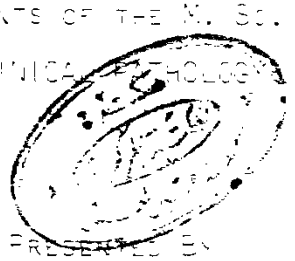
PLATELET AGGREGATION AND VOLUME DISTRIBUTION IN AUTOIMMUNE THROMBOCYTOPENIC PURPURA

THESIS

SUBMITTED IN PARTIAL FULFILMENT FOR THE REQUIREMENTS OF THE M. SC. DEGREE IN CLINICAL PATHOLOGY



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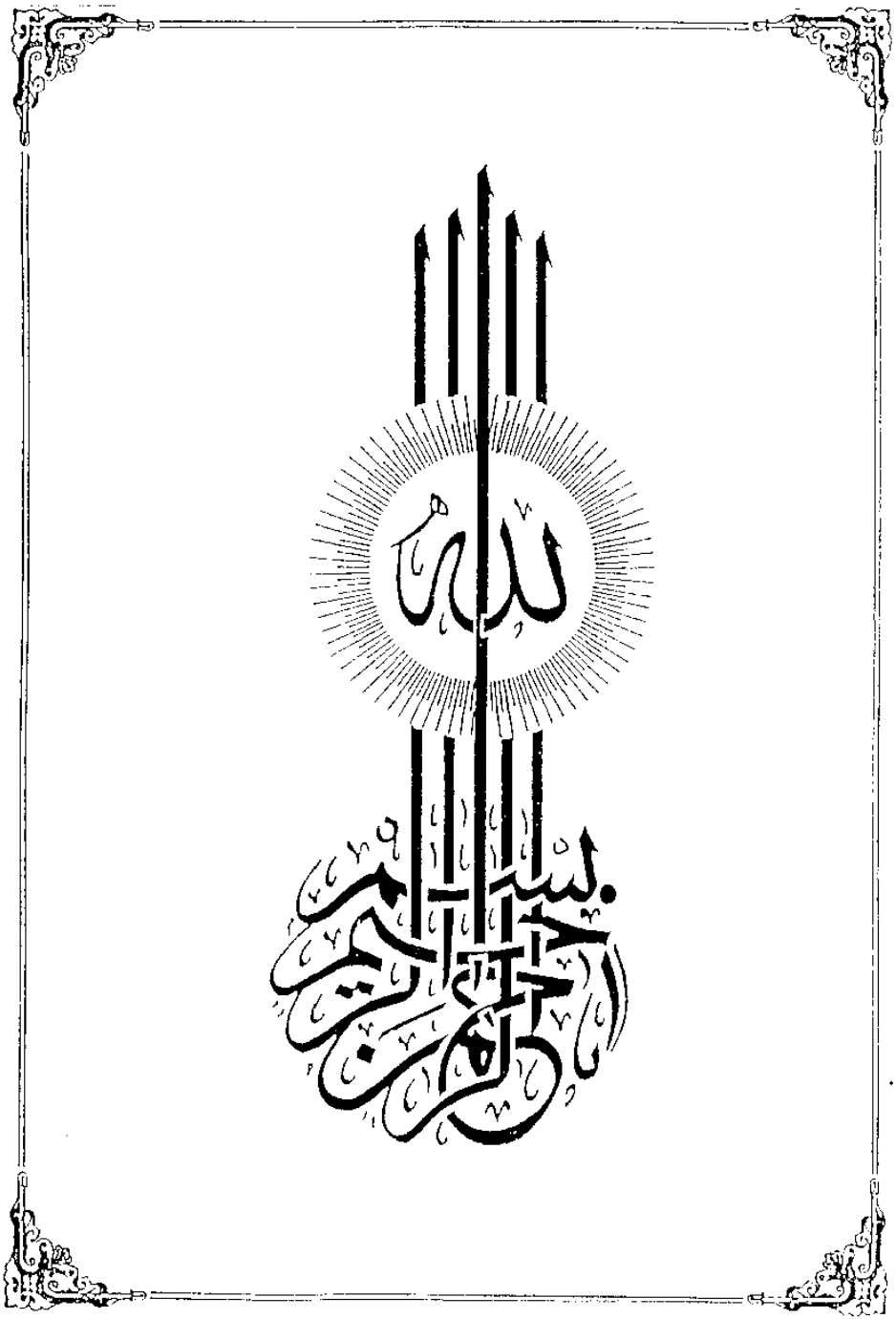

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CONTENTS

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ABBREVIATIONS

ACD	: Acid citrate dextrose.
Ach-E	: Acetyl choline esterase.
ADP	: Adenosine diphosphate.
ADPase	: Adenosine diphosphatase.
ATP	: Autoimmune thrombocytopenic purpura.
ATPase	: Adenosine triphosphatase.
BCG	: Bacille-Calmette-Guerin.
C_1, C_2, C_3, C_4	: Complement components.
C-AMP	: Cyclic adenosine monophosphate.
Ca^{++}	: Calcium ions.
CFU-M	: Megakaryocytic colony-forming unit.
^{51}Cr	: Radioactive chromium.
DNA	: Deoxyribonucleic acid.
DTS	: Dense tubular system.
Fab	: Fragment antigen binding.
Fc	: Fragment crystallizable.
GP	: Glycoprotein.
^{125}I	: Radioactive iodine.
Ig	: Immunoglobulin.
IQR	: Interquartile range.
ITP	: Idiopathic thrombocytopenic purpura.
K_3EDTA	: Potassium ethylene diamine tetraacetate.
Mg^{++}	: Magnesium ions.
MPD	: Myeloproliferative disorders.
MPV	: Mean platelet volume.
Na_2EDTA	: Sodium ethylene diamine tetraacetate.
OCS	: Open canalicular system.
Pct	: Plateletcrit.
PDGF	: Platelet derived growth factor.
PDW	: Platelet distribution width.
Pf-3	: Platelet factor 3.
PG	: Prostaglandin.
PLT	: Platelet count.
PPP	: Platelet poor plasma.
PRP	: Platelet rich plasma.
^{75}Se	: Radioactive Selenium.
TxA_2	: Thromboxane A_2.

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INTRODUCTION
AND
AIM OF THE WORK

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Idiopathic thrombocytopenic purpura (ITP) is a clinical disorder of uncertain etiology associated with thrombocytopenia and purpura. It can manifest clinically in one of two forms, namely acute and chronic ITP.

Evidence for an immunological etiology is ever increasing which probably makes the designation autoimmune thrombocytopenic purpura (ATP) more appropriate (Hirschman and Gralnick, 1974; Rosse, 1975 and Karpatkin, 1971).

The disease was generally believed to be a purely quantitative platelet disorder with normal platelet function. However, Clancy et al. in 1972; Hymes et al. in 1978 and Karpatkin in 1981, reported that platelet antibodies can affect platelet function with impairement of platelet aggregation in patients with chronic ATP.

Furthermore, the recent introduction of platelet measurements in automated blood counters has allowed reliable platelet counts together with platelet volume ditribution profile (MPV, Pct and PDW). However, the significance and hence possible clinical application of these newly measured

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parameters is still somewhat controversial (Bessman et al., 1982 and Rowan, 1983).

Accordingly, the aim of this work is to study ADP-induced platelet aggregation as a representative of platelet function together with platelet volume distribution profile among chronic ATP Egyptian patients.

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REVIEW OF LITERATURE

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MORPHOLOGY OF BLOOD PLATELETS

Platelets are anucleate cytoplasmic fragments originating from megakaryocytes in the bone marrow. They circulate as bi-convex discs (2-4 um diameter; 7-8 fl volume) and do not normally adhere to other platelets, endothelium or other blood cells. In a stained blood smear, the platelet appears formed of 2 zones: a central granular "granulomere" and a clear peripheral "hyalomere".

The main features of platelet ultrastructure as revealed by electron microscopy can be considered under 4 distinct divisions : (figure 1)

I- The peripheral zone.

II- The sol-gel zone.

III- The organelle zone.

IV- The membrane systems (Vermylen et al., 1983).

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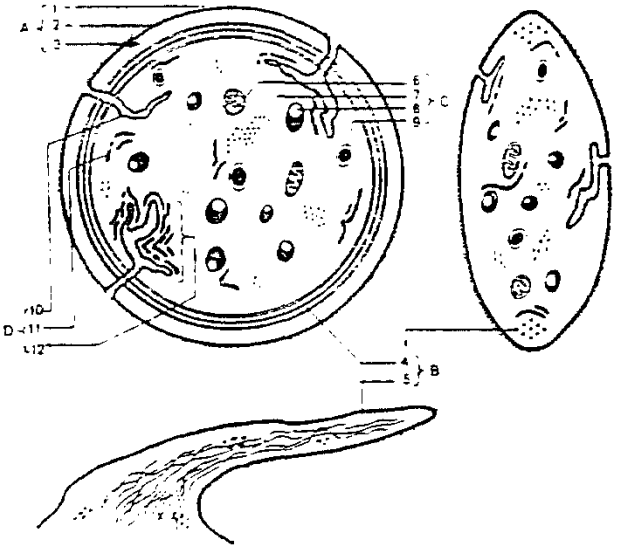
Figure 1



PLATELET ULTRASTRUCTURE
(Vermylen et al., 1983)

* A : Peripheral zone
  - 1 glycocalyx.
  + 2 unit membrane.
  + 3 submembrane area.

* B : Sol-gel zone
  + 4 microtubules.
  - 5 microfilaments.

* C : Organelle zone
  + 6 mitochondria.
  + 7 glycogen.
  + 8 ∝ granules.
  - 9 dense bodies.

* D : Membrane system
  + 10 OCS.
  + 11 DTS.
  + 12 membrane complexes.

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I- The peripheral zone :

The peripheral zone constitutes the platelet wall and invaginates into the interior of the cell to form the surface connecting or open canalicular system.

The peripheral zone plays an important role in platelet function. It maintains platelet integrity, provides receptor sites for various stimulation or inhibitory agents, is responsible for platelet immunological specificity, mediates platelet-platelet and platelet-surface interactions and provides a phospholipid surface to accelerate blood coagulation.

The peripheral zone is composed of 3 morphological domains:

A- The exterior coat (glycocalyx .

B- The unit membrane.

C- The submembrane area.

A- The exterior coat :

It is the outermost component of the peripheral zone which is in immediate contact with the surrounding plasma. It is rich in glycoproteins. Five major platelet membrane glycoproteins (GP I to V) have been well characterized. GP I is necessary for normal adhesion and GP II and III for normal aggregation. The surface glycoproteins contain terminal sialic acid molecules. These result in a large negative surface charge which could

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aid, in preventing platelets from sticking to each other or to normal intact endothelium by electrostatic repulsion (Bull, 1966).

Adsorbed plasma proteins are important constituents of the exterior coat where they participate in blood coagulation.

Coat material retains its structure and remains on platelets before, during and after aggregation (White, 1970).

B- The unit membrane :

It consists mainly of a phospholipid bilayer with randomly dispersed transmembrane protein components. The unit membrane plays an important role in protecting the integrity of the platelet internal milieu. It contains enzymes involved in membrane transport and cyclic AMP metabolism. It also plays an important role in platelet adhesion (Silver, 1968), contraction (Nachman and Marcus, 1967) and in supplying a lipid activator to coagulation (Marcus et al., 1966).

C- The submembrane area :

It constitutes the space between the unit membrane and the circumferential band of microtubules. It contains a system of filamentous elements. Functionally, the submembrane filaments may be involved in maintaining the platelet discoid shape, pseudopod formation and clot retraction.

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II- The sol-gel zone :

This zone comprises the viscous matrix inside the platelet. It is composed mainly of proteins assembled into fibrous elements. Three different fibre systems can be identified : the submembrane filaments, the microtubules, and the microfilaments. These three fibre systems differ only in their state of polymerization, aggregation and site in the cell (Mammas, 1981).

A- The submembrane filaments : (discussed before)

B- The microtubules : form a circumferential band located in the equatorial plane just beneath the cell wall in the non-activated platelet. They are composed of tubulin, polymerized into a coiled single tubule of approximately 250 A° in diameter (White, 1968 a).

C- The microfilaments : about 50A° in diameter. They are essential elements of the contractile mechanism in platelets.

III- The organelle zone :

Different types of organelles and particulate elements are found in the sol-gel matrix of the platelets. They have a random distribution in non-activated platelets but become centralized upon activation.

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A- Granules :

The granules are numerous and represent an important source of substances secreted by platelets during the release reaction They can be subdivided into ∝-granules, lysosomes and peroxisomes (Bentfeld-Barker and Bainton, 1982). The ∝-granules contain platelet factor 4, fibrinogen, B-thromboglobulin, growth promoting factor and other constituents (White and Krivitt, 1965; Marcus et al., 1966; Nachman and Marcus, 1967). The peroxisomes contain catalase. The lysosomes contain hydrolytic enzymes including acid phosphatase, B-glucuronidase and cathepsin (Marcus et al., 1966; Kaplan et al., 1979).

B- Dense bodies :

Although relatively few in number they appear to be very important in hemostatic function (Tranzer et al., 1966; Davis and White, 1968). Dense bodies are inherently electron-opaque, probably because of their content of calcium (White, 1969). Most, if not all, opaque organelles in human platelets may originate from granules (White, 1968 b). The transformation of granules to dense bodies is directly related to the uptake of serotonin (Tranzer et al., 1966).

Dense bodies contain serotonin, non-metabolic ADP and ATP, catecholamines, calcium and pyrophosphate. They are the primary secretory organelles of the platelets.

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