BONE MARROW CULTURE

ESSAY

Submitted for partial fulfillment of

Master Degree in Clinical Pathology

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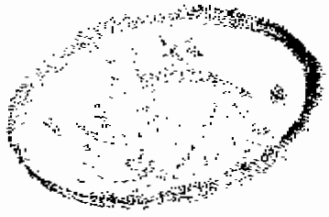


Faculty of Medicine

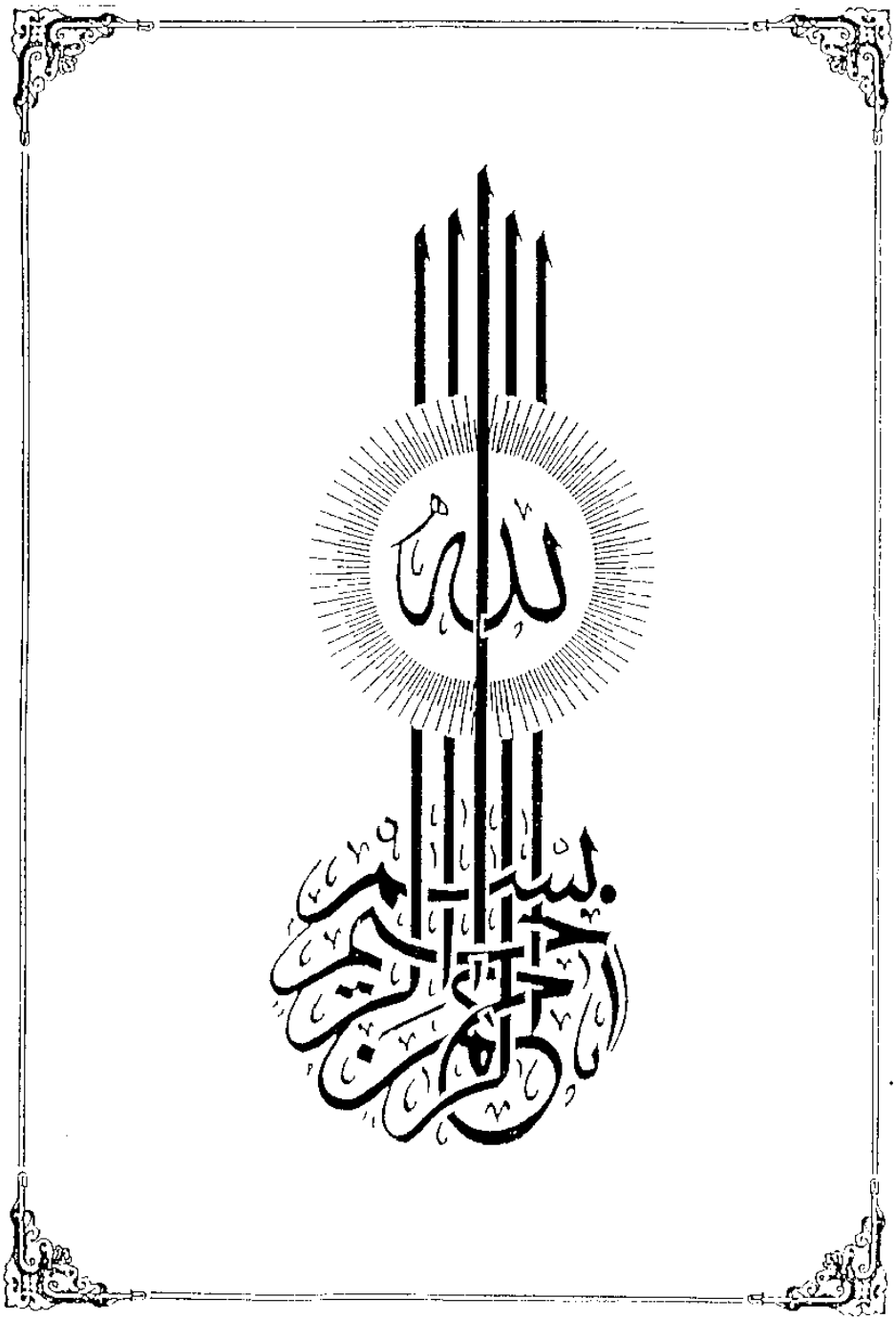
Ain Shams University

1991

بسم الله الرحمن الرحيم

« قالوا سبحانك لا علم لنا إلا ما علمتنا
إنك أنت العليم الحكيم »

صدق الله العظيم
سورة البقرة ، الآية ٣٢



To my family

ACKNOWLEDGEMENT

I would like very much to seize this special opportunity to express my sincere appreciation and great gratitude to **Prof. Dr. Sawsan Abd El Molty Fayaad,** Professor of Clinical Pathology, for giving me the privilege to work under her supervision and also for her valuable suggestions and encouragement.

I would like to express my deep gratitude and thanks to **Dr. Hala Mahmoud Hamdy Abaza,** Lecturer of Clinical Haematology for her energetic help in following every step in this work, to ensure that it would reach an updated valuable level.

ABBREVIATIONS

2-ME	2-mercapto ethanol
AA	Aplastic anaemia
ABMT	Autologous bone marrow transplantation
AL	Acute leukaemia
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloblastic leukaemia
AMS	Anaemic mouse serum
ANLL	Acute non lymphoblastic leukaemia
ATP	Adinosine triphosphate
AUL	Acute undifferentiated leukaemia
BFU-E	Burst forming unit-erythroid
BL-CFU	B-lymphocyte colony forming unit
BM	Bone marrow
BMT	Bone marrow transplantation
CFC	Colony forming cells
CFC-GM	Colony forming cells-granulocyte, monocyte
CFU-C	Colony forming unit-culture
CFU-E	Colony forming unit-erythroid
CFU-G	Colony forming unit-granulocyte
CFU-GEMM	Colony forming units-granulocyte, eosinophil, monocyte, macrophage
CFU-MG	Colony forming unit-megakaryocyte
CFU-Mix	Pluripotent progenitors
CFU-S	Colony forming unit-spleen
CLL	Chronic lymphocytic leukaemia
CMK	Human megakaryoblastic cell line
CML	Chronic myeloid leukaemia
CSA	Colony stimulating activity
CSF(s)	Colony stimulating factor(s)
DNA	Desoxyribonucleic acid
EDTA	Ethylene diamine tetra acetate
FAB	French American British Cooperative Group of Workers
FACs	Fluorescent activated cell sorter
FCS	Foetal calf serum

Introduction and Aim of Work

INTRODUCTION
AND AIM OF WORK

It is now well established that murine multipotential cells can be assayed in-vitro by their ability to produce clones containing multiple haemopoietic lineages. The phenomena of normal and abnormal haematopoiesis appear to be accurately reproduced in culture, thus retaining the same relationship between function and structure as occurs in-vivo (*Yamazaki et al.,* 1989). These in-vitro studies have provided methodological approaches for the assay of similar human cells (*Johnson,* 1984). Chronologically, the assay for human granulocyte-macrophage progenitors (CFU-GM, CFU-C) was the first to be developed, followed by that for human erythroid progenitors (BFU-E, CFU-E), and more recently for megakaryocyte progenitors (CFU-MG), and pluripotent progenitors (CFU-GEMM, CFU-MIX) (*Moore,* 1982).

The clonal cultures of human bone marrow (BM) cells are useful methods for the investigation of the regulatory system of normal and disturbed haemopoiesis (*Muller,* 1989). These in-vitro studies are also reliable for studying human haemopoietic stem cell proliferation and differentiation, providing valuable insights into the mechanism and

possible management of all of the clonal haemopoietic disorders (*Adamson,* 1984).

Colonies formed under selective culture conditions have been used to determine some of the properties of blast progenitors of acute myeloblastic leukaemia (AML) e.g., being uniformly in active cell cycle, their sensitivity to chemotherapeutic agents and capacity to undergo self-renewal, which is correlated with the clinical outcome (*McCulloch,* 1984). Moreover, serial in-vitro BM fibroblast culture can be used to monitor myelofibrosis frequently encountered in leukaemia (*Nagao et al.,* 1983).

Long term BM culture methods (LTBMC) appear to provide a suitable in-vitro model for studying leukaemogenesis. Thus, the cellular and molecular requirements of the developing leukaemia cell and the alterations in the metabolic pathways that results in their abnormal growth, can be dissected (*Umiel et al.,* 1986). LTBMC are also useful in the detection of altered CFU-GM pattern in myelodysplastic BM even in those cases that show normal CFU-GM growth at diagnosis (*Gebbia et al.,* 1989). This technology may also have a major application with the emergence of modulators of growth and differentiation of haemopoietic cell lines (*Stoppa et al.,* 1989). Moreover, LTBMC have potential

applicability in selecting normal haematopoietic progenitors from patients with multiple myeloma. This approach has significant implications for aggressive treatment of multiple myeloma patients, especially in trials involving autologous BM transplantation (ABMT) (*Visani et al.*, 1989). Additionally, BM cells grown in LTBMC can recently be used for ABMT in AML (*Chang et al.*, 1989).

In the field of BM purging for ABMT and T-cell elimination for graft-versus-host disease prophylaxis in allogenic transplants, BM cultures have been used to control BM processing. The use of pure colony stimulating factor (CSF) preparations and cell separators makes the technique well reproducible (*Muller*, 1989). Furthermore, a limiting dilution culture technique using microscopic analysis is proved to be the method of choice for the detection of residual T-cells in T-cell depleted human BM (*Williamson et al.*, 1989).

Aim of the Work

The aim of this work is to review the methods used for BM culture together with their valuable applications.

Review of Literature

CHAPTER I

BM STRUCTURE AND HAEMATOPOIESIS

Bone Marrow

The human BM is the principle site for blood cell formation. Its daily production, in the normal adult, amounts to about 2.5 billion red cells, 2.5 billion platelets, and 1.0 billion granulocytes per kilogram body weight. This rate of production is adjusted to the actual need and can be varied from nearly zero to many times normal (*Erslev and Lichtman,* 1990).

The emergence of cavities within bone occurs in the human being at about the fifth fetal month, and these cavities soon become the exclusive site for granulocytic and megakaryocytic proliferation. Erythropoietic activity at that time is confined to the liver and spleen. At the last trimester, the microenvironment in the marrow becomes attractive to erythroblasts [Fig. 1] (*Erslev and Weiss,* 1983).

At birth, haemopoietic (red) marrow occupies the entire capacity of the bones. From childhood, there is a gradual replacement of the red marrow by fatty (yellow) marrow until, in the adult, red marrow is

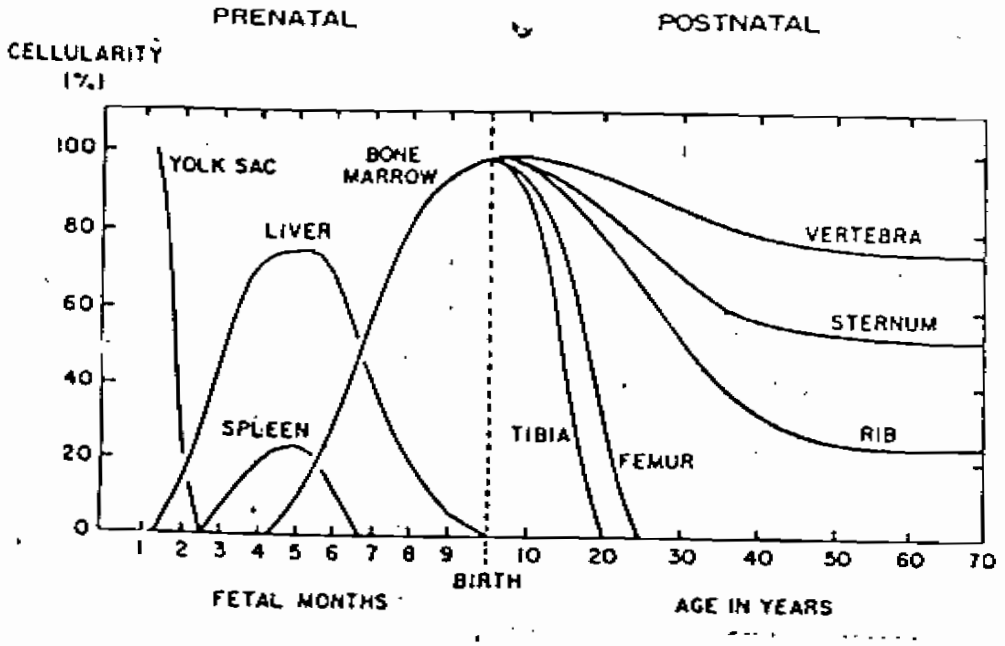


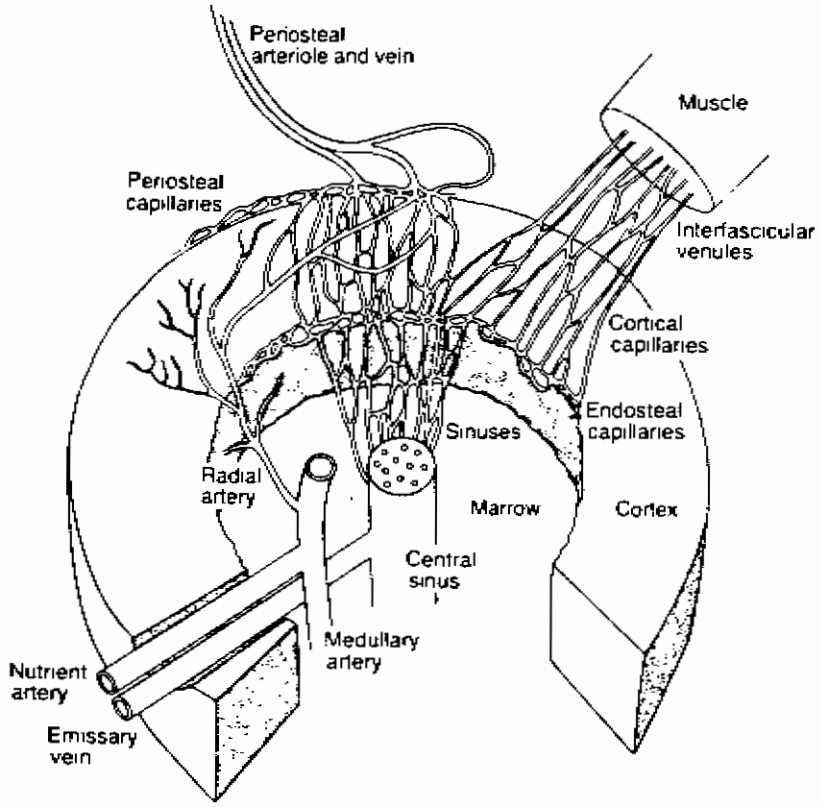
Fig. 1: Expansion and recession of haemopoietic activity in extramedullary and medullary cites.

confined to the sternum, ribs and vertebrae, cranium and pelvis. In old age these sites, too, become increasingly replaced with fatty marrow (*Lewis,* 1989).

Blood supply to the bone is derived from the nutrient artery of the bone and the cortical capillaries, which communicate to from an endosteal network that, in turn, drains through marrow sinuses into a central sinus, from which the blood enters the systemic venous circulation through emissary veins. This arrangement may provide haemopoietic cells with a high concentration of chemicals from the cortical bone or may possibly expose haemopoietic cells to a low O_2 tension (PO_2) claimed to facilitate blood cell formation (*Bradley et al.,* 1978).

Between the sinuses, lie cords of haemopoietic cells, as they are formed extravascularly. The mature cells enter the lumen of the sinuses through apertures in their walls (*Weiss,* 1970), or active transport through the endothelial cells (*Farr and De Bruyn,* 1975), or via the endothelial junctions (*Tavassoli and Shaklai,* 1979).

Large numbers of reticuloendothelial cells occur in the marrow in relation to the sinuses. The role of these cells in haematopoiesis is not known, but there is experimental evidence suggesting that the



Quoted from Quesenberry (1987).