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UNIVERSALITY OF THE ENZYME MOLECULE

By

Olusola Olusoji Shonukan
Professor of Microbiology

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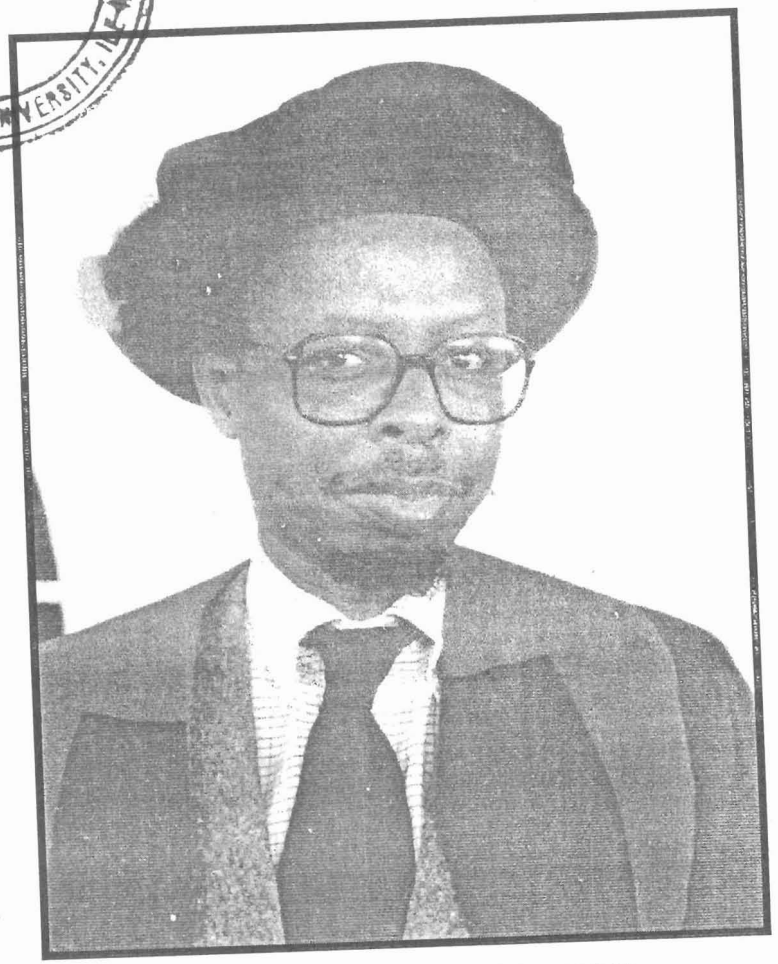

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Introduction

Mr. Vice-Chancellor, Sir, the Registrar, Colleagues, Great Ife Students, Ladies and Gentlemen.I am elated to stand before you this afternoon to deliver my inaugural lecture on the topic that has occupied my attention for many years. There are many areas of Microbiology, but my special interests are Microbial Physiology & Biochemistry and Immunology (Infection & Immunity).

Enzymes are complex proteins that cause a specific chemical change in other substances without being changed themselves. An enzyme is also a protein that is synthesized in a living cell and catalyzes or speeds up a thermodynamically possible reaction so that the rate of the reaction is compatible with the biochemical process essential for the maintenance of a cell. The enzyme in no way modifies the equilibrium constant or the ÄG of a reaction.

As the title of the lecture states, enzymes are found in all living things and perform specific functions in them and they are applied in many disciplines including Medicine, Biochemistry, Microbiology and in Industrial processes.

History of Enzymes

The discovery of enzymes was gradual and occurred during the period that spans the later half of the nineteenth century, (Dixon, 1970). Credit for discovery should go to Payen and Persoz who in 1833 published a report describing an extract of malt which hydrolysed starch to glucose (Dixon, 1970). The true significance of this discovery was appreciated in 1897 when E. Buchner reported an experiment that marked a turning point. An extract of living yeast cells was made by grinding the yeast with an abrasive to break the cell walls. In order to preserve the resulting clear liquid for further study, sugar was added with the surprising result that fermentation occurred, just as if the living yeast cells had been present. It became clear that one did not need intact "living" cell to carry out a typical metabolic process, but that some catalyst or catalysts present in yeast

were capable of bringing about the overall process leading from sugar to alcohol. The work of many investigators including that of brewers was needed before the nature of enzymes was revealed. This early association between enzymes and alcohol made enzymology attractive to many biochemists. Each enzyme in the cell catalyses a particular chemical reaction or group of closely related reactions. This specificity, together with the truly enormous increase in reaction rates brought about by enzymes, is what distinguishes them from inorganic catalysts and gives them their importance in the cell. Enzymes are also different from inorganic catalyst in respect of their fragility. However, in one respect, they are similar. The rate of catalysed reaction is always directly proportional to the concentration of catalyst present. Twice, the amount of catalyst in a given volume gives twice the reaction rate.

The inquisitive student should wonder why enzymes are studied. The reason for studying enzymes is not far fetched as enzymes are involved in many life's processes. An understanding of biology demands familiarity with enzymes. However, there are more important reasons for studying enzymes. For example, in the case of disease caused by pathogenic microorganisms, it has been possible to develop compounds which specifically hit the microbe while missing the man (concept of selective toxicity necessitated by difference in molecular architecture of the prokaryotic microbial cell and that of Eucaryotic mammalian cell). In many cases, this happens because the drug in question inactivates a microbial enzyme, but for one reason or another hardly affects the equivalent enzyme in the human body.

Another reason for studying enzyme is the significant part it plays in the study of cancer. In this case some of the body cells appear to lose part of their essential control mechanisms and proliferate uncontrollably. One can typify cancer as a failure of metabolic regulation in a small number of cells, perhaps only one to start with, what initially causes this imbalance is not yet known, but it is probable that any one of many things may initiate cancer. Mutation induced by radiation, carcinogenic chemicals or a failure of the DNA repair systems may be responsible; or a virus may start the

process by turning off some control mechanism essential for the balance of body cells. Studies of the enzymes of cancerous tissues have shown that many of them appear to be affected, some are missing and others are altered or the control process normally regulating their activity are affected. Whatever the causes of cancer and however it is ultimately understood and brought under control, the study of enzymes will have a contribution to make.

Enzyme Assays

When an experimental work involving the study of any enzyme is to start, one needs to be able to measure its amount or concentration. Whether one is interested in the enzyme as a biologist or a medical biochemist or for whatever other reason, the first requirement is for an enzyme assay. The best way to detect the enzyme is to allow it to catalyse its specific chemical reaction under fixed experimental conditions, where the reaction rate will be proportional to the enzyme concentration. In fact the amount of enzyme is defined in terms of reaction rate under such fixed conditions. The international Unit (i) of enzyme is that amount which will catalyse the conversion of 1i mol of its substrate in 1 minute under defined conditions of temperature and pH that vary from one enzyme to another. Enzyme concentrations are expressed in units/milliliter (µ/ml) or units/litre (µ/l).

The first experimental requirement for making an enzyme assay is a method of following the reaction preferably continuously, so that the initial rate of reaction in the presence of a fixed concentration of substrate can be measured. The initial rate is measured so as to avoid the complications due to the presence of product, and because the enzyme may become inactivated during the course of the assay procedure. In this way, the measured rate will be directly proportional to enzyme concentration in the assay solution. It is usual, however, to employ a substrate concentration at least five times greater than the Km of the enzyme, so that the enzyme will be working on the Michaelis plateau that is in the region of the Michaelis curve. (Fig. 1) where the rate hardly changes with

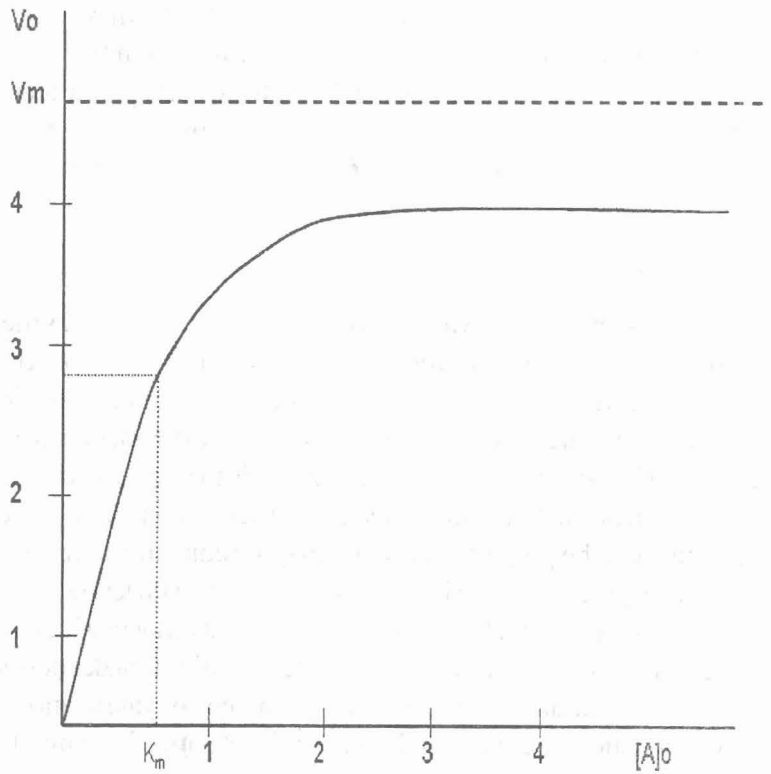


Fig. 1. Michaelis plot of initial rate Vo as a Function of substrate concentration $[A]_o$ for an enzyme obeying the rate equation

$$Vo = \frac{Vm[A]_o}{Km + [A]_o}$$

Where Vm = 5 and Km = 0.5 where Vo is half Vm:

$$\text{i.e. } \frac{Vm}{2} = \frac{Vm[A]_o}{Km + [A]_o}$$

$$4" Km + [A] = 2[A]$$

$$4" Km = 2[A]$$

variations in substrate concentration. This minimizes the possibility of error due to slight variations of the substrate concentration from one assay to another.

The other experimental conditions that must be fixed are the temperature because, like all chemical reactions, enzyme rates vary with temperature. pH of the assay solution because enzymes are susceptible to changes in pH, and the concentrations of all solutes such as buffer ions. The latter restriction applies because enzymes are susceptible to changes in ionic strength and may interact specifically with solute molecules in a way which alters their catalytic activity.

Regulation – cellular

The total metabolic activity of a growing microbe reflects the simultaneous operation of a large number of interconnected pathways, both energy-yielding and biosynthetic. Each pathway comprises some reactions catalyzed by specific enzymes. The product of these reactions is a new cell. The cell is able to make qualitative adjustment in its metabolic machinery in response to changing environmental factors. Microbes have evolved a variety of regulatory mechanisms to take care of these changes.

The fact that microbial metabolism is highly regulated can be inferred from several observations. One of them is the changes in macromolecular composition of bacteria that occur in response to the kinds of nutrients available to the cells. The first study of this aspect of regulation was made about 40 years ago by Maaloe and his associate (Maaloe, and Kjeldgaard, with enteric bacteria, 1965) principally *Salmonella typhimurium*. Enteric bacteria can synthesize all cellular components from a single carbon source and inorganic salts, and they can utilize a number of organic compounds as carbon sources. Certain carbon sources such as acetate are metabolized slowly and support growth at a low rate; others, such as glucose, are metabolized more rapidly and support more rapid growth. If precursors of macromolecules (e.g. amino acids) are added to the medium, growth is even more rapid. By changing the composition of the medium while maintaining temperature constant, one

can obtain growth of *S. typhimuriium* with doubling times that vary between 20 minutes and several hours (at 37°C) Moreover, the size and composition of the cells vary systematically with growth rate (Table 1 below) cells growing at high rates are richer in RNA, poorer in DNA, and larger than cells growing at lower rates.

Table 1: Size and composition of *Salmonella typhimurium* growing exponentially at various rates.

Doubling Time (Min)	Average Wt. of a cell (ug)	Content of			70s ribosomes (Number/cell
		DNA (%)[a]	Total RNA (%)[a]	ãRNA[b](%)[a]	
25	0.77	3.0	31	25	69,800
50	0.32	3.5	22	14	16,300
100	0.21	3.7	18	9	7,100
300	0.16	4.0	12	4	2,000

a. Calculated as a percentage of the dry weight of the cell

b. RNA contained in ribosomes

*Data Adapted from Microbial World (Stanier, *et al.*, 1976)

These changes in cellular composition are dependent on growth rate alone, provided that temperature is not changed. The variation of the macromolecular composition of cells with growth rate can be interpreted as follows: a cell growing at a high rate must synthesize protein much more rapidly than does a slowly growing cell. This higher rate of protein synthesis requires that the cell contain more ribosomes, since the rate of protein synthesis per ribosome is constant.

The ability of a bacterium to regulate its content of ribosomes is of great importance for the maintenance of high growth rates under changing environmental conditions. An insufficient complement of ribosomes would clearly restrict growth rate; an excess of ribosomes would also do so because the cell would be engaged in non-productive synthesis of ribosomal protein and RNA.

The biosynthesis and degradation of small molecules by bacteria are similarly subject to close regulation as shown by the following observations on enteric bacteria.

(a) When, growing in synthetic media containing a single organic compound as source of energy, bacteria synthesize all the monomeric precursors (e.g., amino acid) of macromolecules at rates which are precisely coordinated with the rates of macromolecule synthesis.

(b) The endogenous synthesis of any one of these monomeric precursors is immediately arrested when the same compound is added to the medium, provided that the exogenously furnished monomer can enter the cell.

(c) Formation of the enzymes that mediate biosynthesis of the monomers in question is also arrested.

(d) Bacteria frequently synthesize enzymes responsible for the dissimilation of certain organic substrates only if the compounds in question are present in the medium.

(e) When presented with two organic substrates, a bacterium first synthesizes the enzymes required to dissimilate the compound which supports the more rapid growth, only after this compound has been completely utilized are the enzymes required to dissimilate the second compound synthesized – Protein sparing action of microbes.

Two different regulatory mechanisms operate in the cell – The regulation of enzyme synthesis, and the regulation of enzyme activity. Both of them are mediated by compounds of low molecular weight, which are either formed in the cell as intermediary metabolites or enter it from the environment. Both regulatory mechanisms involve the operation of a special class of enzymes, called allosteric proteins. The fundamental importance of allosteric proteins as the key components of regulatory systems was first perceived by J. Monod in 1963.

Allosteric proteins are proteins whose properties change if certain specific small molecules, effectors are bound to them. Hence, allosteric proteins are mediators of metabolic change which is directed by changes in concentration of the small effector molecules. There are two classes of allosteric proteins: allosteric enzymes whose activities are either enhanced or inhibited when combined with their effectors, and regulatory allosteric proteins, devoid of catalytic activity, which regulate the synthesis of specific enzymes. Regulatory allosteric proteins attach to the bacterial chromosome near the specific structural genes whose repression they control. This attachment can be modified by the binding of small effector molecules to the regulatory proteins, thereby changing the rate at which specific messenger RNAs are synthesized.

The best studied allosteric proteins are the allosteric enzymes, exemplified by aspartic transcarbamylase (ATCase). ATCase catalyses the first reaction in the pathway of biosynthesis of pyrimidines. Its activity is inhibited by an end product of the pathway, cytidine triphosphate (CTP).

Thus, elevated intracellular concentrations of CTP inhibit the activity of ATCase and consequently the formation of more CTP until its concentration decreases to an optimal level. ATP_1 a second effector of ATCase, activates the enzyme and therefore, serves to coordinate the synthesis of purine and pyrimidine nucleotides.

Role of Enzyme in the Physiology of Microorganisms

Practically, most of, if not all the activities of living organisms are mediated by enzymes. Needless to say that the activities of microorganisms are no exemptions. Enzymes are implicated in the following processes:

- Enzymes are involved in bacterial cell wall biosynthesis.
- Enzymes are implicated in the cell membrane transport process and in energy metabolism
- Nucleic acid metabolism require the participation of enzymes.
- Attack on non-penetrating nutrients
- Cytoplasmic activities.

A. Enzymes Involved in Cell Wall

The assembly of cell wall structures as outer shells external to the plasma membrane involves a number of enzymes in the membrane so that the biosynthetic intermediates can be translocated across the membrane to an externally located acceptor site.

Peptidoglycan Biosynthesis

Particulate enzymes are involved in all of the steps in wall peptidoglycan synthesis subsequent to the formation of N-acetyl muramyl pentapeptide-C_{55} isoprenol pyrophosphate from UDP-muranyl pentapeptide and poly-isoprenod phosphate with concomitant release of UMP.

Newhaus (1971) has proposed the name "translocase" for the enzyme transferring the precursor molecule from uridylic acid to the undecaphenyl phosphate carrier in the membrane. Another membrane enzyme which catalyses dephosphosylation of the C_{55}-isoprenyl-pyrophosphate is essential for re-initiation of another cycle of peptidoglycan synthesis. This phosphatase has been purified from the membrane particles of *Micrococcus lysodeikticus* following solubilization with Triton X-100.

Moreover, one of the terminal reactions in wall-peptidoglycan synthesis involves a transpeptidase responsible for the cross-linking of adjacent peptides.

B. Membrane Associated enzymes in Bacteria

(1) Membrane AT Pase(s)

$$ATP \xrightarrow{ATPase} ADP + Pi$$

Functions
- Oxidative phosphorylation
- ATP synthesis
- ATP degradation for membrane functions
- Transport of cations and amino acids

ATPase occupies an important position in the chemiosmaotic theory of Mitchell. Most bacteria have an ATPase which is localized in the membrane of the cell and has a paramount role in the interconversion of chemical and osmotic forms of energy. The membrane ATPase in prokaryotes is now believed to specifically translocate protons like the ATPase in mitochondria and chloroplasts. The proton-translocating ATPase is a central feature of the chemiosmotic hypothesis which was formulated by the English biochemist, Peter Mitchell in 1961.

Brief Summary of the Chemiosmotic Theory as Applied to Procaryotes

The export of protons by the bacterial cell generates what is referred to as a proton motive force (Pmf) across the cell membrane, which is relatively impermeable to protons. The Pmf is the composite of the transmembrane pH gradient (ÄpH) and electrical potential (Äø). Some bacteria which lack a respiratory chain and a photosynthetic system, for

example *Strp. faecalis* use the membrane ATPase to export protons as the only available means of producing a (Pmf). The Pmf is the form of energy which bacteria use to drive transport of nutrients as well as for the reduction of NADP+ by NADH and motility.

Bacteria which have a respiratory chain or a photosynthetic system for example *E. coli* or *Halobacterium halobium* use these electron transport systems as an alternative to the ATPase to eject protons from the cell. In addition, Pmf produced during the transfer of electrons may be used in the synthesis of ATP from ADP and inorganic phosphate. The synthesis of ATP in response to the Pmf generated by substrate oxidation or photosynthesis occurs via the reversal of the proton translocation by the ATPase. The processes in prokaryotes which produce and consume a Pmf are summarized below:

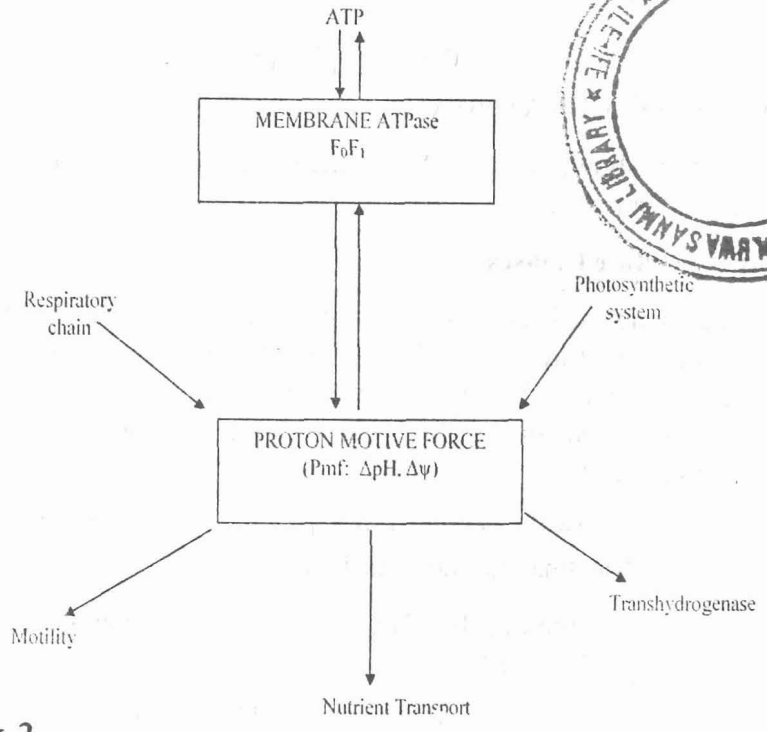


Fig. 2

Polymerases: Three DNA polymerases (I,II, and III) have been isolated from *E. coli;* enzymes with similar properties exist in other microorganisms and in animal cells. In vitro these enzymes catalyse the reaction:

n(dATP, dCTP, dTTP, dCTP)

↓ Enzyme Polymerase

↓ DNA template

(dAMP, dGMP, dTMP, dCMP)n

Polynucleotide
+
N(PP) pyrophosphates

The main role of DNA polymerase I is repair synthesis.

T. Kornberg showed that DNA polymerase III probably carries out replicative synthesis.

Polynucleotide Ligases

These enzymes which join polynucleotide chains are present in all cells. *E. coli* ligase restores a phosphodiester bond break in single-stranded DNA, between a 3'OH and 5'P end (such as produced by pancreatic Dnase). The restoration is perfect, as shown by return of biological function in transforming DNA.

(1) NAD + Enzyme ⟶ Enzyme-AMP + NMN
(Nicotinamide mono nucleotide)

(2) Enzyme – AMP + Nicked DNA ⟶ Enzyme + Nicked DNA - AMP

(3) Nicked DNA – AMP ⟶ AMP + Joined DNA

D. Attack on Non-Penetrating Nutrients

Microbes can take up foods of low molecular weight, including oligo-peptides, nucleosides, and small organic phosphates, e.g. glycerol phosphate. Nucleotides generally cannot penetrate at a substantial rate. But the organic matter initially returned to the soil in dead plants and animals is predominantly macromolecular. Preliminary extracellular hydrolysis is therefore necessary, just as in higher animals. For this purpose various bacteria and fungi elaborate a variety of exoenzymes. Many of these are secreted into the medium (especially by gram positive rods) as extracellular enzymes.

In pathogens these often play an important role by attacking tissue constituents. Extracellular enzymes include: Proteases and peptidases, polysaccharides (amylase, cellulase, pectinase); mucopolysaccharidases (hyaluronidase, lysozyme etc).

E. Enzymes in the Cytoplasm

Practically all living organisms operate the known major pathways – Unity of Biochemistry. The glycolytic pathway/Embdem Myer of pathway, citric acid pathway/Krebs citric acid pathway and these pathways make use of enzymes to metabolise glucose to pyruvate and to other pathways because pyruvate is at the hub of metabolism. Kinases, dehydrogenases, isomerases and aldolase are some of the enzymes encountered in this pathway.

Fermentation

Fermentation is defined as the anaerobic breakdown of carbohydrates resulting in the formation of stable fermentation products. Examples of useful fermentation products include ethyl alcohol, lactic acid, acetic acid, glycerol, butylene glycol, acetone, butanol, butyric acid. It is pertinent here to state that ***all fermentations are enzymatic.*** Moreover, many of our local food(s) here in Nigeria are products of fermentation. The list

include, Fufu, Ogi, Garri, condiments like Iru, Soydaddawa, Ugba, Pito, Burukutu etc.

Bioremediation

Bioremediation is the act of adding fertilizers or other materials to contaminated environments to accelerate the natural biodegradation process. Bioremediation is used in sewage treatment, aquatic and terrestrial oil spills and for hazardous wastes. The strategies being used in bioremediation for treatment of oil spills include addition of petroleum degrading microorganisms to contaminated sites, addition of nutrients and petroleum degrading microbes to the affected sites. Bioremediation is therefore the augmentation or stimulation of biodegradation. Biodegradation, however suggests the breakdown of materials through biological processes and enzymes are therefore implicated in the process of Bioremediation. The enzymes are of necessity excreted into the environment by microorganisms so as to assist in the process of remediation of the polluted environments.

Enzymes in Medicine and Medical Sciences

The application of enzymes in medical practice is well seen and demonstrated by the functions of the chemical pathologist in assessing the levels of enzymes of diagnostic importance in body fluids and clinical uses of isoenzymes. Phosphatases do tell tales in the diagnosis of diseases. Phosphatase is an enzyme capable of hydrolysing monophosphoric esters. It is present in highest concentration in renal tissue, intestinal mucosa and growing bone. Phosphatases which exhibit maximum activity in alkaline and in acid media exist and are called alkaline and acid phosphatases. Serum acid phosphatase is increased in patients with carcinoma of the prostate. The level of alkaline phosphatase in the serum is fairly constant but it is increased in skeletal diseases associated with marked osteoblastic activity, and in certain other conditions e.g., Jaundice.

Between 1969 and 1971, I had the opportunity to work in the Laboratory of Dr. B.S. Vandeheiden at Eastern Pennsylvania Psychiatric

Institute, Department of Biochemistry as a Research Assistant. In that laboratory, we were working on Inosine triphosphatase in the red blood cells of Normal and Psychiatric (Schizophenic) patients. Accumulation of ITP in red blood cells indicated ITPase deficiency (Vanderheiden, 1969). Further work has since been done by Vanderheiden and other investigators (Vanderheiden, 1975; Sumi *et al*., 2002).

Chymoral, an enzyme preparation composed of Trypsin and chymotrypsin is used in clinical practice as an anti-inflammation remedy. Moreover, enzyme also plays a role in immunodiagnosis. Enzyme-linked immunosurbent assay (Elisa) is a very familiar assay in immunological methods. The four-chain molecular model for Immunoglobulin G, proposed by Dr. R. Porter in 1962 was aided by the use of the enzymes papain and pepsin

Restriction Endonucleases

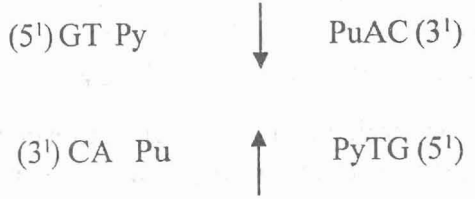
Restriction endonucleases are very important in recombinant DNA technology and molecular biology. In fact, it is a *sine qua non* for studies in this area. They are molecular scalpels that cut DNA at specific sites. All the nucleases, the enzymes that were first found to break the phosphodiester bonds of nucleic acids, showed very little sequence dependency. The most specific was the T1 Rnase, which was found to cut only next to Guanine residues.

Then in the late 1960's Stewart Linn and Werner Arber, working in Geneva found in extracts of cells of *E. coli* strain B both a specific modification enzyme that methylated unmethylated DNA and a "restriction" nuclease that broke down unmethylated DNA.

These enzymes cleaved DNA at random locations far removed from the specific unmethylated sites. However, in 1970, Hamilton, Smith of Johns Hopkins University, accidentally discovered that *Haemophilus influenzae* rapidly broke down foreign phage DNA. These degradative activity subsequently observed in cell free extracts and shown to be due to a true restriction nuclease, because the enzyme broke down *E. coli*

DNA, whereas it failed to cut up the DNA of Haemophilus cells from which it was extracted.

Hind II binds as follows



Hind II cut specific sequences some restriction enzymes recognize specific group of 4 buses, whereas many others recognize groups of six. Restriction enzymes are now isolated from about 230 bacterial strains e.g. Bam HI from *Bacillus amyloliquefaciens*, ECORI from *E. coli*, PST1 from *Providencia stuartii et. al.*.

My Contribution

Studies on Enzymes

Adenosine triphosphatase(s) ATPase(s) of *Agrobacterium tumefaciens* were studied (Shonukan, 1982, and Shonukan, 1982). The following background information explains the reason for this study.

A virulent strain of *Agrobacteirum tumefaciens*, a gram negative rod, is responsible for the induction of crown gall, a non-self limiting neoplastic disease of many dicotyledonous plants. Early in the twentieth century smith and Townsend (1907) identified the causative agent of the crown gall tumor disease as *A. tumefaciens*. It is the only bacterium known to independently cause tumours in a eucaryote. The Agrobacteria are of practical importance because of their pathogenicity, and also because they offer a model system for the study of cancer. Moreover, in this organism one sees the uniqueness of a procaryote causing a transformation in eucaryotes, apart from offering an excellent example of a host-parasite relationship.

The substance responsible for induction of crown gall tumor was diligently searched for without success. The tumor-inducing principle (TIP), as it was referred to by investigators of the field, was not characterized until a breakthrough in 1974, when Zaenen *et al* and Van Larebeke *et al.* (Zaenen *et al.*, 1974; Van Larebeke *et al.*, 1974) demonstrated that a large closed circular DNA plasmid (T1-plasmid) present in *A. tumefaciens* strains, is essential for the crown-gall tumor-inducing ability of such strains. T1(tumor-inducing) plasmid of *A. tamefaciens* is 1.1×10^8 Daltons in size. This is about 5% of the *A. tumefaciens* genome. Comparatively, *Escherichia coli* plasmids average about 3×10^6 Daltons.

Crown gall is incited by inoculation of a wound site with *Agrobacterium tumefaciens*. In an excellent review, Lippincott and Lippincott (1975), pointed out that a wound is essential because the pathogen lacks the ability to invade. In addition, it contributes the following essential components for tumor induction;

(1) A site at which the bacterium must attach.

(2) A medium that supports the metabolic activities of the bacterium and

(3) Changes in cells surrounding the wound (conditioning) leading to a susceptible stage (competence).

Although the mechanism by which living bacteria transform plant cells in the healing wound is unknown, it is, however, clear that the viable bacterial cell is essential in the initiation of crown gall and that wounding of the host plant is necessary for the initiation of the tumorigenic process. From the wounded plant tissue, some juice is released, which stimulates the intercellular growth of bacteria. It is, therefore, suggested that the plant cells, sharing a common external milieu with bacteria, pick up certain substance(s) released into the environment by bacteria. It is further suggested that the released substance transforms or mediates the transformation of plant cells which proliferate into a tumor. Obviously, transport is a very important consideration in this problem. However, the

concept of transport cannot be fully discussed without a discussion of adenosine triphosphatase, an enzyme which has been implicated in the transport of substances (Shonukan, 1971; Rosen, 1973).

Adenosine triphosphatase also occupies an important place in the chemiosmotic theory of Mitchell. It was, therefore, on the basis of the preceeding background that Adenosine triphosphatase was studied in *Agrobacterium tumefaciens*. Chromatographic, electrophoretic, catalytic and immunochemical studies of the ATPase(s) have been done in both the membrane and the cytoplasmic soluble fractions of *Agrobacterium tumefacicus* strain C-58. Both enzymes were inhibited 25% and 34% respectively by $1x10^{-2}$M mercuric chloride. Both enzymes were activated by trypsin (Shonukan, 1979).

The enzymes are cold-labile but could be stabilized by storing them in a buffer solution containing 5mM Mg++ and 20% methanol (Shonukan, 1979). Shonukan (1985), also implicated the membrane ATPase in the transport of valine in *Agrobacterium tumefaciens* while, the uptake of proline does not require the participation of membrane ATPase.

Regulation of Enzyme Synthesis

Regulatory mutations affecting the synthesis of alpha-amylase in *Bacillus stearothermophilus* has been studied (Isola and Shonukan, 1997) using ethyl methane sulfonate (EMS) mutations in the regulatory system controlling alpha-amylase synthesis in Bacillus stearothermophilus were isolated. Depression of the alpha-amylase gene (amyE) at various levels was achieved in these mutants resulting in differential rate of the enzyme biosynthesis. Four classes of mutants were identified. One of the groups was constitutive for alpha-amylase synthesis and partially free of glucose repression while another group designated as Cri (catabolite repression insensitive) mutants were non-constitutive but free of glucose repression. In another group of mutants classified as super-repressed, the alpha-amylase gene (amyE) appeared repressed under all conditions of growth.

Campestris was studied (Boboye and Shonukan, 1993) Four classes of mutants were identified with respect to pectate lyase. Pectate lyase production in the wild-type and classes I and IIb mutants was partially dependent on the growth phase whereas in classes IIa and III it was totally dependent. Enzyme activity in some of the mutants was constitutive and resistant to catabolite repression.

Regulatory mutations affecting the synthesis of cellulase was also studied in both *Bacillus pumilus* (Kotchoni and Shonukan, 2002) and *pseudomonas fluorescens* (Bakare *et al.*, 2005a). A wild strain of *Bacillus pumilus* was investigated for cellulase production, and putative mutants of this strain were screened for catabolite repression insensitivity after chemical mutagenesis using ethyl methane sulfonate (EMS) as a mutagenic agent. Out of four classes of mutants studied and classified according to their cellulase induction rate and level of cellulase production in the presence of high concentrations of glucose (2.6%([w/v]), classes III and IV exhibited cellulase production up to 6.2 mg cellulase and 11.4mg cellulase per gram of dry cell mass respectively.

These mutants were referred to as catabolite repression-insensitive when compared to the wild strain which exhibited a total repression of cellulase synthesis under the same conditions. How EMS triggered the catabolite repression insensitivity in these mutants was not established. However, this mutation brought out new strains of cellulase hyperproducers (mutants 6 and 11) in the presence of glucose when compared to other cellulase producers such as *Aspergillus terreus* (Ali & Sayed, 1992) and *A. nidulans* (Bagga *et al.*, 1989).

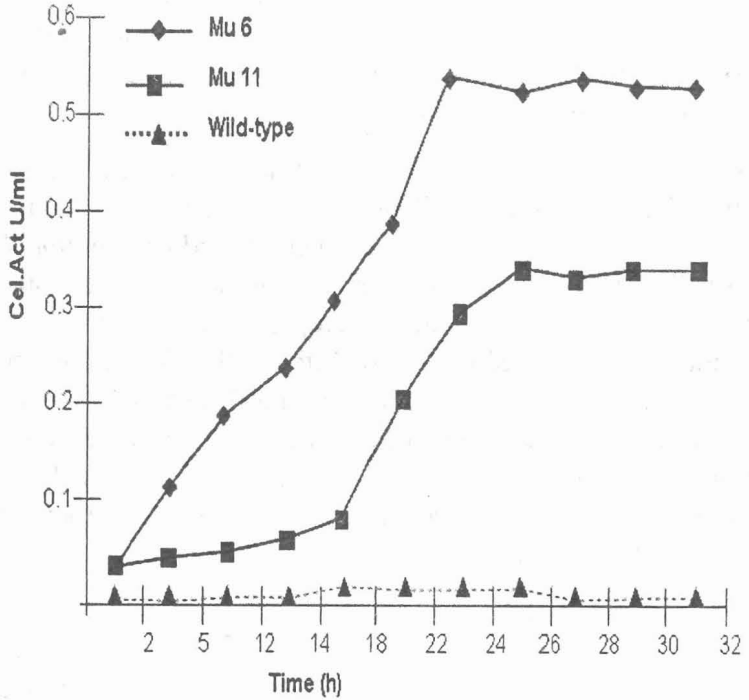


Fig. 3: Cellulase synthesis in cultural media by improve cellulase producer strains of *B. pumilus* (class III mutants: Mu.11. class IV mutants: Mu 6) and the wild strain (a) Using high concentration of glucose as carbon source [2.6%(w/v)]. (b) Using glycerol as carbon source [2.6%(w/v)]. Mu. – mutant of *B. pumilus*. Cel. Act. – cellulase activity.

The EMS chemical mutagenesis approach of (Kotchoni and Shonukan, 2002) has been recently used to successfully generate catabolite repression resistant mutants (CRRmut) in *Pseudomonas fluorescens* (Bakare *et al.*, 2005a). They showed that cellulase activity in selected *P. fluorescens* mutants namely CRRmut4 and CRRmut24 is higher in presence of 1% (w/v) glucose than in wild type *P. fluorescens* (Bakare *et al.*, 2005a), indicating, therefore the reproducibility of the method originally proposed (Shonukan and Nwafor, 1989), and its subsequent modification (Kotchoni and Shonukan, 2002). The regulation of carbon metabolism in bacteria is generally mediated by a regulatory protein termed catabolite control protein (Ccp) via a well-known mechanism (Boisset *et al.*, 2000., Miwa *et al.*, 2000; Lopez-Contreras *et al.*, 2004). The Ccp binds to a specific cis-acting element known as CRE (catabolite responsive element), repressing or activating gene expression (Lopez-Conteras *et al.*, 2004).

Studies on Fermentation

Microbiological and biochemical changes in the traditional fermentation of soybean for 'soy-daddawa' was investigated. Although the information on the microbiological, biochemical and nutritional changes during the fermentation of African locust bean for 'daddawa' are well documented (Eka, 1980, Odunfa, 1981, 1985, Abiose *et al.*, 1988) but there is practically no information on the biochemical changes that occur during the fermentation of soybean for 'soy-daddawa' production. As a result of the above mentioned scarcity/lack of information, the study on microbiological and biochemical changes in the fermentation of 'soybean' for "soy-dadawa" was embarked upon (Omafuvbe, 1998). The major group of microorganisms involved in this fermentation of 'soybean' for soydaddawa were found to be *Bacillus* species especially *B. subtilis, B. licheniformis* and *B. pumilus*. Amylase activity increased rapidly with fermentation attaining a peak at 48h with a large decrease in total soluble sugar level (Omafuvbe *et al.*, 2002). It was surmised that the utilization of the soluble sugars by the increase population of fermenting organisms was probably responsible for the steady drop in the total sugar level.

The rapid rise in the total free amino acids in the early stages of fermentation coincided with the increased protease activity which peaked at the 36th hour of fermentation. The high level of free amino acids is a reflection of the high protein content of soybean as protease activity has been reported to be abundant in the fermentation of similar protein rich foods (Abiose *et al.*, 1988). It was believed that the identification of the roles of the different isolates of the bacillus species in monoculture fermentation of soybean for 'dadawa' would assist in the production of starter culture to improve the traditional method of production, Bacillus species (*B. subtilis, B. licheniformis* and *B. pumilus*) previously isolated from samples of soy-dadawa prepared traditionally were tested singly and in combinations (as starters) for their ability to ferment soybeans for acceptable soy-dadawa. Out of the three Bacillus species, only *Bacillus subtilis* as single or member of a mixed starter produced soy-dadawa which was not different from the naturally fermented soy-dadawa in their sensory attributes. (Omafuvbe *et al.*, 2002).

Immunological Studies

The role(s) of Macrophages, Antibody and T-lymphocytes in the protection of mice against *Shigella dysenteriae* were investigated.

Protection against bacterial infections depends on many factors. Phagocytes seem to be the most important because of their rapid accumulation into the affected area, trapping and ingesting the invading organisms. The degree of contribution of phagocytes in the protection against various infections differ for various bacteria. *Listeria monocytogenes, Pseudomonas aeruginosa* were shown to be eliminated by macrophages and polymorphonuclear cells (PMN) respectively (Mitsuyama *et al.*, 1978) and Tatsukawa *et al.*, 1979). Salmonella typhimurium is mainly eliminated by macrophages and antibody. We carried out an experiment to determine the contribution of macrophages to protection of mice against *S. dysenteriae* type 1 infection of mice.

The work was carried out by depleting the total population of macrophages with carrageenan. Carrageenan selectively damages cells of the macrophage series but not PMN (Catanzaro *et al.*, 1971).

Female albino mice (24-30g) were used for the study. The bacteria, shigella dysenteriae type 1 was maintained on Nutrient agar (Difco) and cells were harvested in phosphate buffered saline solution (PBS) pH 7.2. The viable count of the challenge dose was assessed by plating appropriately diluted bacterial suspension on nutrient agar plates. The LD_{50} of this strain was approximately 10^7 viable bacteria by the intraperitoneal route. A set of mice were inoculated with 10^5 viable shigellae suspended in PBS interperitoneally. At intervals, the livers and spleens were dissected out and the organs were homogenized individually in PBS. The homogenates were diluted for colony counts as described by Tatsukawa *et al.* (1979). The number of viable bacteria in the whole organ was expressed as $\log_{10}$ (bacterial count). The carrageenan dose was calculated using 200mg per kilogram body weight and was administered 24 hours prior challenge with the bacteria. (Onifade and Shonukan, 1990). The effect of carrageenan treatment on bacterial count in normal mice is shown in Fig. 4 below.

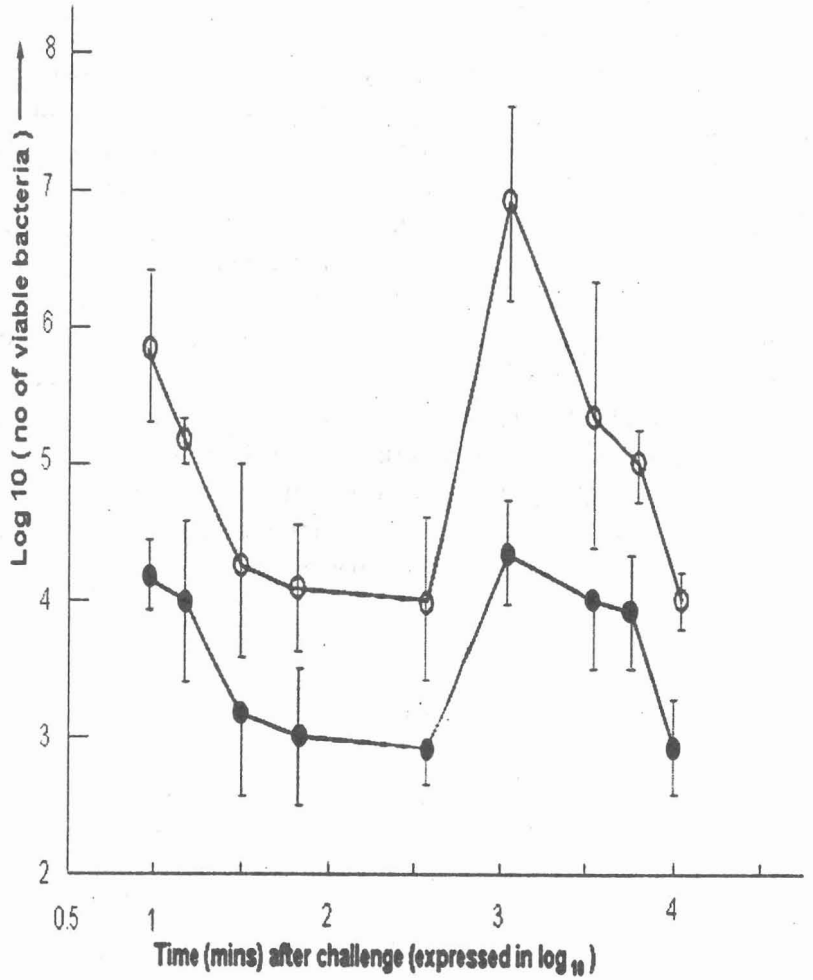


Fig. 4: Bacterial counts in the liver of carrageenan treated mice (o) and control mice (o) after challenge with 10^5 *S. dysenteraie*. Each point and bar indicates the mean result for 3 mice ± S.E.M.

The relative contributions of Thymus derived Lymphocytes and antibodies to protection against mouse shigellosis was also examined. Bacterial growth of shigella dysenteriae type 1 infection of mice was enhanced by the immunosuppression of T-lymphocytes of normal mice with anti-thymocytic serum (ATS) (Fig 5&6). Immunization with antishigella antibody raised in actively immunized Rabbits had no protective role on passive transfer to normal mice (Onifade & Shonukan, 1996).

Bacterial Lipopolysaccharides and Effects in Protection of Mice against Bacterial Diarrhoea.

Shigellosis remains highly endemic in many areas of the world. The control of this disease has proved difficult because shigellae have been reported to be increasingly antibiotic resistant. (Farrar & Edison, 1971). Since Lipopolysaccharide (LPS) a major surface antigen present on the surface of gram negative bacteria has been reported to be a B-lymphocyte mitogen (Anderson *et al*,., 1973), it became of interest to investigate whether an increase in resistance to shigella flexneri infection

- The major aetiologic agent of Shigellosis in Nigeria (Onifade, 1993), could be achieved by immunizing with LPS of homologous strain.

Two groups of mice were actively immunized with the lipopolysaccharide (LPS) extracted from shigella flexneri-01 cells and live cells of the same organism respectively. The immune status of the immunized mice was determined on the 7th day by challenging them with the 50% lethal dose (LD_{50}) of the organism alongside the non-immunized ones. It was observed that all of the immunized mice, that is, those immunized with LPS and those immunized with the cells were protected from the disease but the non-immunized ones recorded only 40% survival.

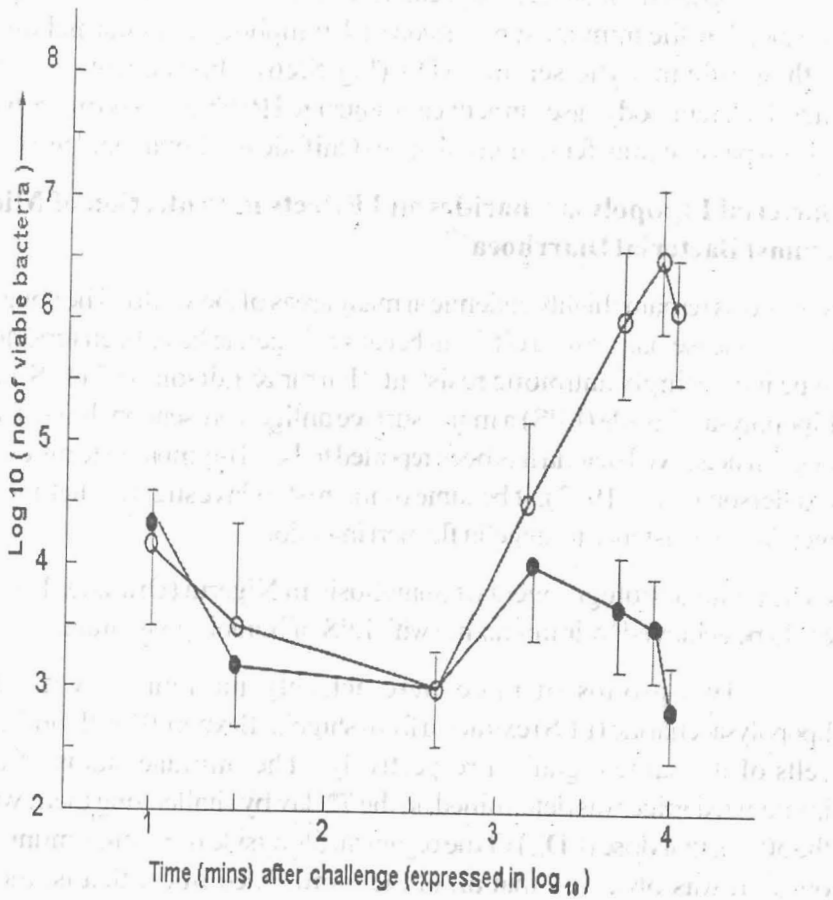


Fig. 5: The effect of T-lymhocytes immunosuppression on bacterial counts in the liver (o) compared with the control mice (**o**) after intraperitoneal challenge with 10^5 *S. dysenteriae.* Each point and bar indicates the mean for 3 mice ± S.E.M.

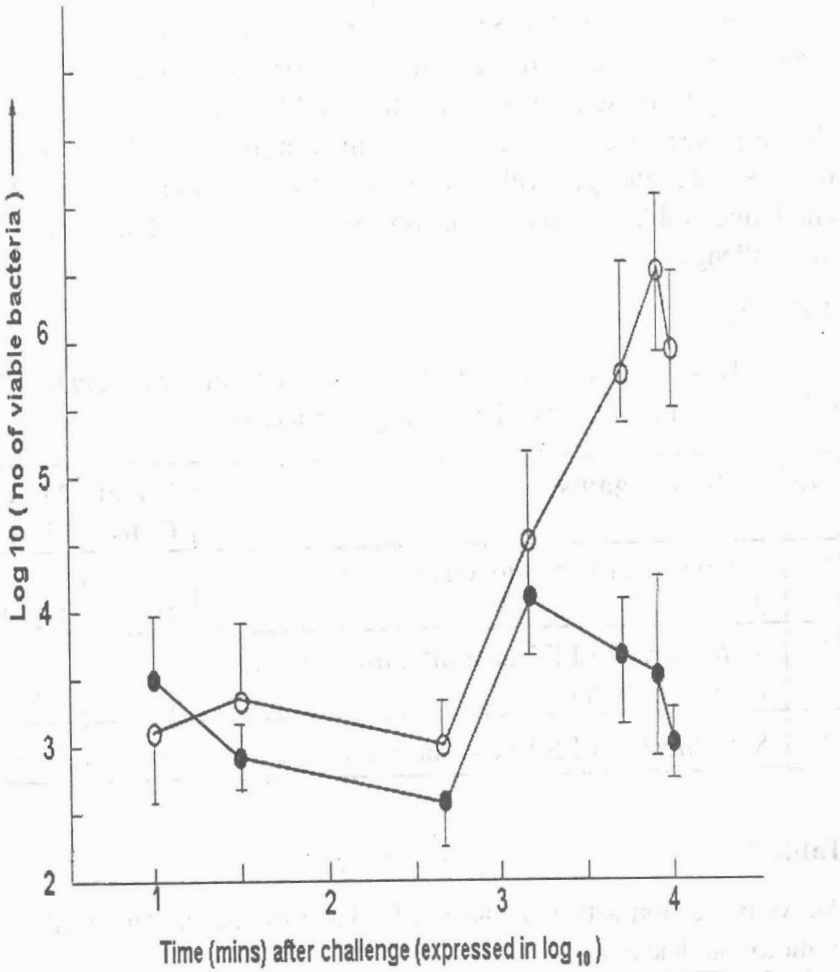


Fig. 6: The effect of T-lymhocytes immunosuppression on bacterial counts in the spleen (o) compared with the control mice (**o**) after intraperitoneal challenge with 10^5 *S. dysenteriae.* Each point and bar indicates the mean for 3 mice ± S.E.M.

The anti-shigella flexneri 01LPS titres of sera from mice actively immunized with *S. felexneri* 01 LPS prior challenge with homologous infection ranged from 1:128 to 1:256 giving a mean titre of 1:213. After the challenge however, the titre increased to a mean titre of 1:4, 096 at day 7 and decreased considerably to 1:384 by the 14th day. (Table 2). For the protection experiment, all of the mice immunized with *S. folexneri* 01 LPS and challenged with pathogenic homologous strain survived the challenge (Table 3). In contrast, only 40% of the control mice survived the challenge.

Table 2:

Antibody titres of sera from mice immunized with *S. flexneri* 01 LPS prior and after challenge with homologous bacteria.

S/n	Antisera against	No. of Cells	Mean titre
1	*S. flexneri* 01 LPS prior challenge with the bacteria	10	1:213
2	*S. flexneri* 01 LPS 7 days after the challenge with the bacteria	10	1:4,096
3.	*S. flexneri* 01 LPS 14 days after the challenge	10	1:384

Table 3:

Active protection with *S. flexneri* 01 LPS against the challenge with homologous bacteria.

Pre-treatment with	Challenge with	No. of mice	Percentage survival after 24h,	48h,	72h
S. flexneri 01 LPS	*S. flexneri* 01 LD_{50}	10	100	100	100
Non-immunized	*S. flexneri* 01 LD_{50}	10	40	40	40

The Influence of Age-Sex on the Pattern of Immuno-depression following Surgery

We investigated the influence of age-sex on the pattern of modulation of selected immune parameters, following surgery (Ajayi *et al.*, 2005). Male patients of different ages, without any known immune depression but requiring minor or moderate dissection were randomly recruited into the study following informed consent. Two groups were delineated (group 1) = ages 01 to 12 years and (group 2) = ages 13 to 50 years.

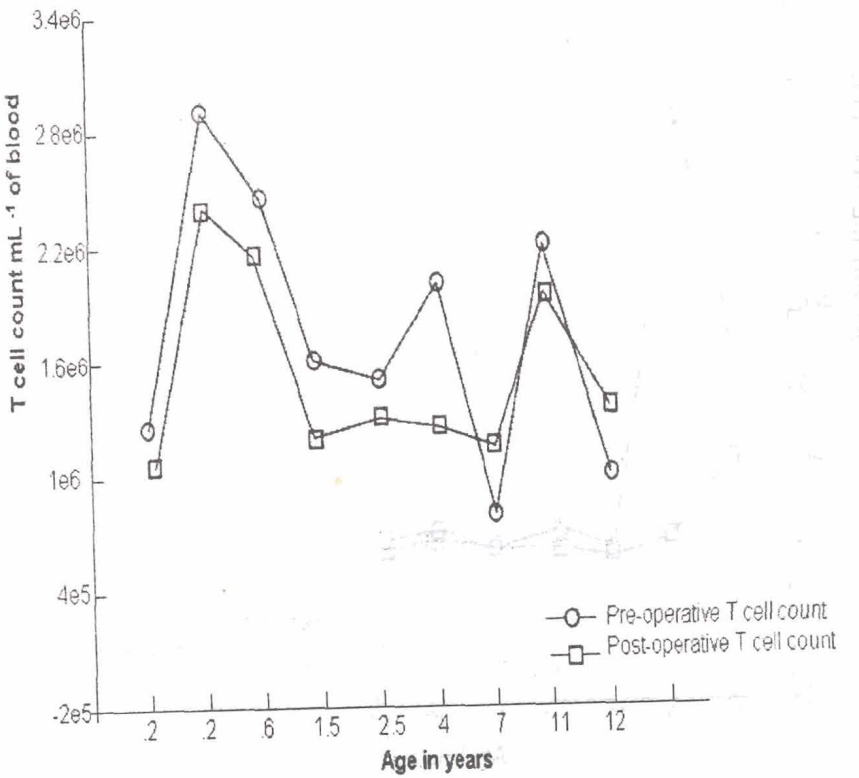


Fig. 7: Pre and post-operative T cell count for group 1

Blood samples were obtained immediate pre-operatively and immediate post operatively in every case and assessed for T-cell count, lymphocyte viability, B-cell count, total leucocyte count, total lymphocyte count and percentage lymphocyte transformation. T-cell counts appear higher in the age range 0.2-12 years when compared to the older patients (Fig. 7 and 8). Post-operative counts were, however, lesser (Fig. 7) with respect to their corresponding pre-operative values. Post-operative T-cell counts were significantly different between Groups 1 and 2 only ($P<0.03$) (Fig. 9).

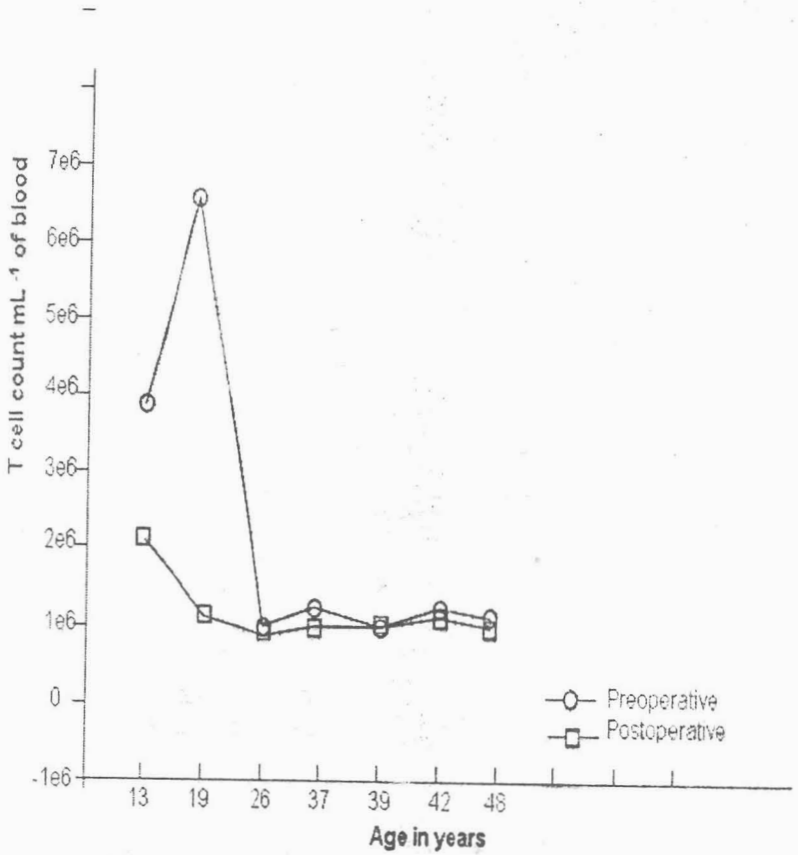


Fig. 8: Pre and post-operative T cell count for group 2.

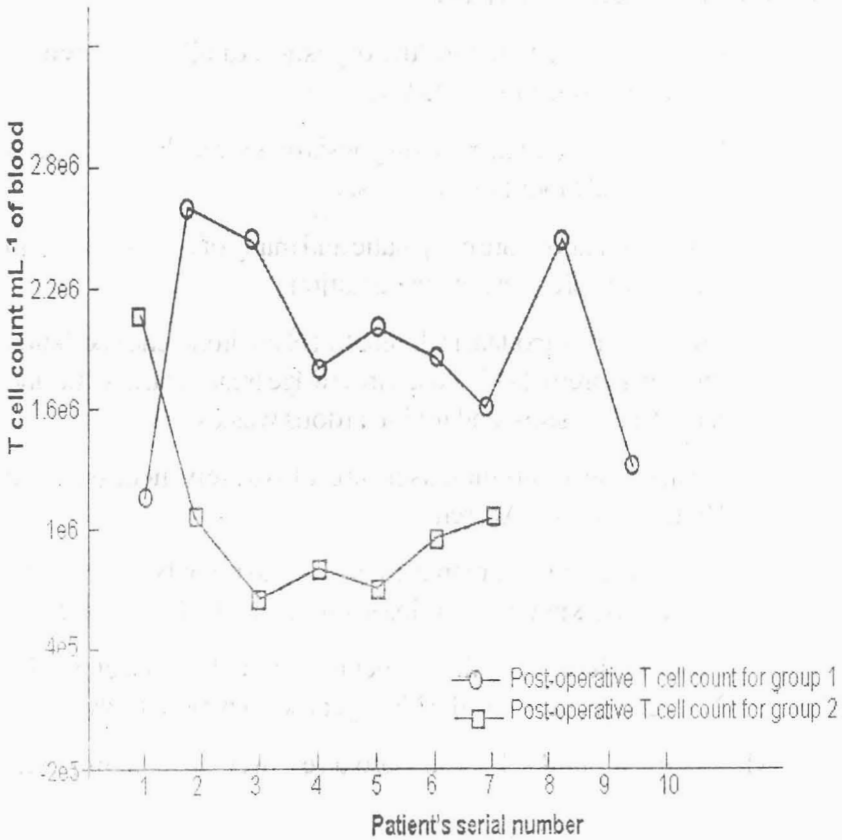


Fig. 9: Post-operative T cell count for groups 1 and 2.

Pre-operative counts were, however, not significantly different between the groups. The result here have shown that the age-sex influences immune parameters such as T-cell population and function after, but not before, surgery suggesting the possible involvement of background gonadal neuroendocrinologic influences in the immune response to stress.

Mr. Vice-chancellor Sir, I submit that enzymes are very important for the existence of man on this planet.

- Enzymes are needed for the digestion of all food eaten by man and all other living things.
- Enzymes are needed in the diagnosis of diseases by the medical doctors and laboratory scientists.
- All fermentations are enzymatic and many of our local food in Nigeria are products of fermentation.
- Enzymes are important in the control of environmental pollution through bioremediation used in sewage treatment, aquatic and terrestrial oil spills and for hazardous wastes.
- Restriction endonucleases are absolutely necessary in Biotechnology Research.
- In the movement of man from point A to point B, the enzyme ATPase is expended to release energy locked up in ATP.
- Food production on this planet is intimately connected with Nitrogen fixation and the Nitrogenase enzyme activity.

Moreover, about 8 – 10 years ago a research group composed of:

- Prof. A., Afolayan - Biochemistry Department
- Prof. P.O. Olutiola - Microbiology Department
- Prof. S.K. Layokun - Chemical Engineering Department
- Prof. O.O. Shonukan - Microbiology Department
- Prof. B.O. Solomon - Chemical Engineering Department

because of the importance of enzymes submitted a proposal to the Raw Materials Research Institute on the topic concerning Enzyme Technology

and the production of very key and important enzymes in ;this country. The group was subjected to a fairly rigorous interview by an officer of the Institute, the proposal was acknowledged but we are still waiting for the approval and funding of this project.

In view of all the above concerning the importance of enzyme, I want to suggest the following recommendations.

RECOMMENDATIONS

1. As a result of the importance of enzymes highlighted in this lecture, the government should fund research in universities, especially research on enzyme technology and production of enzymes needed to pursue Biotechnology research (Restriction endonucleases for example).
2. The Government should endeavour to establish a National Institute of Enzyme Technology, fully equipped with equipment, material(s), dedicated and well-trained and experienced staff.

   If the Institute is established and well funded, this will reduce the foreign exchange spending and possibly increase our earnings as we export our products.

I thank God for the opportunity to be alive today to give this inaugural lecture. Moreover, my modest contribution is possible through the cooperation of my students. Some of whom are listed here-under.

- Joshua B. Owolabi, Ph.D., MBA - Assistant Director,
Technical Assessment Discovery and
Assessment Group,
TAP Pharmaceutical, Lake Forest, IL. U.S.A.

- Prof. Olusola Oyewole	-	Prof. Of Food Science and Technology, Federal University of Agriculture, Abeokuta.
- Prof. Israel Jideani	-	Dept. of Food Science & Technology, University of Bauchi.
- Dr. Tinuola Tokunbo Adebolu	-	Snr. Lecturer, Department of Microbiology, Federal University of Technology, Akure.
- Dr. B.O.; Omafuvbe	-	Snr. Lecturer, Department of Microbiology,Obafemi Awolowo University, Ile-Ife.
- Dr. Bola Boboye	-	Lecturer, Department of Microbiology, Federal University of Technology, Akure.
- Isola, O.O.	-	Evans Medical, Plc, / Pharmadeco Plc.
- Dr. Simeon O. Kotchoni	-	Postdoctoral Fellow, West Virginia University Morgantown, West Virginia.
- Dr. M.K. Bakare	-	Lecturer, OSUTECH, Esa-Oke.
- Dr. A.O. Ajayi	-	Lecturer, Department of Microbiology, Obafemi Awolowo University, Ile-Ife.
- Bunmi Ojo	-	Warri
- Uju Eze	-	Graduate student, Washington, DC.

- Mr. N. Obiallo	-	Evangelist, Nsukka/Port-Harcourt.
- Adeyinka Ajayi	-	Medical Doctor, New Jersey, U.S.A

Mr. Vice-Chancellor, distinguished ladies and gentlemen, I thank you for your presence.

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