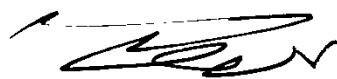


BIOCHEMICAL STUDIES ON THE EFFECT OF NEW
BIOLOGICALLY ACTIVE COMPOUND ON THE BEHAVIOUR
OF LIVING CELLS *IN VIVO*

Thesis

Submitted

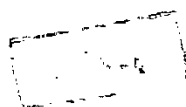
By



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(B. Sc. Biochem., 1983)

In Partial Fulfilment for the Degree of
M. Sc. in *Biochemistry*

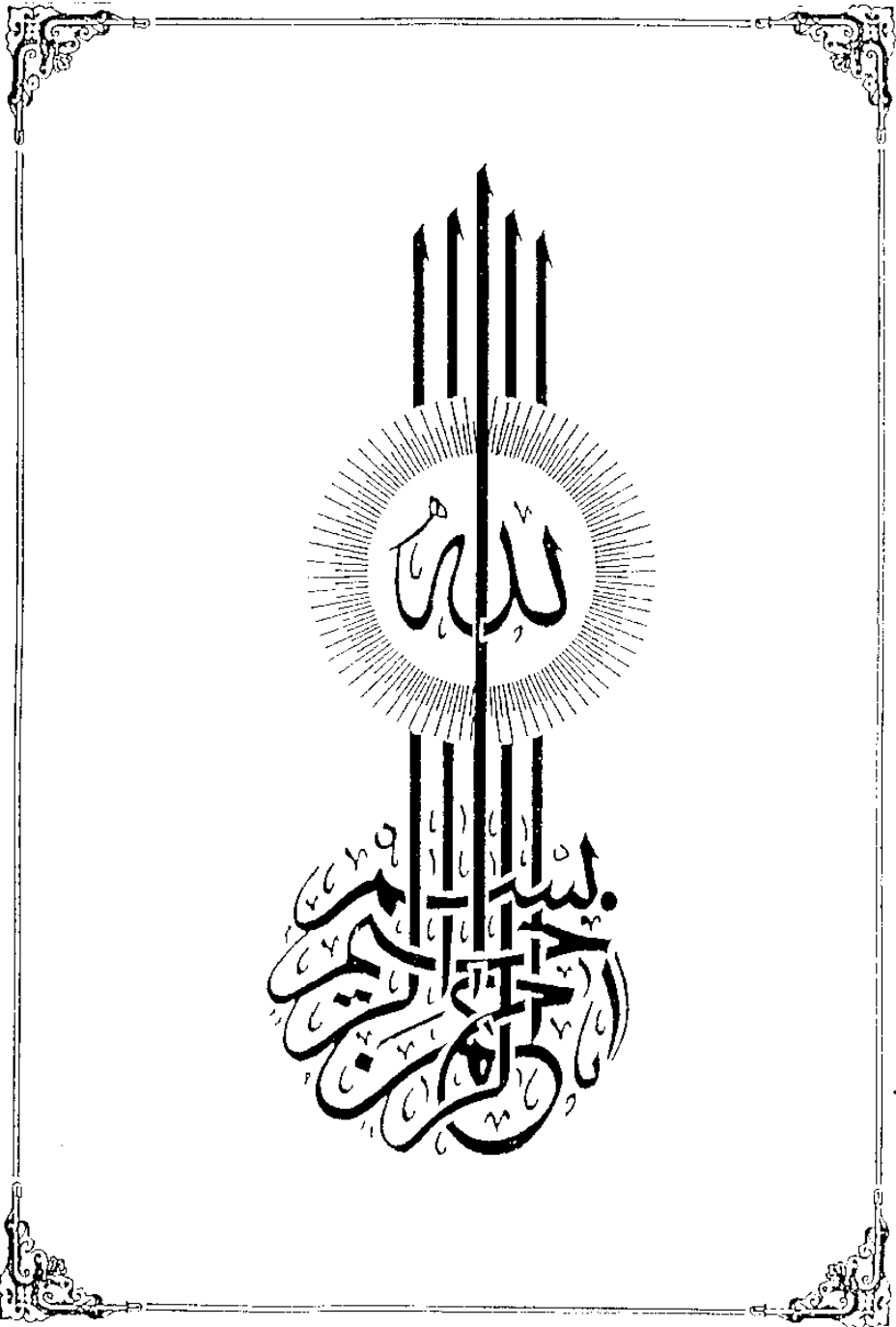


Ain Shams University
Faculty of Science
Department of Biochemistry



1987

بِسْمِ الرَّحْمَنِ الرَّحِيمِ
وَحَمْدُهُ سَائِلُهُ تَعْلَمُ
وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا
.. صدق الله العظيم ..



To My Parents

THIS THESIS HAS NOT BEEN SUBMITTED FOR
A DEGREE AT THIS OR ANY OTHER UNIVERSITY

Magda A. Amin

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ABBREVIATIONS

A/G ration	Albumin per globulin ratio
ALT	Alanine transaminase
AST	Aspartate transaminase
<u>B. C.</u>	Bacillus cereus
<u>B. S.</u>	Bacillus subtilis
CE	Cholinesterase
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
DNPH	Dinitrophenylhydrazine
<u>E. coli</u>	Escherischia coli
Fig.	Figure
g	Gram
i.p	Intraperitoneal
I.U.	International unit
i.v	Intravenous
Kg	Kilogram
LD ₅₀	Lethal dose ₅₀
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
µg	Microgram
mg	Milligram
MIC	Minimum inhibitory concentration
mmol	Millimole
N.S.	Nonsignificant
NAD ⁺	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
O.D.	Optical density
P. value	Probability
r.p.m.	Revolutions per minute
RNA	Ribonucleic acid
<u>S. typhosa</u>	<u>Salmonella typhosa</u>
S.D.	Standard deviation
S.E.	Standard error
<u>St. epidermids</u>	<u>Staphylococcus epidermids</u>
<u>St. aureus</u>	<u>Staphylococcus aureus</u>
t-RNA	Transfer RNA
TCA	Trichloroacetic acid
U	Unit
VLDL	Very low density lipoprotein

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INTRODUCTION

INTRODUCTION

One of the major triumphs of medical science in the twentieth century has been the virtual eradication of many infectious diseases by the use of chemotherapeutic agents. Antimicrobial drugs are those chemotherapeutic agents which are toxic to microorganisms. They are amongst the most important, widely used, and effectiveness compounds in medicine. They are multitude agents available in treatment of fatal and severely disabling illness places. Infections unlike many of the other major categories of diseases, can attack the perfectly normal person and curing of this infection can restore the patient to his original state of health. Thus, the synthetic antimicrobial agents play a large part in the control of various infectious diseases. The use of these agents is indicate the rapid development of the synthetic techniques (Franklin, 1981).

The synthetic compounds represent the largest group of the therapeutic agents. They are compounds neither occurring naturally, nor derived from natural products. Many antimicrobial agents are manufactured synthetically rather than extraction from the tissues (Briggs & Briggs, 1977).

The chemotherapeutic agent is a synthetic chemical substance, designed to treat infections by destroying the infecting organisms when administered to the host without injuring the tissues of the host. The range effects of these agents extend from unicellular viruses to multicellular bacteria (Busch and Lane, 1967).

The earliest chemotherapeutic agents known to man were of plant origin. The Greeks used the extracts of *Cinchona bark* to treat malaria. Mercury, was the first substance used to treat syphilis. It is not of plant origin

to be used chemotherapeutically. Until the beginning of the twentieth century preparations such as those were the only agents available.

Chain and Florey (1941), demonstrated the effectiveness of penicillin in man as a chemotherapeutic agent. The search for other antimicrobial agents was undertaken in many centres and to date about 5000 different substances have been discovered, many of which have found a use in chemotherapy of human infection (Williams and Kruk, 1983).

The concept of cell surface receptors were defined as sites which were recognised by chemicals of a particular structure and to which the chemical become attached. Different cells might have different receptors to which chemicals could bind selectively. These chemicals which bound to and were toxic towards pathogenic cells or organisms would be ideal drugs to treat infections.

Biochemists are concerned with the basic cellular processes against which the antimicrobial agents are effective, and often introduce the teaching of the subject by referring to the more important drugs when describing the metabolic processes that they affect. Pharmacologists are concerned with the absorption, distribution, metabolic transformation and the effects of the antimicrobial agents amongst many other drugs (Williams and Kruk, 1983).

The mechanisms of action of antimicrobial agents may be discussed within molecular and cellular levels which involve, the interaction of the drug and parasite exclusively. Carter and McCarty (1985), reported that the antimicrobial agents in current clinical use may be classified into four major categories as follows:

I. Agents that Alter Structure and Function of the Cell Wall

The cell wall is a component of the cell envelope of the most micro-organisms and plants but it is absent from outer structures of animal cells. All cell walls contain a rigid component "Sacculus", the chemical composition of which is characteristic of taxonomical group of organisms (Perkins, 1963).

The sacculi of plant cell walls consist of cellulose, these of most fungi are composed of chitin, where as most bacteria contain peptidoglycan in their cell wall. Several antimicrobial agents are known to inhibit the biosynthesis of cell wall polymers in the bacteria or in fungi such as penicillins and cephalothin which are bactericidal for sensitive bacterial cells (Zahner & Mass, 1972) and prevent the cross-linking of the glycopeptide (Anderson *et al.*, 1965).

Strominger *et al.* (1975) reported that cycloserin is a structure analogue of D-alanine, which is one of the substrates of the peptide side chain within the cell wall. This drug appears to prevent assembly of the side chain containing D-alanine by competitive inhibition of one or both of the enzymes, alanine racemase or D-alanyl-D-alanine synthetase (Strominger *et al.*, 1976; Neuhaus & Lynch, 1977).

Tipper and Strominger (1976), found other antimicrobial agents which inhibit the action of the enzyme glycopeptide synthetase, which is responsible for condensation of the glycopeptide backbone bone of the cell wall.

II. Agents that Restrict Function of the Cell Membrane

Gale *et al.* (1981) stated that several antimicrobial agents produce their effect by acting upon the cell membrane by at least three mechanisms:

The first appears to act by producing a disorganization of the membrane structure. The second which inhibits the membrane-bound protein involved in transport processes. The third which produces specific changes in the permeability towards specific ions.

Cell membranes have very similar constituents throughout the phylogenetic ladder from bacteria to mammalian cells; but with one important difference which is the lack of sterols in bacterial cell membranes, zymosterol and ergosterol are present in the cell membranes of fungi and plants and cholesterol in the mammalian cells (Lancini and Parenti, 1982).

The ratio of proteins to lipids in the bacterial membranes tends to be higher than that observed in other biological membranes with the exception of the inner mitochondrial membranes (Wallash, 1977).

Teuber and Cerny (1970), observed leakage of periplasmic ribonuclease from *E. coli* cells in the presence of "polymyxin B." Sebek (1967) suggested that polymyxin reacts with lipophilic and lipophobic groups of the cytoplasmic membrane and its molecule is oriented in the interphase between the lipid and protein layers of the membrane.

Two infrequently antimicrobial agents used, gramicidin and tyrocidin, uncouple oxidative phosphorylation, thus decrease respiration with leakage of aminoacids from the cells (Hotchkiss, 1977). Polymyxin E acts as cationic detergent with affinity for phosphate radicals and thereby alter the osmotic barrier function of the cell membrane (Newton, 1964).

D'Agnolo *et al.* (1973) stated that the the antimicrobial agents which inhibit the biosynthesis of lipids; generally affect membrane functions. Cerulenine was known to inhibit fatty acids synthesis in bacteria. Citrinin was

characterized as an inhibitor for sterol and fatty acids synthesis in rat liver. While citrinin was inactive in cell-free polymerization system, it was assumed that its inhibition for nucleic acids and proteins is a secondary effect which is related to its known effects on cell membranes and lipids synthesis (Kuroda & Endo, 1976; Betina *et al.*, 1980).

III. Agents that Impede Replication and Impair Translation into Protein Synthesis

A variety of antimicrobial agents are known to be inhibitors of nucleic acids synthesis and can thus, interfere with replication and/or transcription at various stages.

Betina (1983), stated that an antimicrobial agent may interfere with the function of DNA as a template by at least one of the following interactions: Cross-linking by covalent bonds; intercalation; non-covalent interactions and degradation of DNA molecules. Acridines are classical intercalating drugs, they were among the earliest antimicrobial agents known and long been in use as antimicrobial agents; their toxicity to bacteria and malarial parasite involve inhibition of their DNA and RNA synthesis (Albert, 1973 and Bryant, 1980).

Most of the interchelating agents in addition to their antimicrobial activity have cytotoxic carcinostatic; mutagenic or carcinogenic properties as actinomycin D and adriamycin (Betina, 1983).

Nalidixic acid specially inhibit the supercoiling of the two strands of DNA during the replication of circular DNA of *E. coli* (Gellert *et al.*, 1976 and Suingo *et al.*, 1977). Griseofulvin which is structurally related to purine nucleotides blocks DNA synthesis in sensitive micro-organisms, its structural