# RECENT TRENDS

# IN

# ANTIPLATELET THERAPY

Thesis Submitted for the Partial Fulfilment
of the M.Sc. Degree in
Cardiology

By

Samia Anwar Mishriki
MB.B.Ch.

Supervised By

Prof. Dr.
Khairy Abdel-Dayem
Professor of Cardiology

Dr.
Wagdy Abdel-Hamid
Lecturer of Cardiology

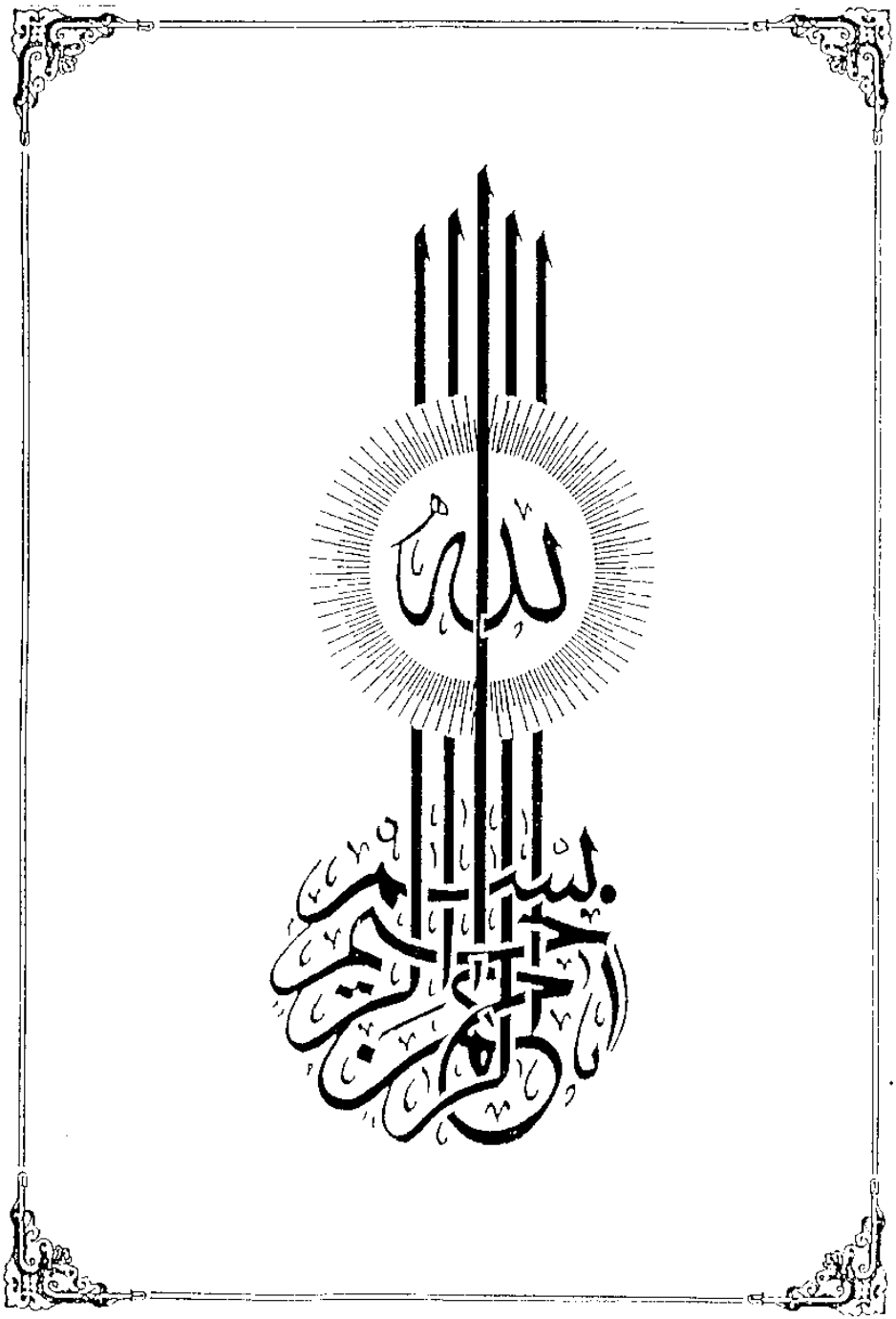
Faculty of Medicine
Ein Shams University

A C K N O W L E D G E M E N T

I am genuinely grateful to Professor Doctor Kairy Abdel-Dayem. He has taught me to be accurate in choosing my words and to be clear in expressing my meaning. I am deeply touched that despite his numerous responsibilities, he always gave me prompt advice and gentle welcome.

Dr. Wagdy Abdel-Hamid was the continuous source of gentleness, kindness and encouragement. He had immense patience and forbearance in supervising my work.

I deeply thank them both.



CONTENTS

# INTRODUCTION

# INTRODUCTION

Platelets, the once forgotten stepchildren of the blood components have now been assigned a central role in haemostasis and thrombosis.

Their role in the primary arrest of bleeding is well established; however, the properties of adhesion, aggregation, and secretion that make the platelets useful in haemostasis also permit them to be deposited as thrombi in blood vessels, on heart valves, and on the surfaces of prosthetic devices and artificial membranes used in clinical medicine. In addition, products that are stored in platelet granules ( such as adenosine diphosphate, serotonin, smooth muscle mitogens, proteolytic enzymes ) or are synthesized during platelet activation ( prostaglandins, thromboxane ) are extruded from the cell during platelet secretion. These agents may mediate a variety of biological processes such as vascular permeability, smooth muscle proliferation; immune reaction and atherogenesis.

A growing body of evidence indicates that platelet activation and its consequences may be inhibited by a wide range of drugs. These drugs are generally referred to as anti-platelet or platelet-inhibitory drugs. The possibility that these drugs may be clinically useful has received widespread attention recently.

The aim of this study on platelets is to review their biological and biochemical pharmacology and to survey the present status of antiplatelet therapy in clinical medicine.

# CHAPTER I

# C H A P T E R I

## FUNCTIONAL PHYSIOLOGY OF PLATELETS

### GENERAL PROPERTIES of PLATELETS

Platelets are 1.5 um in diameter and from 0.5 to 1 um in thickness. They originate from the cytoplasm of bone marrow megakaryocytes. They have no nucleus and their cytoplasm has many azurophil granules which in blood films tend to be concentrated in the centre. On election microscopy, platelets are seen to be bounded by plasma membrane, and in their cytoplasm there are prominent dense granules which correspond to the azurophil ones seen on light microscopy. These granules contain hydrolytic enzymes and also lipoprotein which is important in blood clotting. They are probably the main site at which 5-HT is bound ( Weiss, 1975 ). They also bind histamine and catecholamines.

Platelets contain microtubules, a few mitochondria, and some vesicular elements which may be derived from the endoplasmic reticulum or the Golgi complex of the megakaryocyte from which they originate.

The normal platelet count varies from 140,000 to 440,000 per ul with an average of 250,000 per ul. A count below 100,000 per ul can be taken as thrombocytopenia. There are comparatively few physiological variations in platelet count; neither the newborn infant nor the menstruating woman has any

alteration in count. It tends to be reduced in later pregnancy, but during the puerperium there is a moderate rise in count. The administration of adrenaline produces an immediate increase in count up to 50 percent, and is presumably due to splenic release, since it does not occur in individuals with splenectomy. On the other hand, the increased count that follows exercise is uaffected by splenectomy and may be the result of release of platelets from the lungs ( Marcus, 1969 ).

## PLATELET SURVIVAL and LIFESPAN

Platelets circulate in the blood for an average of 9-10 days and during this time undergo changes in both biochemical composition and function.

For example, as platelets age, they undergo a progressive reduction in protein, phospholipid, cholesterol, ATP, rate - limiting enzymes involved in metabolism, and membrane glycoproteins ( Karpatkin, 1972 ). In addition, young platelets generally show greater functional capcity, such as the ability to aggregate in response to collagen, and are heavier than older platelets. Whether they are also larger under normal circumstances is the subject of some controversy. The biochemical and functional changes that occur in platelets during aging are probably related to their eventual removal by reticuloendothelial system in spleen and liver.

That platelet removal is, like that of red cells, age-dependent ( rather than random ) is suggested by the linear disappearance curve obtained when a mixed population of platelets from a normal subject is labelled with $^{51}Cr$ and reinjected into the donor ( Harker, 1978 ). Further supporting evidence has come from studies showing that cohorts of young $^{51}Cr$ labeled platelets survive longer than cohorts of older platelets ( Harker, 1978 ). Although aging is clearly involved in platelet removal under normal circumstances, the mechanisms involved have not been clearly established. In almost all instances, a decreased survival of platelets implies that they have been injured in some way.

## Methods for Studying Platelet Survival

Platelet survival in the circulation is most commonly studied by measuring the disappearance of isotopically tagged platelets ( Harker, 1978 ).

In one method, a cohort of platelets is labeled in vivo with $^{32}P$, $DF^{32}P$, $^{35}S$, or $^{75}S$-methionine, and loss of radioactivity from newly formed platelets is measured. This method has the disadvantage that the period the isotopes remain available for labeling megakaryocytes is long compared with the mean platelet survival time. Therefore, the appearance of radioactively labeled cells from the marrow in circulating platelets proceeds concurrently during the period of platelet removal.

A second method which is extensively used at present is to label platelets in vitro and to follow the loss of radioactivity after reinjection, generally into the donor. Isotopes previously used in this method include $^{32}P$-orthophosphate, DFP labeled with $^{32}P$, $^{3}H$, or $^{14}C$, and $^{14}C$-serotonin. At present, the isotope most frequently used to label platelets is $^{51}Cr$, and technical improvements for obtaining and processing the platelets have made this a useful procedure for studying platelet survival. $^{51}Cr$-platelet disappearance from normal individuals is essentially linear, indicating that platelet removal is predominantly an age related process.

Recently $^{111}In$ has been introduced as a platelet label, and the results of animal studies have shown that the kinetics compare favourable with the results obtained using $^{51}Cr$-labeled platelets. In addition, $^{111}In$ permits quantitative gamma camera imaging of both the in vivo distribution of labeled platelets and the localization of labeled platelets accumulating at thrombogenic foci ( Hansen et al., 1983 ).

## RHEOLOGIC ASPECTS of PLATELETS

The flow of platelets within the circulation and their attachment or non attachment to other cells depend primarily on rheological factors determining platelet movement and on the properties of the vessel wall, notably the endothelial cell .

Under normal conditions, platelets are carried in streamlines parallel to the surface of blood vessels and must diffuse perpendicular to these streamlines inorder to reach the surface ( Goldsmith, 1972 ).

A variety of factors are important in determining the diffusion of platelets to the vessel wall. Studies have shown that platelet diffusion in whole blood is determined not by Brownian movement, but rather by local movements of fluid induced by rotation and oscillation of erythrocytes in shear fields ( Goldsmith and Karno, 1979 ). For example, cinematographic studies showed that the mean square radial excursions of latex microspheres of diameter similar to that of platelets increased when red cell membranes were present. That platelet diffusion does, indeed, depend on both shear rate and red cell concentration has been demonstrated by Turitto et al., (1979) who found both a 100-fold increase in platelet diffusion in the presence of red cells and an increase in platelet deposition on vascular surfaces with increasing shear rate.

The importance of red cells in enhancing platelet diffusion as well as other red cell properties that may directly influence the reactivity of platelets at the vessel wall, could be of great importance in mediating thrombosis in conditions characterized by higher than normal red cell concentration ( Turitto and Weiss, 1980 ). The critical importance of shear rate in determining platelet reactivity at vessel

surfaces has been demonstrated in the bleeding disorder known as von Willebrand's disease, where a defect in platelet adhesion which is highly shear rate-dependent has been observed.

The movement of platelets under conditions of non laminar flow is considerably more complicated. Such conditions may be created in a vicinity of obstructions within the vascular system where areas of separated flow ( vortices ) may be created proximal and distal to the obstruction ( Dormandy, 1980 ). Such areas of separated flow may occur at bifurcations of vessels in extracorporeal shunts and around platelet-fibrin thrombi. Direct observations of platelet behavior in vortices have been made by Goldsmith and Karno, who showed that the relative concentration of platelets within a cortex was greater than in the bulk of a suspension ( Goldsmith, 1972; Goldsmith and Karno, 1979 ).

## STRUCTURAL BIOCHEMISTRY of PLATELETS

### 1- Membranes

The trilaminar plasma membrane of platelets, similar to that of other cells, is covered with an amorphous coat 100 - 200 $A^{\circ}$ in thickness ( Behnke, 1970 ). The function of this amorphous coat which is not present in red cells or leucocytes ( Holmsen, 1972 ) is unknown, but some role in platelet aggregation has been inferred from the observation that the gaps between aggregating platelets contain a similar substance ( Behnke, 1970 ).

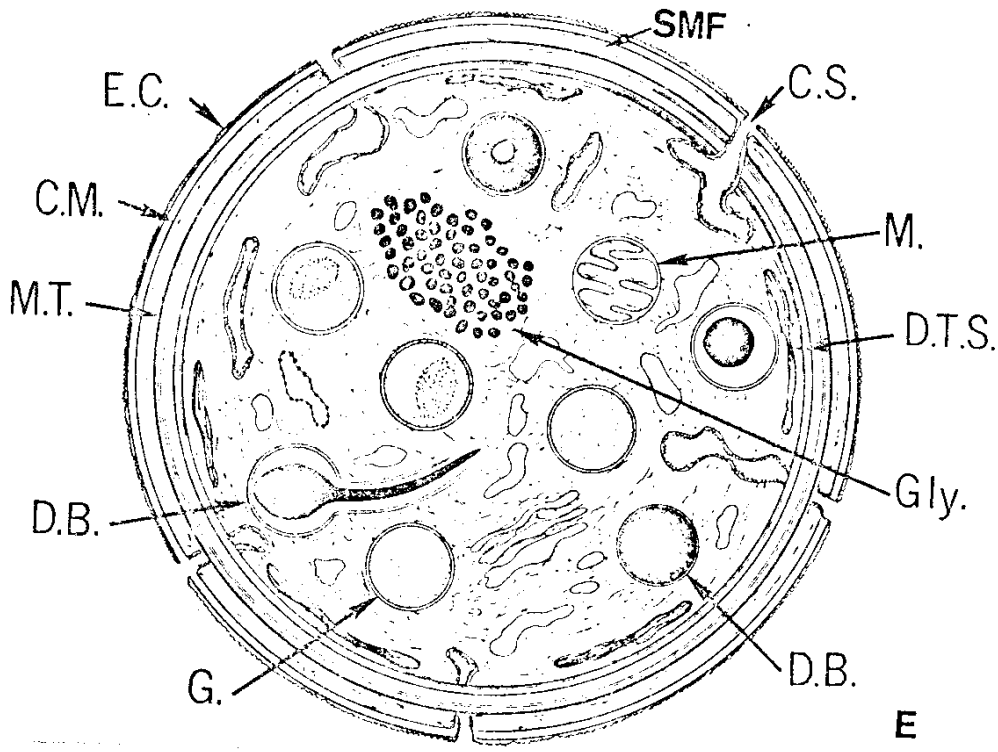



Fig. 1 :Ultrastructural features observed in thin sections of discoid platelets cut in equatorial plane,exterior coat (EC), trilaminar unit membrane (CM),sub-membrane area containing specialized filaments (SMF),surface-connected cana-licular system (CS),mitochondria (M), granules (G), dense bodies (DB),surface connected canalicular system (CS), dense tubular system (DTS), Golgi apparatus (GZ), circumferential band of microtubules (MT),glycogen (gly)
( White,J.G. : Am.J.Clin.Pathol., 71 : 4, 1979 ).