INTER-RELATIONSHIP OF THE COULTER S MEASURED RED CELL INDICES

Thesis

Submitted for partial fulfilment

of master degree in

Clinical Pathology

By

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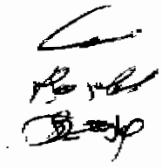

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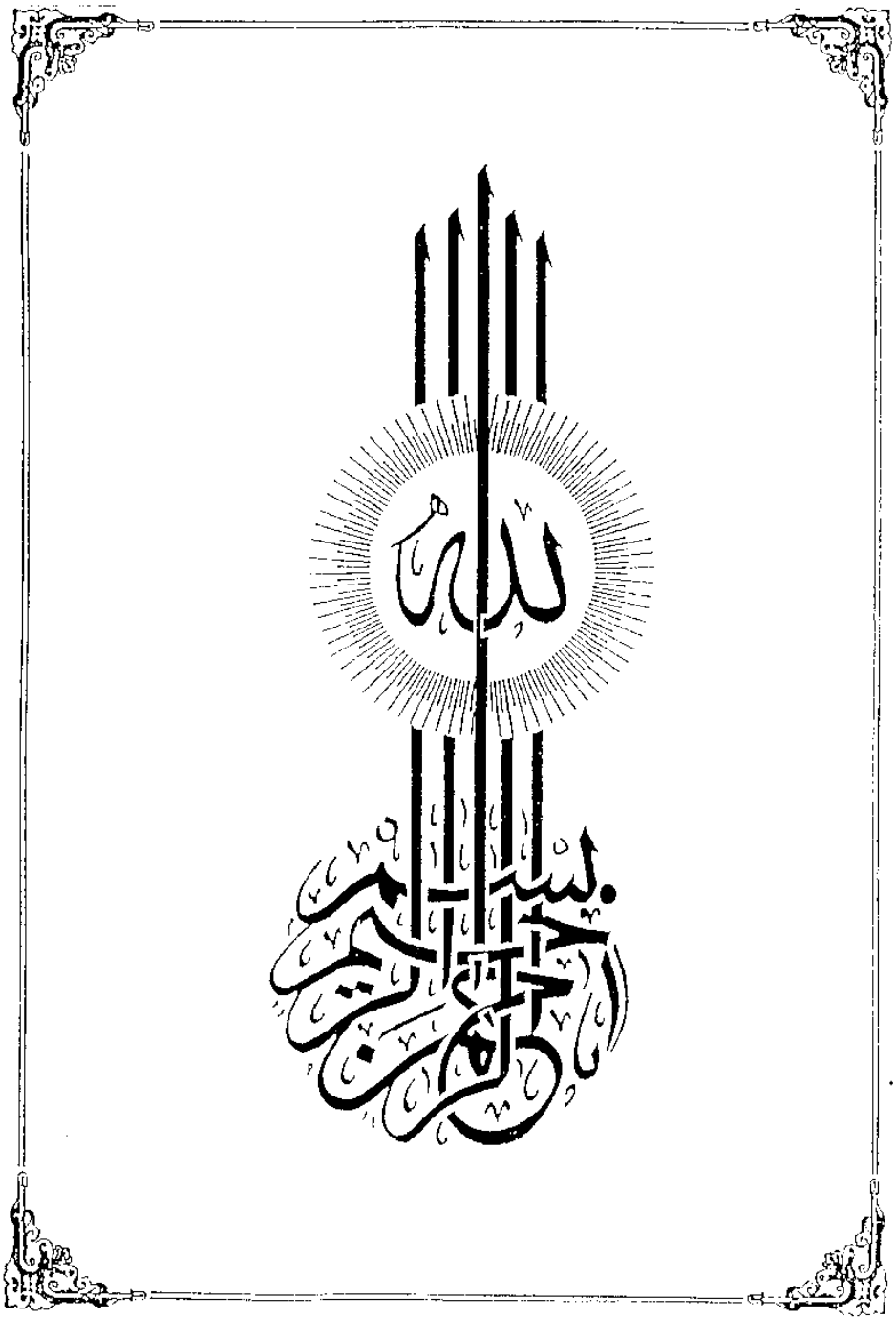

TO MY PARENTS

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to Professor Dr. Wageeh Naguib Ibrahim, Professor of Clinical Pathology, Ain Shams University, for his fatherly attitude, close supervision and moral support.

I'm greatly indebted to his valuable remarks and suggestions provided through this work.

I'm also sincerely grateful to Professor Dr. Fadila Hassan Sabry, Professor of Clinical Pathology, Ain Shams University for her valuable guidance, useful remarks and great help.

My heartful thanks to Dr. Nevine Ahmed Kassem, Lecturer of Clinical Pathology, Ain Shams University for her constant kind help and immeasurable efforts that lead to the completion of my thesis.

Thanks to my colleagues in the hematology laboratory especially Azza Sadek for her useful advice and intelligent notes.

INTRODUCTION
and
AIM OF THE WORK

Introduction

In 1932, Wintrobe devised the red cell indices using the red cell count, the hemoglobin concentration and the hematocrit value. Two years later (1934) he proposed a classification of anemias based on the differences in average volume and hemoglobin content of erythrocytes. However, the recognition of the inherent error of red cell count by visual techniques has minimized the value of these indices (Dacie and Lewis, 1984).

The introduction of electronic cell counters, the standardization of hemoglobinometry and microhematocrit have created a great advance in the use of these indices. Such advance has been greatly reinforced by the use of fully automated blood analyzers such as the Coulter S counter (Hall and Malia, 1984).

The widespread use of these fully automated instruments with capability of handling large numbers of samples at a high speed and with a maximum degree of analytical precision, has created a desire for a new interpretation of these indices and their related diagnostic significance (Klee et al., 1976).

A study of interrelationship and correlation between red cell indices was performed by Hamilton and Davidson (1973)and they derived the following regression line: MCV = 2.5 MCH+16

Since that time no further study or criticism of this formula could be traced in the available literature. Besides, this correlation between MCV and MCH is lost in certain well known circumstances such as spherocytosis, marked dehydration and hyperosmolarity states. (Hall and Malia, 1984).

Our aim of work is to study and statistically analyze the relation between various blood indices and recognize any deviation after applying the formula of Hamilton and Davidson (1973). Special attention will be paid to the relation between red cell morphology and the above mentioned indices.

Meanwhile, an internal quality control system based on the internal consistency of the mean values of patient data will be performed.

REVIEW OF LITERATURE

THE COULTER S COUNTER

PRINCIPLE AND DESCRIPTION

The Coulter impedance principle:

Coulter principle depends on counting cells by measuring their ability to alter electrical resistance (Coulter, 1956). Cells suspended in an electrically good conductor diluent are forced to flow regularly through a small aperture. This aperture is made in an insulated bridge placed between two platinum electrodes. The whole system is immersed in the same diluent, and a regulated constant current is passed between the two electrodes. As cells are relatively good electrical conductors, their passage will cause a measurable change in electrical flow. This produces a voltage pulse of short duration having a magnitude proportional to particle size (Nelson and Morris, 1984).

Coulter counters can discriminate between particles of different sizes. Small particles, which are below the lower limits of cell size can thus be excluded (Wintrobe, 1981).

DESCRIPTION:

The Coulter counter model S7* is a fullyautomated analytical instrument which provides simultaneous measurement of seven blood parameters. It performs all necessary dilutions on whole blood sample or accepts a prediluted sample. Cell counts and mean corpuscular volume (MCV) measurements are made applying the Coulter principle of voltage pulse counting and size analysis. Hemoglobin is measured by the cyanomethemoglobin method. While, hematocrit, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are calculated by computation (Brittin et al., 1969a).

The instrument consists of two chambers for counting and sizing red cells and two chambers for counting white cells; in addition there is a photosensitive device for measuring hemoglobin concentration (Coulter manual, 1981)(Fig.1)

A pneumatic power supply provides vaccum and pressure to aspirate the sample and move the diluting fluid and the dilutions through the system. The time taken from aspiration of the sample into the apparatus until presentation of the data from the printer is 40 seconds. However, the machine can accept a new sample every 20 seconds as it counts one sample while printing the other (Pinkerton et al., 1970)

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When analysis is performed on whole blood the instrument aspirates about 1 ml of anticoagulated blood. The major part of this amount is used for flushing away the previous sample, while only 44.7 µl is used for the estimations. This volume is diluted 1 :224 in isotonic particle free fluid and the dilution is splitted into two parts; the first part is further diluted 1:224 and from the resulting 1:50,000 dilution the red cell count and the mean cell volume are determined. The second part is mixed with a lysing agent which destroys the red cells, converts hemoglobin to cyanomethemoglobin and brings a final dilution of 1:250. This dilution is used for white cell count and, by passing through a photosensitive device, for hemoglobin estimation. The white and red cell counts and the mean corpuscular volume measurments are made simultaneously in duplicate and each group is averaged. These data are printed out unless one result disagrees with the other by 3 standard deviations from the mean (Couler manual, 1981). The hemoglobin is automatically zeroed on a diluent rinse before each hemoglobin is measured.

A manually prediluted sample of 44.7 µL in 10 ml diluent can be presented to the instrument through a separate aspirator. This aspirator by-passes the first diluting step. This process is of much value in dealing with capillary blood or with samples of small volume.

The data from each of the four counting pathways are monitored electronically and corrected for coincident particle passage. The MCH, MCHC and hematocrit are calculated and the seven results are received through an automatic digital printer on special cards (Nelson and Morris, 1984).

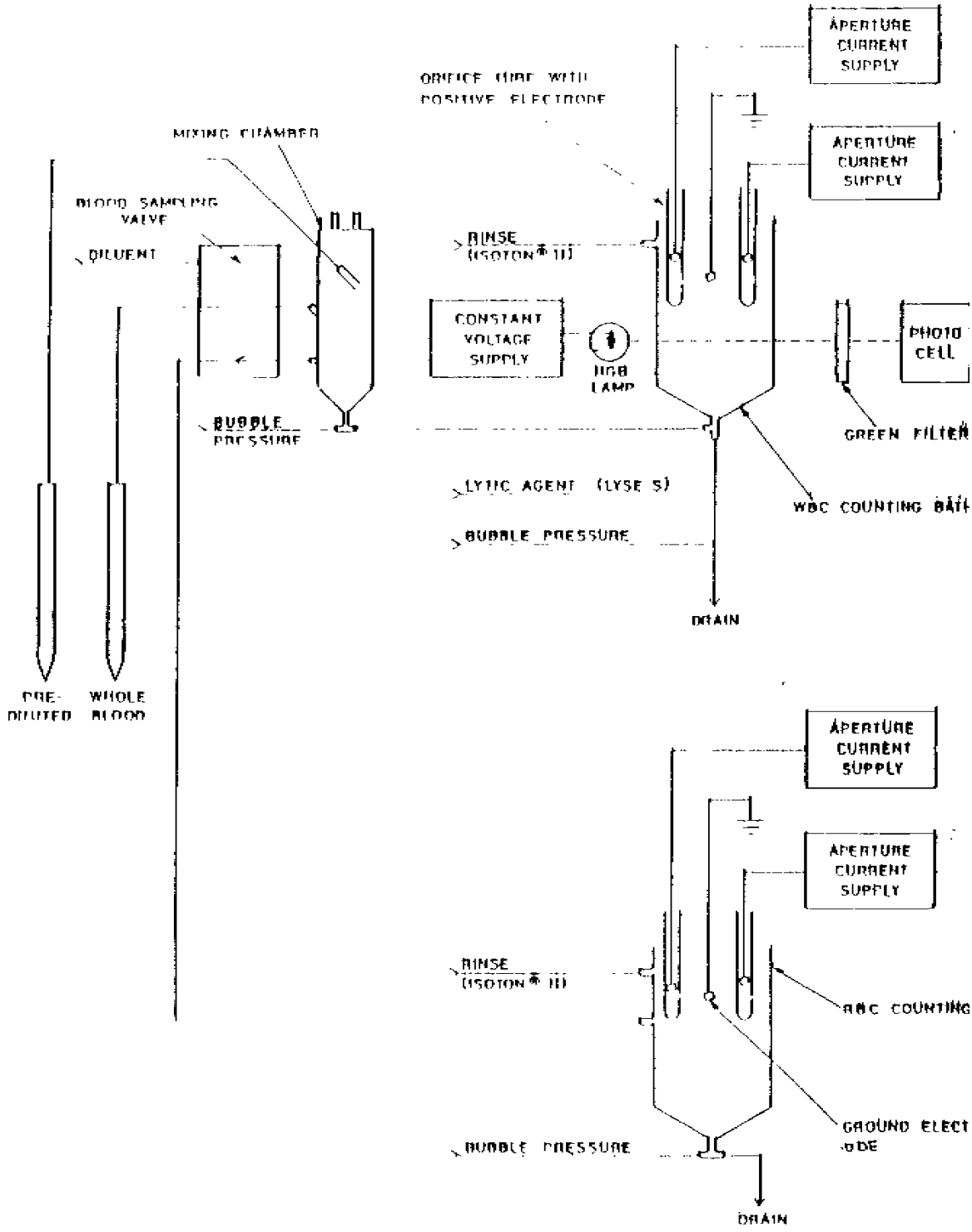

Fig. 1 : Illustrative diagram of the Coulter S7 counter (Coulter manual, 1981)

Factors that influence the electric impedance method of cell sizing:

The original electrical impedance theory states that particle volume and impulse size are directly related to each other (Coulter, 1956). However, it is now accepted that the pulse produced by a particle in the aperture results from disturbance of electrical lines of force in addition to displacement of the conducting liquid (Rowan, 1983). The following factors are known to influence the pulse size:

1- Cross sectional area of the orifice:

This variable is the most important one to be considered. Even slight blockage of the orifice will produce considerable increase in pulse size which will be misinterpreted as larger particles (Keopke, 1984)

2- Resistivity of the suspending medium:

Increased resistivity of the suspending medium will lead to false perception of small sized pulses. When a suspending medium of known conductivity is used, the important factor affecting resistivity will be changes in temperature. Elevation of temperature will lead to decreased resistivity with consequent increase in pulse size (Young and Lawrance, 1975)