platelets performs a specific biochemical function, thus eliciting suitable physiological responses of the cell. Ultrastructural studies of platelets may contribute to a further understanding of platelet activity in the healthy, as well as the pathological state.