PLATELET COUNT

BY

DIFFERENT METHODS

Thesis Submitted for Partial fulfilment,
of Master Degree in Clinical Pathology

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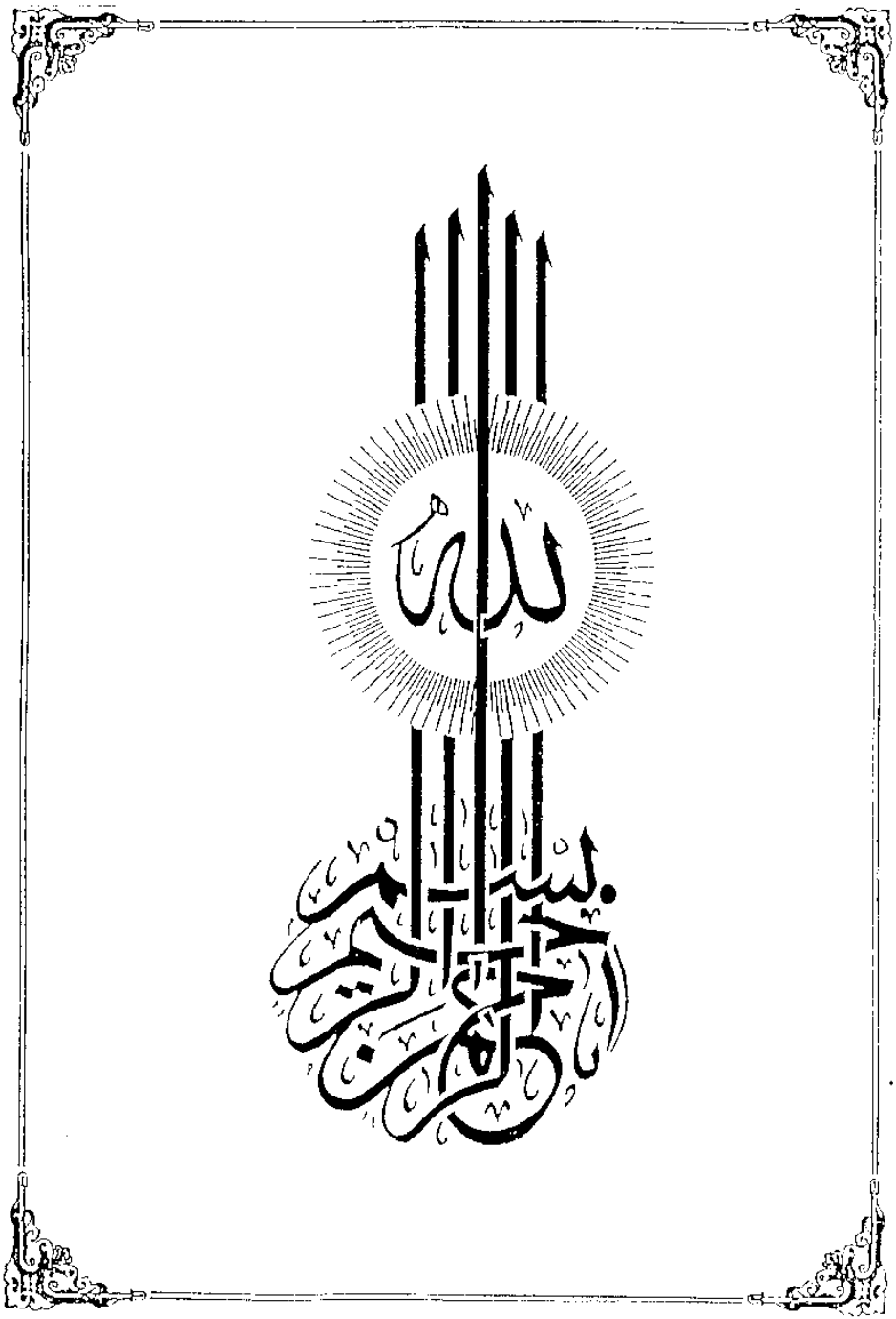
(1982)

INTRODUCTION AND AIM OF THE WORK



INTRODUCTION
AND THE AIM OF THE WORK

In view of the possible technical difficulties and sources of error associated with performing an accurate platelet count, many methods have been published, and adopted by various worker and laboratories.

This is particularly true when the assessment is made by visual or manual methods in which the coefficient of variation ranges from 10-20%.

On the other hand, automated methods do not suffer from this drawback and it is estimated that the coefficient of variation in this situation is usually around 4% (Bull et al., 1965).

Unfortunately electronic counters are not always available in most laboratories and reliance is made on visual techniques.

The object of this study is to compare the results of two popular and recognized methods of visual platelet counting with the results of the electronic Coulter Counter C (Coultronics).

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ACKNOWLEDGEMENT

I would like to express my sincere gratitude and thankfulness to Prof. Dr. WAGUIH NAGUIB IBRAHIM Professor of Clinical Pathology for his faithfull supervision, guidance and encouragement through the accomplishment of this study.

My best thanks and gratitude go to Dr. NADIA Mewafy lecturer in Clinical Pathology, for her supervision and revision of this work.

To all members of the Clinical Pathology Department, Faculty of Medicine, Ain Shams University I should pay my gratitude and thanks.

TO MY PARENTS

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HISTORICAL INTRODUCTION

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Platelets were first recognized as a distinct morphologic element in the blood by the French scientist Donné in 1842. In a report before the Paris Academy of Sciences, he stated that there were three elements in the blood, the red cells, the white cells and thirdly, the globulins of chyle. Two years later, in his book "Cours de Microscopie", he repeated and amplified his original description, nevertheless, his interpretation of the origin of "the globulins" (the lymph) was erroneous.

In 1860, Zimmermann described certain bodies which he believed were the precursors of red blood cells and remarked on their tendency to gather in clumps. Those bodies were undoubtedly platelets.

Among the first attempts to attribute the origin of platelets to other blood elements was the work of Schultze (1865). He showed that those small elements had a strong tendency to clump and form "granular masses". He thought that platelets resulted from the destruction of white corpuscles. Schultze's theory was supported in 1872 and subsequent years by Riess, who maintained that during anaemias and cachectic states the white corpuscles fragment and give rise to smaller corpuscles which he called (disintegration bodies).

Throughout the third quarter of the 19th Century, platelets were frequently described as bacteria or artefacts. Their morphologic variability, depending on the conditions of collection and observation of the blood, was perhaps the reason for regarding them as extrinsic matter, peculiar to certain pathologic conditions.

In 1873, Vulpian noted the presence in the blood of colourless corpuscles having the properties of sticking to the cover glass and accumulating in clumps. In the same year, Ranvier claimed that granular masses, which from their size and appearance obviously were platelets, probably determine the coagulation of blood, very much in the same way as the crystal of salt brings about crystallization of saturated solution of that salt. Osler (1874) pointed out that the "granular masses" of Schultze were the result of agglutination of small bodies which were found as single units in the circulation. The important role of platelets in thrombus formation, was stressed by Hayem (1878), Bizzozero (1882), Ebert., &Schimmelbush, (1886) among many others. In spite of the limited resolving power of their microscopes, these workers gave a brilliant description of the morphology and kinetics of the haemostatic and thrombotic process.

During the first half of the 20th Century, relatively little platelet research was reported in the literature, whereas studies on plasma coagulation increased considerably. However, since the early fifties, research on haemostasis and thrombus formation has gradually shifted from blood coagulation to platelet functions. This research has centred on the morphology by electron microscopy and on platelet reactions of adhesion, aggregation and release which are initiated by vascular injury and have important roles in the mechanism of haemostasis, arterial thrombosis and thromboembolism, and atherogenesis.

Attempts to count the number of platelets in the blood seemed to have been made first in 1872 by Reiss. Technical difficulties must have interfered with the accuracy of his determinations since he reported increases in the number of platelets in diseases which we know today are accompanied by thrombocytopenia. He did, however, observe that in pernicious anaemia there was a diminution. Osler (1883) confirmed this finding.

The credit goes to Hayem in 1878 for the first accurate account of platelets Fig. 1. The figure for the average number of platelets per cubic millimeter of blood generally accepted today as representing

DÉSIGNATIONS.	HÉMA-TOBLASTES.	HÉMATIES.	GLOBULES blancs.
Nouveau-né de 24 heures.....	222 000	5 022 000	16 000
— de 3 jours.....	200 000	6 138 000	7 580
— de 5 jours.....	216 000	5 487 000	9 000
— de 8 jours.....	231 000	5 022 000	11 000
— de 13 jours.....	317 000	5 115 000	11 300
Enfant de 8 mois, non sevré.....	316 000	3 906 000	14 156
— de 8 mois, » »	276 000	4 960 000	21 340
— de 15 mois 1/2, sevré.....	318 000	3 906 000	10 000
— de 4 ans.....	260 000	5 177 000	12 000
Homme de 25 ans.....	231 000	5 487 000	5 500
— de 36 ans.....	231 000	3 979 000	6 830
— de 72 ans.....	318 000	4 991 000	7 700
Femme de 26 ans.....	231 000	4 557 000	5 300
— de 32 ans.....	220 000	4 278 000	6 500
— de 65 ans.....	216 300	5 363 000	5 650

. ▮. TABLE GIVING THE RESULT OF THE FIRST ACCURATE PLATELET COUNTS BY HAYEM (1878)

the normal does not differ significantly from that found by Hayem.

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ORIGIN OF THE PLATELETS

Origin of the Platelets

The first morphologically recognizable precursor is the megakaryoblast which arises from a pluripotent haematopoietic stem cell that becomes an intermediate committed precursor (Odell and Jackson 1968) .

The megakaryoblast is a large cell (15-50 µm) in diameter containing a large, oval or kidney shaped nucleus with several nucleoli. The cytoplasm is scanty, non granular and intensely basophilic. As the megakaryoblast enlarges, cytoplasmic granules appear, and the promegakaryocyte, a cell 20 to 80 µm in diameter, is formed . The nucleus of this cell is oval or irregular in shape; its cytoplasm is more abundant than that of the megakaryoblast and contains granules that develop first in the perinuclear zone. From this precursor the mature granular megakaryocyte, described above, is formed. The morphology of the megakaryocyte normally is quite variable in stained smears. Some observers distinguish "reserve" megakaryocytes from platelet producing forms on the basis of absence of granules in the periphery of the cell.

It was Wright in 1906 who concluded that platelets are detached portions of the cytoplasm of megakaryocytes.