بسم الله الرحمن الرحيم

وما اوتيتم من العلم إلا قليلا

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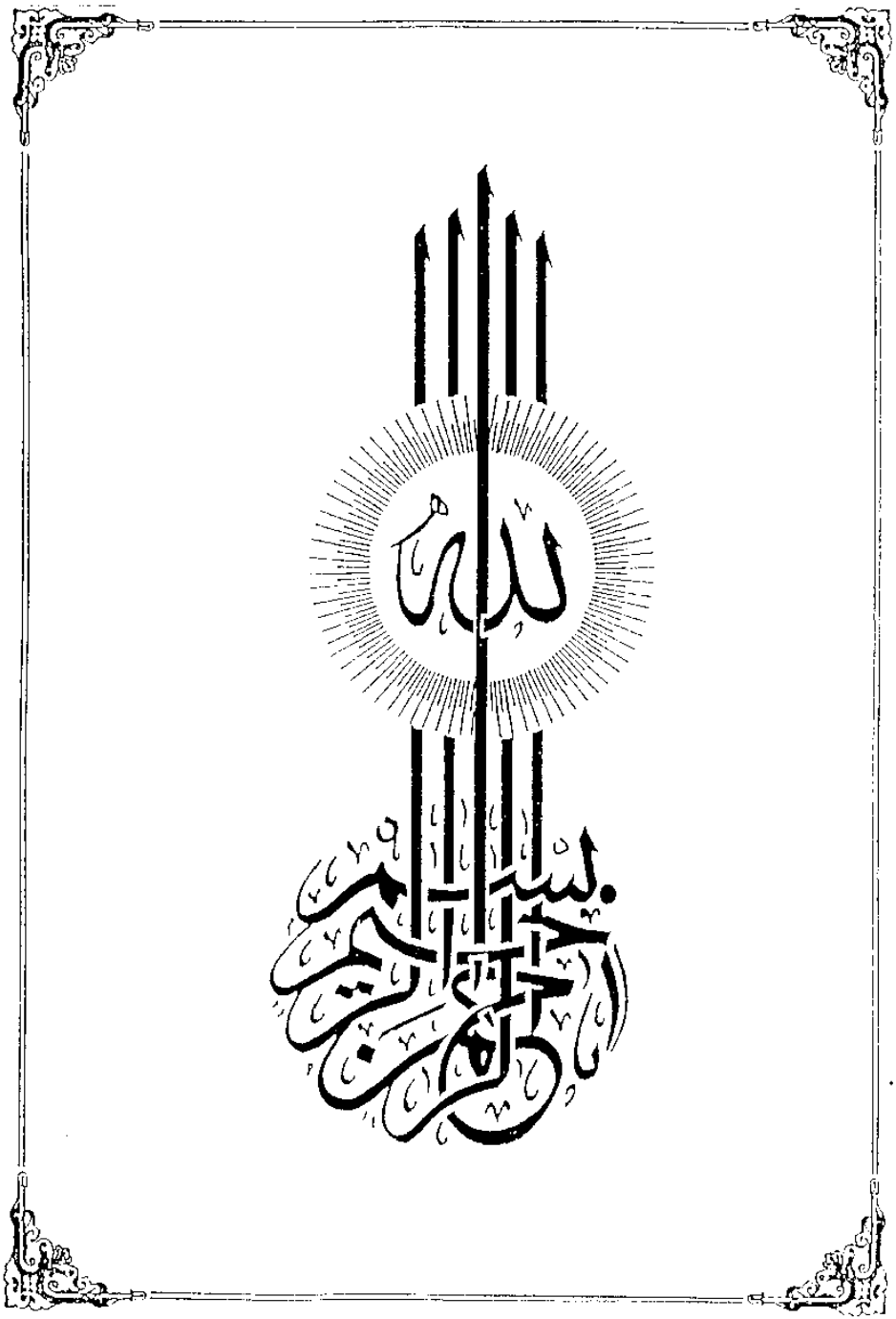

NITROBLUE TETRAZOLIUM REDUCTION BY NEUTROPHILS IN PREMATURE INFANTS

T H E S I S

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Introduction

and

Aim of the Work

INTRODUCTION & AIM OF THE WORK

Neonatal infection remains a major clinical problem in many newborn nurseries, with a high risk of mortality and morbidity (Wilson and Eichenwald, 1974).

Neonates, especially when premature and of low birth weight, have an increased susceptibility to infection. The basis of this infectious tendency has been the subject of considerable investigation and has been attributed to defects in both humoral and cellular defence mechanisms (Al-Hadithy et al., 1981).

Among the various defence mechanisms available to the host in its struggle with invading microorganisms, polymorphonuclear neutrophil leucocytes (PMNLs) play an important role in the critical early stages of bacterial infections and, thereby, are a significant determinant of the eventual outcome of these infections. Effective phagocytic activity by PMNL, early in the course of bacterial invasion, may limit the spread of bacteria and prevent ongoing infection while ineffective phagocytosis may lead to uncontrolled bacterial multiplication and overwhelming infection (Winkelstein and Drachman, 1974).

The nitroblue tetrazolium test (NBT) has been promoted

in the last 10 years as a useful laboratory aid in differentiating systemic bacterial infections from other disease states. It has also been strongly favoured as a screening test for chronic granulomatous disease of childhood and for detecting neutrophil dysfunction (Lace et al., 1975).

Aim of the Work :

Since there is an increased susceptibility to infection in premature infants, it will be of value to screen the neutrophil function among those infants.

The aim of the present work is to evaluate the neutrophil function in premature infants by the extent of nitroblue tetrazolium reduction by neutrophils to find out if there is defective polymorphonuclear phagocytic activity which would be added to the causes of increased susceptibility of these preterm babies to infection.

Review of Literature

POLYMORPHONUCLEAR NEUTROPHIL LEUCOCYTES

Neutrophil development :

The major site of neutrophil development from primitive myeloblast to the fully mature PMNL occurs entirely within the bone marrow (Vincent, 1977).

The myeloblast is the earliest recognizable precursor of granulocyte progenitor cell. Myeloblasts are about 15 u in diameter, have 2 to 5 relatively large nuclei, little cytoplasm and no granules. They divide and differentiate to produce promyelocytes. These are larger cells, have rounded nuclei and are the first granulocytic cells with recognizable granules. Promyelocytes divide by mitosis to give myelocytes. Myelocytes are smaller, about 12 u diameter and have slightly idented nuclei. They multiply and differentiate into metamyelocytes then give the band and segmented forms (Lichtman and Weed, 1972).

Under normal circumstances, only differentiated neutrophils (bands and segmented) are released into the circulation from their reserves in the bone marrow. The formation of these cells is under stimulating effect of a glycoprotein substance called colony stimulating activity (C.S.A) elaborated by monocytes and macrophages (Cline et al., 1974).

Morphology and Ultrastructure :

In fixed preparation stained with Romanwisky dyes under the light microscope, the mature neutrophil has pale pink cytoplasm which contains neutrophilic granules and a nucleus that is deeply purple in colour and consists of up to 5 lobes joined by fine strands of chromatin. The average diameter of the mature neutrophil is about 10-12 u (Junquiera et al., 1977).

At least 4 physically and biochemically distinct populations of neutrophilic granules exist : (Spitznagel et al., 1977)

1 and 2 : Two populations of azurophil or primary granules, they arise from the proximal concave side of the Golgi apparatus, stain blue and are formed during the promyelocyte stage (Bainton et al., 1971). They contain a number of enzymes and other substances, such as acid phosphatase, myeloperoxidase, esterase, B-glucuronidase, B-galactosidase, aryl sulfatase, 5 nucleosidase, sulfated mucosubstance, lysozyme and other basic proteins (Bainton et al., 1971; Dewald et al., 1975). The 2 populations of the azurophil granules differ in the fact that one population contains more myeloperoxidase, neutral protease and B-glucuronidase, and the other contains more lysozyme (Spitznagel et al., 1974).

3 : The specific or secondary granules, they arise from the distal convex surface of the Golgi apparatus, formed during the myelocyte stage and are peroxidase negative. They contain alkaline phosphatase (Bainton et al., 1971), aminopeptidase, collagenase (Murphy et al., 1977), lysozyme and lactoferrin (Leffell and Spitznagel, 1972).

4 : A population of tertiary granules that are poorly defined (Bretz and Baggiolini, 1974) thought to appear during the late myelocyte, metamyelocyte and segmented neutrophil stages (Payne and Ackerman, 1977). Some regard them as merely a morphologic variation of primary and secondary granules (Bainton et al., 1971). They contain acid hydrolases, glycerophosphatase and acetyl-b-glucosaminidase (Scott and Horn, 1970).

Thus the life history of PMNL occurs in 3 environments. In general, the contents of the primary granules have a lower pH optimum than the contents of the specific granules. Careful studies using differential centrifugation techniques permit further separation of these two granules into subtypes (West et al., 1974).

During maturation, the nucleus becomes segmented and microfilaments and microtubules appear, the latter structures are important for motility and degranulation and

have been referred to as the cytoskeletal components. Microfilaments are thought to be composed of polymers of actin, a contractile protein. It has been estimated that actin may comprise 10% of the total cellular protein. Myosin, another contractile protein, accounts for only 1% of the cellular proteins. Although these proteins have clearly been implicated in cell motility, the actin to myosin ratio is higher in neutrophils than in muscle cells and the details of how their interaction may produce movement in phagocytic cells have not yet been clarified. Microfilaments are most readily observed in the subcortical regions of the cell but probably form a meshwork throughout the entire cell (Stossel, 1977).

In contrast to microfilaments, microtubules are larger hollow structures which course straight through the cytoplasm for long distances. They are composed of dimers of tubulin which can be rapidly polymerized to form the microtubules (Bucher, 1972).

Neutrophil Kinetics :

The bone marrow produces large numbers of neutrophils (1 to 2 $x10^9$ cells/kg/day) to compensate for their short life span in the circulation which is about 6 to 7 hours as a half time of disappearance (Dancey et al., 1976).

Once in the circulation, the granulocytes are nearly evenly divided between those in the circulation and those along the walls of smaller vessels, the so-called marginal pool. The granulocytes in the marginal pool are easily released into the circulation by epinephrine or exercise, indicating that from a kinetic point of view the circulating and marginal granulocyte pools can be considered as one (Davis and Quie, 1980).

The marrow contains large reserves of mature cells which may reach up to 10 times the normal daily neutrophil requirements. The numbers of neutrophils in the blood stream increase within a few hours in response to inflammatory stimuli, as cells are released from these large marrow reserves and move from the marginated to the circulating pool of cells (Vincent, 1977).

Fate of neutrophils :

Neutrophils either die in the tissues after performing their function in cellular defence or are lost from mucosal surfaces. Once they have migrated to tissues, they probably live for only 1 or 2 days (Golde, 1983).

Normal values of neutrophils :

(in the first 2 years of life)

1st day	:	57%
2nd day	:	55%
6th day	:	50%
2 weeks	:	34%
1 month	:	34%
2 months	:	33%
3 months	:	33%
6 months	:	36%
1 year	:	39%
2 years	:	42%

(O'Brien and Hammond, 1982).