UNIVERSITY OF PORT HARCOURT

"CONTINUING THE PRAISE OF ENZYMES"

An Inaugural Lecture

By

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ACKNOWLEDGEMENTS

Vice-Chancellor Sir, I would wish to start by acknowledging the Almighty God who made this day possible. May His name be glorified. Having done this, I would then express my gratitude to my late father, Pa Rufus, who laid a solid foundation for us his children quite early in our lives. Indeed, his fatherly care and vision propelled us to the great heights of life. He was a school administrator, having headed a number of Teacher Training Colleges in the then Eastern region/East Central State of Nigeria and passed the external degree of London University in History, Law and Theology in 1964, among other earlier academic successes. Next would be my late mother, Ma Paulina, who taught my siblings and I many attributes of life that enabled us go through challenges later in life.

I would not fail to mention our dear elder sister, Prof. (Mrs.) Patience C. Onakala of the Dept. of Geography and former Dean of Social Sciences, University of Nigeria, Nsukka, who was a strong leader when our mother passed on in 1976, two days to Christmas in an auto accident. She guided me and my siblings through our pursuits. It was her effort that made me go to Canada for my postgraduate studies. My siblings and close relations cannot be forgotten here as they did their best to hold forth the 'home front' and encouraged me, especially during my 6-year 'lonely' sojourn in Canada.

I acknowledge the contributions of some of my teachers/lecturers at various points of my academic training, but notably Professors A. Ogan, G. Umezurike together with Professor Emeritus E. O. Anosike. I must not forget Prof. L. C. Eze, my undergraduate project supervisor who coached me to my first international journal publication. My thanks also to Dr. Neil Madsen, now retired, my supervisor at the University of Alberta (U of A), Edmonton, a renowned Enzymologist, who encouraged me during my training in Canada. I acknowledge the guidance I got at U of A from the Graduate students' adviser, Dr. D. Scraba. I must not fail to appreciate the Alberta Heritage Trust Fund for Medical Research Fellowship awarded by the Province of Alberta, Canada, which enabled me complete my Ph.D. programme with

minimal financial stress after the initial Graduate Assistantship from the U of A.

My colleagues and students of the Department of Biochemistry, University of Port Harcourt deserve mention too as they were part of my success story. To my St. Nicholas (Ang.) Church, Aluu – University of Port Harcourt family I say many thanks for your prayers and encouragements. I will not forget my Government Secondary School, Owerri Old Boys' Association, Port Harcourt Branch for the support even as one continues with life activities. Also, I acknowledge Agulu Grammar School, Agulu, Anambra State, the 'providential vehicle' that enabled me complete my secondary education. I deeply appreciate my amiable wife, Dr. (Mrs.) Nkechinyere, and our children (Okechukwu, Chukwudi – both Civil Engineers from Uniport, and Chinenye – 2nd Year Geology student of Uniport) for the understanding and conducive home atmosphere that allowed me to reach the level of academic achievements that provided the knowledge I will impart in this lecture. To my students (undergraduate and postgraduate) and colleagues in the Department, and all those I could not mention due to exigencies, thanks for being there for me. Thanks a million!

Preamble:

I wish to thank the Vice Chancellor for giving me the opportunity to deliver the 124th Inaugural lecture of this unique University today.
As is global practice, an academe that has attained the peak of a Professor is expected to deliver an Inaugural lecture soon after to profess the academic activities that resulted to the esteemed elevation. Some do this almost immediately while others take their time mostly due to administrative and other distracting engagements that do not allow them time to organise their thoughts. I would say I belong to the latter group and it is gladdening that I have been able to pay my dues!
While tinkering with what slant to give my lecture, I pondered over my academic paths towards the specialty of Enzymology/Protein chemistry, a subdivision of Biochemistry, and could not but reflect on the 6th Inaugural lecture of the University of Port Harcourt given by my teacher and mentor, Professor Emeritus Emmanuel O. Anosike in 1987. Those who could recollect will remember that his lecture was titled 'In praise of enzymes'. I had wanted to title mine differently but could not run away from the fact that the praise of these 'wonder' macromolecular structures of living things can never be enough, hence, this lecture title - 'Continuing the praise of enzymes'. I do hope to convince you at the end that it was apt not to succumb to the usual temptation of taking a completely unique/personalised focus in a lecture like this.

OUTLINE:

Introduction
General overview of enzymes
Nature and characteristics of enzymes
Applications of enzymes:
- Industry
- Medicine

Specific contributions
Conclusion
Recommendations

INTRODUCTION

At the early stages in the development of science, there was general agreement that a remarkable characteristic of living things was their ability to carry out complex chemical reactions which enable them to sustain their living. This characteristic distinguished them from non-living things. However, the nature of the major players in this important characteristic was plagued with disagreements as some scientists proffered that they were chemical in nature while other supported the view that they were biological matter. The term 'enzyme' was introduced in 1877 by Wilham Friedrich Kũhne, a Professor of Physiology at the University of Heldelberg. It stands for the Greek word 'in leaven' (Kuhne, 1877, cited by Neelam *et al*., 2013) but could also stand for 'in yeast' as later reports stated, and represented the vital force for the various chemical reactions that characterised living things.

Many centuries before this important development many observations on the activity of this biological matter were known but no definite agreement was reached on their nature and characteristics. On historical note, as far back as 400 B. C., Ancient Egyptians were reported to have used processes of preservation of foods and beverages, as well as cheese making, all driven by what we now know as enzymes. In 1783, the famous Catholic priest, Lazzan Spallanzain, working on biogenesis of biomolecules, concluded that a force exists which makes living organisms to create self if given sufficient time in certain kinds of organic matter and called it 'life-generating force' (Vallery and Devonshire, 2003). By 1812, Gottlieb Sigismund Kirchhoff, during investigation of the conversion of starch to glucose, demonstrated the involvement of this biological matter (Asimov, 1982). The action of diastase was reported in 1833 by the French chemist, Anselme Payen (Payen and Persoz, 1833, cited by Neelam *et al*., 2103), and this was quickly followed in 1835 by the report of the hydrolysis of starch by the same material by Swedish scientist, Jōns Jacob Berzelius. Subsequently, Dubonfout demonstrated the action of invertase in 1846 (Neelam *et al*., 2013).

A major breakthrough in the discoveries was seen in 1862 in the work of Louis Pasteur, along with Ferdinand Cohn and Robert Kuch, which concluded that fermentation of sugars to alcohol by yeast was done by a vital force contained in the yeast cell called 'ferments', thought to function only within living organisms (Wang and Liu, 1996). By 1884, the Japanese Jokichi Takamine discovered takadiastase, a form of diastase from the microorganism *Aspergillus oryzae*.

Eduard Buchner demonstrated the conversion of glucose to ethanol by a cell-free extract from the yeast cell in 1897, suggesting that the so-called 'living force' or 'ferments' could be active outside living systems. In 1908, Otto Rohn, a German scientist, introduced application of pancreatic enzyme with inorganic salt in tanneries for 'bating' of hides (Neelam *et al*., 2013). Despite these advancements, the nature of this biological matter remained controversial.

A feat by James B. Sumner of Cornell University in 1926 provided the strong evidence on the nature of enzymes. Sumner and other colleagues purified in pure form and crystallized the enzyme, urease from jack bean. This achievement earned him a Nobel Prize which he shared with John H. Northrop and Wendell M. Stanley in 1947. The latter two discovered a complex procedure for isolating pepsin, a procedure since applied to crystallize several enzymes (Bennett and Frieden, 1969). With this development, the controversy on the nature of the 'vital force' or 'ferments' reported earlier by several researchers, was laid to rest. Since then, over 10,000 different enzymes have been described.

As stated by Anosike (1987), "the story of enzymes is the story of the subject 'Biochemistry', for it is impossible to talk about biochemical reactions without the enzymes that catalyze or speed them up"".

NATURE OF ENZYMES

Following the purification to purity, it was deduced that enzymes are biological macromolecules called proteins which are made up of amino acids that are linked to each other by peptide bonds to form polypeptides. These polypeptides are folded in nature to produce the 'native' or 'natural' structure of enzymes. These biological catalysts

convert substrates to products through well-know chemical events. The region where the enzyme interacts with its substrate is the ‘active site’. Some enzymes contain non-protein materials, required for their activity and these are called ‘prosthetic groups’ or ‘coenzymes’. Several factors such as enzyme concentration, concentration of the substrate, hydrogen ion concentration (pH), temperature and effector molecules (activators and inhibitors), are known to affect enzyme activity. These factors have been exploited in many ways towards modulating the activities of enzymes to solve a number of industrial and medical issues and may become evident as this lecture progresses. It is pertinent to point out that enzymes have been shown to catalyze more than 4,000 biochemical reactions involved in the maintenance of living organisms. Nature has ‘packaged’ these reactions in form of metabolic pathways, inter-linked in so many ways that makes the whole process intriguing to an appreciative mind.

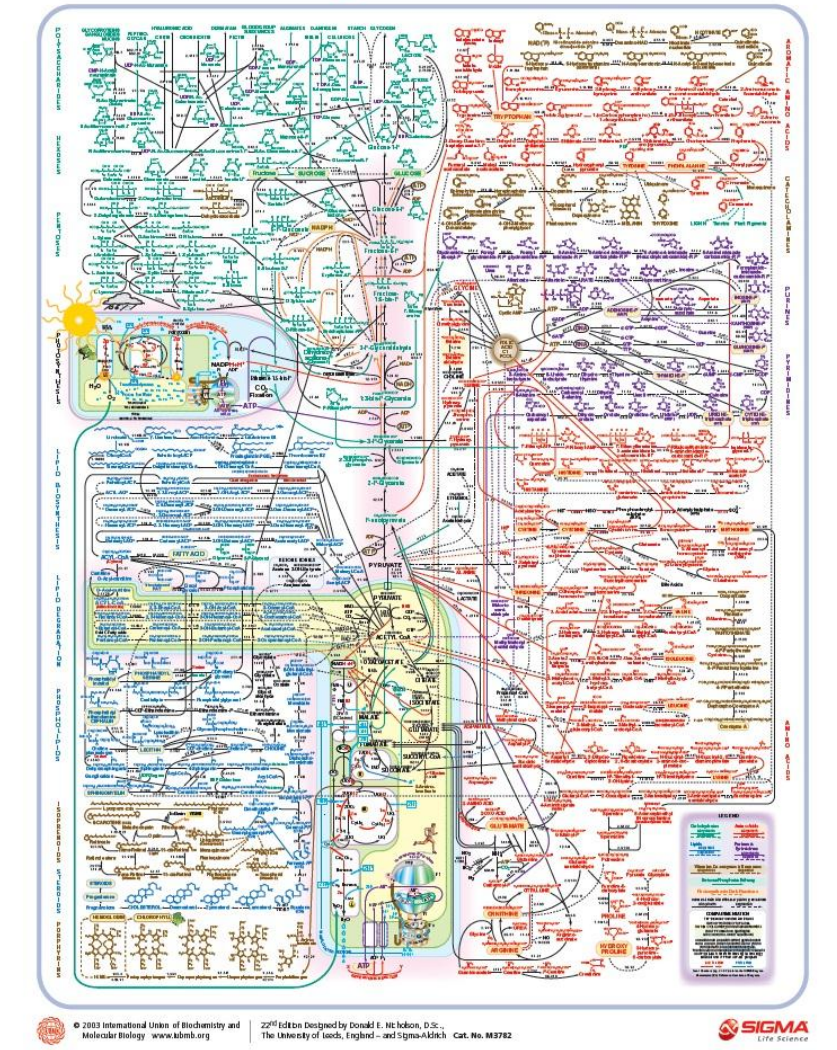


Figure 1. *A map of different metabolic pathways identified in living organisms*

APPLICATIONS OF ENZYMES

A wide range of applications have been described for enzymes in different biotechnology products of food/beverages, cleaning supplies, clothing, paper product, transportation fuels, pharmaceuticals and monitoring devises. Enzymes have also been applied in medicine.

Industrial applications

a. Animal feeds – plant materials, cereal, vegetables proteins that are found in feeds are not fully digested without addition of external enzymes. This arises from non-starch polysaccharides (NSP, commonly called fibre) and complex proteins. Examples of enzymes applied include α-amylase, xylanase, protease, β-glucanase, phytase, cellulose, in various combinations

b. Baking – basic materials is flour which contains gluten, starch, NSP and lipids, among other compounds. Dough preparation requires the action of yeast which ferments the sugars present into alcohol and CO_2 which causes the dough to 'rise'. These events are enzymatic (involving starch-degrading enzymes like amylase). For biscuits/crackers, 'soft flour' is used with low protein content. Weakening of gluten requires introduction of reducing agents, the common one being sodium bisulphite (damages vitamin B1). This agent has been banned in many countries including Nigeria. Protein-degrading enzymes are softer alternatives (bacterial/fungal protease).

c. Detergents – various compounds known to soil fabrics are best removed by enzymatic action
    i) grass, blood, egg, human sweat by protease as non-enzymatic detergents produce oxidation/denaturation caused by bleaching and drying
    ii) grease spots are problems with blends of cotton and polyester; also fats, butter, salad oil, and sauces but removed easily by lipases; also in dish-washing detergents

iii) starch-based foods like potatoes, spaghetti, custard, gravies, chocolate, removed by amylase

iv) fluffy-look on cotton/cotton blended cloths arise from micro fibrils but brightening can be achieved with cellulose which degrade these micro fibrils; also removed are dust particles

d Textile/Leather- hides and skins contain protein and fats in between collagen fibres, and must be removed before tanning. Protease and lipase used for soaking, bating and enzyme-controlled un-hairing. Bating de-swell swollen pelts and prepares leather for tanning (makes leather pliable). Starch and size lubricants is added to yarn before fabric production for fast and secure weaving. De-sizing achieved by amylase and lipase actions

e. Brewing – traditional beer production begins with 'mashing', a process in which crushed barley malt and hot water is mixed at raised temperatures. The product is called 'wort'. Apart from malt, other starch-based cereals like maize (corn), sorghum, rice or pure starch are added as adjuncts. Proteins also play roles as part of the fermentation of the wort by providing soluble nitrogen compounds needed by the yeast to react. The addition of proteases could add more soluble products for the yeast. Furthermore, slow filtration of wort and the final beer is a common problem arising from certain polysaccharides (β-glycans and pentosans). The addition of β-glucanases during the marshing circumvents the problem. The enzymes applied in brewing include heat-stable α-amylase, glucoamylase, bacterial proteases, and β-glucanases.

f. Alcohol production – the starting materials for fermented alcohol drinks include maize/corn, rye and barley, and wheat for whisky, and other cereals for grain spirits. Starch is the basic ingredient of these materials, and is acted on by yeast to form alcohol. The process occurs in two stages (liquefaction and saccharification). Commonly, enzymes are provided for the

process by adding malt, but industrial enzymes offer advantages over the malt. Liquefaction (ie. gelatinization of starch by pressure-cooking) which has been replaced in modern times by lower temperatures using α-amylase, and saccharification using glucoamylase to break down the starch molecules and dextrin. When other cereals are used, the low soluble nitrogen gives rise to slow yeast growth which is overcome by the addition of β-glucanase and pentosanase. Other enzymes applied include α- and β-amylases for liquefaction, glycoamylase and fungal α-amylase for saccharification, bacterial proteases for better yeast growth and reduced fermentation time.

g. Food industry- the modification of food flavour is achieved by the presence of synthetic esters of short-chains fatty acids and alcohols. Lipases are applied as natural modes of fragrance and flavour determinations. Other roles for lipases include the fermentation step in sausage manufacture which requires lipases; fat-removal during the processing of meat and fish (biolipolysis); refining rice flavour; modifying soya bean milk, enhancing aroma and speed of fermentation of apple wines. Mention has to be made of the research done by Anosike and others which provided the basis for the control of browning process of foods through addition of inhibitors of the browning-causing enzyme, polyphenol oxidase.

Applications in medicine

A number of enzymes are used as 'diagnostic enzymes' and this arises because of the specific actions of enzymes that make it feasible to determine their activities even when other compounds and proteins are present. Normally, very few enzymes are found in body fluids, with most of them arising from normal cell death in a living organism. There are some whose occurrence in body fluids is natural (eg. enzymes involved in the blood clotting process). In disease conditions, cell death rate increases leading to rise in amount and number of enzymes in body fluids. The measurement of the levels of such markers enzymes known to be specific to the organs and tissues play significant roles in medicine. Two classifications are made:

Use in diagnosis

	Disorder/disease state	**Enzyme(s)**
1.	Bone disorders, autoimmune and Inflammatory disorders	Alkaline phosphatase, Cathepsin D, Gelatinase B, Lysozyme
	Rheumatoid arthritis	Tartarate-resistant acid phosphatase
	Periprosthetic joint infection	Leucocyte esterase
2.	Cancer	
	Breast	Cathepsin D, Lactate dehydrogenase
	Bone metastasis	Alkaline phosphatase Tartrate-resistant acid phosphatase (isoform 5b)
	Hepatocellular carcinoma	Alanine Transaminase
	Gastric cancer	Glucose-6-phosphate dehydrogenase
	Prostrate cancer	Prostatic acid phosphatase (PAP)
	Premalignant lesions in colon, thyroid, brain, liver, breast, prostate	Cystheine cathepsins
	Germ cell malignancy	Lactate dehydrogenase
3.	Diabetes (type-2)	Alkaline phosphatise
4.	Gaucher's disease	Acid phosphatise
5.	Liver disease	
	Jaundice/hepatitis	Alanine transaminase
	Obstructive liver	Alkaline phosphatase
	Liver fibroisis	Aspartate transaminase
	Liver damage	Lactate dehydrogenase -5
6.	Myocardial infarction	α-amylase, creatine kinase (MB), gelatinase A and B Glycogen phosphorylase b Lactate dehydrogenase -1

7.	Pancreatitis	Amylase and Lipase
8.	Dental disorders	Aspartate transaminases, Cathepsin D
9.	Renal disorder	Urinary lysosomal glycoside Urinary lysozyme
10.	Skin disorder	Lipase
11.	Schizoprenia	Butyl choline esterase
12.	Intracerebral haemorrhage	Aspartate transaminase

Diagnostics (biosensors):

The emergence of biosensors (devices made up of biological sensing element that is linked to a transducer that could produce detectable data of the event) has gone a long way to assist in many industrial but mostly biomedical applications. Some examples are:

Glucose - a quick and reliable measurement of glucose levels in fluids involves the interactions of three enzymes (hexokinase, glucose oxidase and glucose-1-dehydrogenase). The actions of these enzymes are linked to develop a colour reaction that can be used to quantify the glucose levels in fluids, a valuable tool for diagnosis of diabetes mellitus.

Lactate - blood lactate level is a sensitive determinant of oxygen deprivation of tissues arising from ischaemia, trauma and haemorrhage. A number of lactate sensors have been described based on immobilized lactate monooxygenase and lactate oxidase.

Creatinine - estimation of creatinine is vital for renal, thyroid and muscle function evaluations. A number of biosensor developed generally employ creatinine deaminase.

Urea - fast and accurate measurements of urea in urine and blood samples are necessary to evaluate renal and liver disorders. Biosensors based on urease, which catalyses the conversion of urea to hydrogen carbonate and ammonium ion has been developed. In fact, the action of urease is crucial for the effective renal dialysis for patients with kidney disease or failure.

Other diagnostics (biosensors) are listed in the following table:

Compound detected	Enzyme(s)	Disorder/disease state
1. Creatinine/Creatine	Creatinine midohydrolase, Creatinine amidinohydrolase, Sarcosine oxidase	Renal/thyroid and muscle function
2. Glutamate	Glutamate oxidase	Neuropathology
3. Carnitine	Carnitine dehydrogenase & diaphorase	Carnitine deficiency, renal insufficiency, diabetes mellitus, etc
4. Theophylline	Theophylline oxidase	Determination of theophylline (e.g. asthma)
5. Cholesterol	Cholesterol oxidase	Artherosclerosis
6. Amino acid (D-serine)	D-amino acid oxidase	Brain disorder (e.g. schizophrenia)
7. Acetyl choline & choline	Acetyl choline esterase & choline oxidase	Neurological problems
8. Bilirubin oxidase	Haemoglobin & glucose	Jaundice
9. γ-amino butyric acid oxidase	Gabase & Glutamate	Neurological problems
10. H_2O_2	Horse radish peroxidise	Determination of cerebral peroxides

Enzyme therapy:

This deserves mention here as it is currently an important area of application of enzymes in medical practice. In focus are lesions that arise due to either lack of or presence of defective enzymes in the human body. Several factors could produce this. The most logical way of treatment is to make available the enzyme that is either lacking or defective. Ways to introduce this include oral administration, but several difficulties occur that include ensuring target sites are reached in addition to antigen-antibody reactions. Encapsulation has been introduced as a way of shielding the exogenous enzyme. In fact, successes has been made by the Canadian commercial product, Caelyx,

licensed for treatment of Karposi's sarcoma, a cancer found more commonly in AIDS patient (the stealth technology).

SPECIFIC CONTRIBUTIONS:

GLUCOSE HOMEOSTASIS

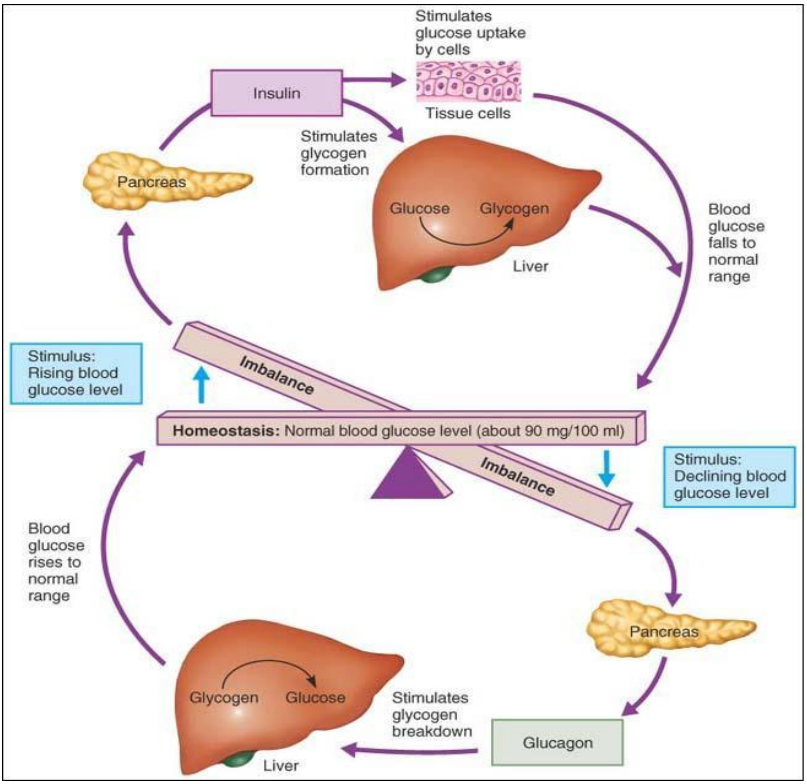


Figure 2. Illustration of glucose homeostasis

A hypothesis had been advanced by Hers and Stalmans (Stalmans, *et al*., 1974) that glycogen phosphorylase *a* was the glucose receptor of the liver cell, by the demonstration of activation of a modulating enzyme, glycogen phosphorylase *a* phosphatase by increased concentrations of glucose. By the time I made foray into Enzymology

under the tutelage of Neil Madsen, his laboratory had made important discovery that suggested a molecular basis for this hypothesis in their demonstration of a glucose-binding site on glycogens phosphorylase *a* that enhanced its activation by the phosphatase (Madsen *et al*., 1978; Fletterick, *et al*., 1979). In addition, a new inhibition site specific for nucleoside derivatives, which act in synergy with glucose in inhibiting glycogen phosphorylase from both muscle and liver, was defined (Kasvinsky *et al*., 1978). This nucleoside-binding site on muscle glycogen phosphorylase was adduced to play a role in the regulation of glycogen metabolism. While the natural effector for this site was unknown, caffeine was effective with glucose in accelerating the action of the phosphatase on glycogen phosphorylase *a* in intact hepatocytes (Kasvinsky *et al*., 1981).

Previous workers had deciphered a cascade regulation of glycogen in animals involving interplay of the synthetic enzyme, glycogen synthase, and the degrading enzyme, glycogen phosphorylase. Both enzymes were co-ordinately regulated by a cycle of phosphorylation /dephosphorylation, achieved through the action of phosphatases as in Figure 4. The un-phosphorylated glycogen phosphorylase (*b*-form) is inactive while the un-phosphorylated glycogen synthase (*b*-form) is active. Other forms of regulation occur through allosteric and hormonal mechanisms.

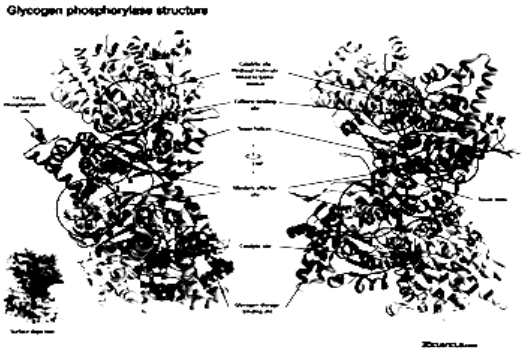


Figure 3. X-ray deduced structure of glycogen phosphorylase showing the nucleoside-binding site

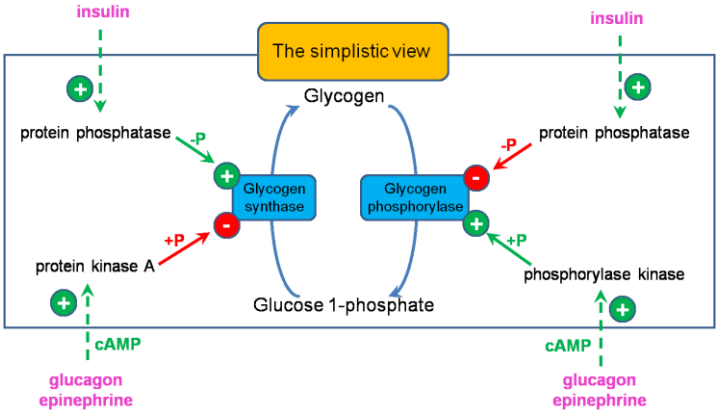


Figure 4. Scheme showing the inter-conversions of glycogen phosphorylase and synthetase

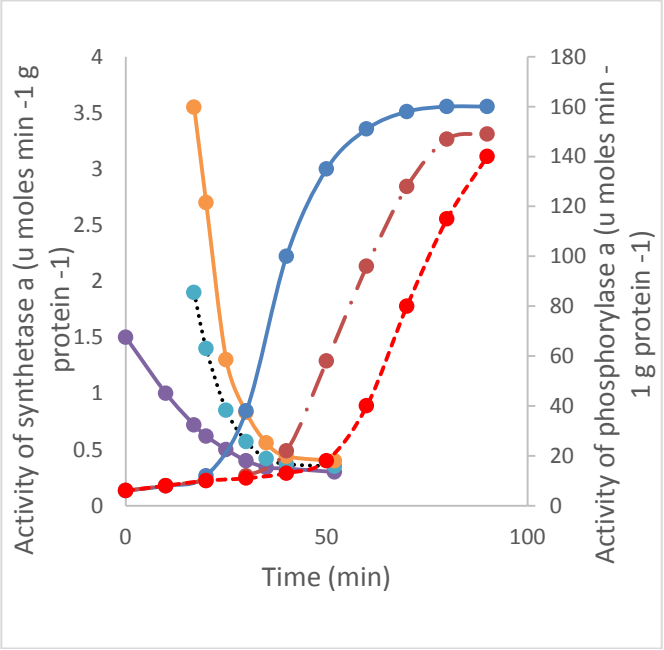


Figure 5. The effect of liver glycogen phosphorylase *a*, added before end of lag period, on the activation of glycogen synthase in rat liver gel filtrate.

Published report had indicated the occurrence of multiple molecular forms of protein phosphatase by the '80s, from skeletal and liver of rabbit with action on a large number of substrates including glycogen synthase and phosphorylase (Lee *et al*., 1980; Li, 1982). These phosphatases where classed into protein phosphatase-1 and -2, later shown to contain a catalytic subunit of molecular weight 35,000M_r. Cohen and collaborators demonstrated that protein phosphatase-2 accounted for a large amount of phosphatase activity on numerous substrates while protein phosphatase-1 was not the predominant form of phosphorylase *a* phosphatase in the liver (Martensen *et al*., 1973; Ingrebitsen *et al*, 1981; Ingrebitsen and Cohen, 1983).

Due to technical limitations most research on understanding the different phosphatase forms had used glycogen phosphorylase *a* from skeletal muscle as substrates for liver phosphatises. There was therefore the need to investigate the action of liver phosphorylase *a* phosphatase using liver substrate. My main preliminary research was to develop a novel procedure for isolating in large amount, glycogen phosphorylase from rabbit liver to be used as substrate for liver glycogen phosphorylase *a* phosphatase. This venture was tasking but with determination, I succeeded (**many bunnies were sacrificed for this venture!**) and was able to perform a number of experiments to further address the role of the nucleoside-binding site on glycogen phosphorylase *a* and the modulation of the protein phosphatase action by effector molecules including glucose and caffeine, alone and in combination. It was no mean task to isolate the 'holo' forms of protein phosphatases due to the fact that they were easily denatured following purification from cellular structures (Monanu and Madsen, 1985 & 1987).

Phosphorylase *a* phosphatase activity of three different molecular forms of rabbit liver glycogen phosphorylase *a* phosphatases (catalytic subunit of 35,000 M_r, proteins phosphatases-$2A_1$ and $2A_2$) were determined by measuring the release of ^{32}P from ^{32}P-labelled glycogen phosphorylase *a* at 30^o C. Some of the results obtained were as follows:

Effects of Mg^{2+}, glucose and caffeine on K_m and V_{max}

Variable effects were seen for glucose and caffeine, acting alone or in combination, reflected by increase in V_{max} for the different phosphatase forms. These increases were in most cases associated with small (for the 35,000 M_r catalytic subunit) to appreciable (for phosphatases $2A_1$ and $2A_2$) increases in K_m. With liver substrate, 10-fold and 25-fold increase in K_m and V_{max}, respectively, over the value obtained with muscle substrate was observed for 35,000 M_r phosphatase. The holo-phosphatases, $2A_1$ and $2A_2$, exhibited K_m's for the liver enzyme which were four-fold lower than was obtained for the catalytic subunit, explainable by noting that the holo-phosphatases represent more closely the 'native' enzyme form. Mg^{2+} had an activating effect on phosphatases $2A_1$ and $2A_2$. The independent and additive effects of glucose and caffeine on V_{max} for these two phosphatase forms were more significant with Mg^{2+} present while there was a smaller increase in Km in the presence of Mg^{2+} for phosphatase $2A_2$. The 1.2-fold increase in V_{max} for phosphatase $2A_1$ in the presence of both glucose and caffeine increased to about six-fold with Mg^{2+} present, suggesting almost a complete dependence on Mg^{2+} by this phosphatase form.

Table 1. Effects of Mg^{2+}, glucose and caffeine, in different combinations, on K_m and V_{max} of three rabbit liver phosphatase forms using rabbit liver and muscle ^{32}P-labelled phosphorylase *a*

	Phosphatase forms							
	35000 M_r phosphatase				Phosphatase $2A_1$, using liver substrate		Phosphatase $2A_2$, using liver substrate	
	Using muscle substrate		Using liver substrate					
Conditions	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
				Experiment A				
Control	4.3 ± 0.29	91.8 ± 2.4	54.6 ± 5.7	2281 ± 123.0	17.1 ± 4.5	0.2 ± 0.03	12.6 ± 3.5	1.68 ± 0.3
Glucose	7.3 ± 0.65	234.1 ± 9.9	56.8 ± 8.3	3589 ± 279.0	8.5 ± 2.1	0.1 ± 0.01	35.6 ± 12.6	6.08 ± 1.7
Caffiene	6.6 ± 0.65	264.0 ± 11.7	89.8 ± 8.1	5168 ± 291.0	13.2 ± 2.4	0.2 ± 0.02	77.3 ± 19.0	16.10 ± 4.4
Glucose-caffeine	7.7 ± 0.81	326.5 ± 16.3	74.1 ± 4.3	4876 ± 165.0	25.8 ± 4.3	0.4 ± 0.04	123.7 ± 33.4	33.50 ± 11.6
				Experiment B				
Control					19.8 ± 3.5	0.26 ± 0.02	16.4 ± 4.8	2.6 ± 0.5
$MgCl_2$					30.5 ± 5.2	0.74 ± 0.07	25.7 ± 6.8	7.2 ± 1.2
$MgCl_2$-glucose					31.6 ± 4.7	0.88 ± 0.08	65.8 ± 13.8	26.8 ± 6.0
$MgCl_2$-caffeine					47.4 ± 7.6	1.56 ± 0.17	45.9 ± 10.3	24.2 ± 4.3
$MgCl_2$-glucose-caffiene					35.4 ± 2.2	1.62 ± 0.06	47.8 ± 6.2	29.5 ± 3.2

Relief of inhibition by glucose and caffeine

Glucose and caffeine, acting alone or in combination, relieved inhibition by the various inhibitors tested. Using muscle substrate, the

inhibition by 2.5 mM ATP of the 35, 000 M_r catalytic subunit was relieved by combined activations of glucose (1.5 mM) and caffeine (25µM). Glucose-1-P (1 mM) inhibition was relieved by glucose and caffeine acting independently and together (Fig 6). Using liver substrate, ATP inhibition at 0.2 mM could be overcome by glucose (25 mM) and caffeine (1 mM), acting alone and in combination. AMP (0.3 mM) or ATP plus AMP, required the presence of both activators of the phosphatase for relief of inhibition. At the presumed physiological concentration of 7 mM for ATP, relief of inhibition was not seen. With excess Mg^{2+}, some relief was seen, however. Glucose-6-P and UDP-glucose inhibitions could be overcome significantly by glucose and caffeine acting alone or together (Fig 6).

The inhibition of phosphatase-$2A_2$ by ATP could not be overcome by glucose and caffeine acting alone or together in the absence of Mg^{2+}. Some relief of AMP inhibition was afforded by both ligands. The combined presence of glucose and caffeine was needed to relief the combined inhibition by ATP and AMP, although the activity was below the control value. Mg^{2+} significantly affected the response of this phosphatase to ATP inhibition (Fig. 3). ATP inhibition of phosphatase-$2A_1$ was relieved by glucose and caffeine, acting alone or in combination (Fig. 4). The combined presence of glucose and caffeine abolished the inhibition by AMP, while only the combination of glucose and caffeine could relief the combined inhibition by ATP and AMP. It is notable that the combined effect of glucose and caffeine was most potent in relieving inhibition of the different phosphatase forms by the inhibitors tested in the study.

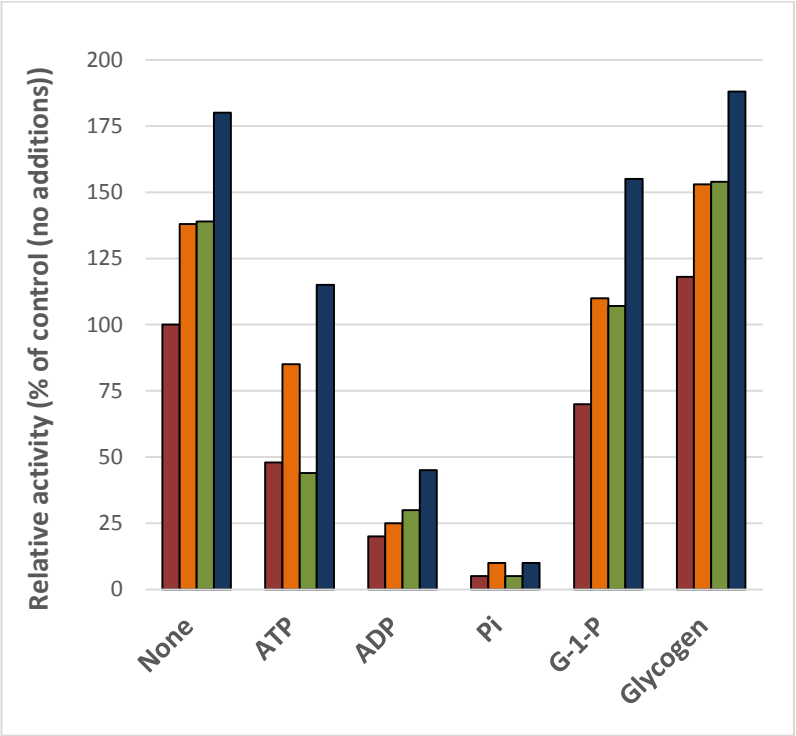


Figure 6. Effects of different combinations of ligands on the activity of 35,000 M_r phosphatase towards rabbit skeletal muscle ^{32}P-labelled phosphorylase *α*. Final concentrations were phosphorylase *a* (10μ*M*), ATP(2.5m*M*), ADP(2.5m*M*), glycogen(1%), glucose-1-P(G-1-P)(1m*M*), P_i(50m*M*), glucose (1.5m*M*), and caffeine (25μ*M*). Values are expressed in relative activity with control at 100%.

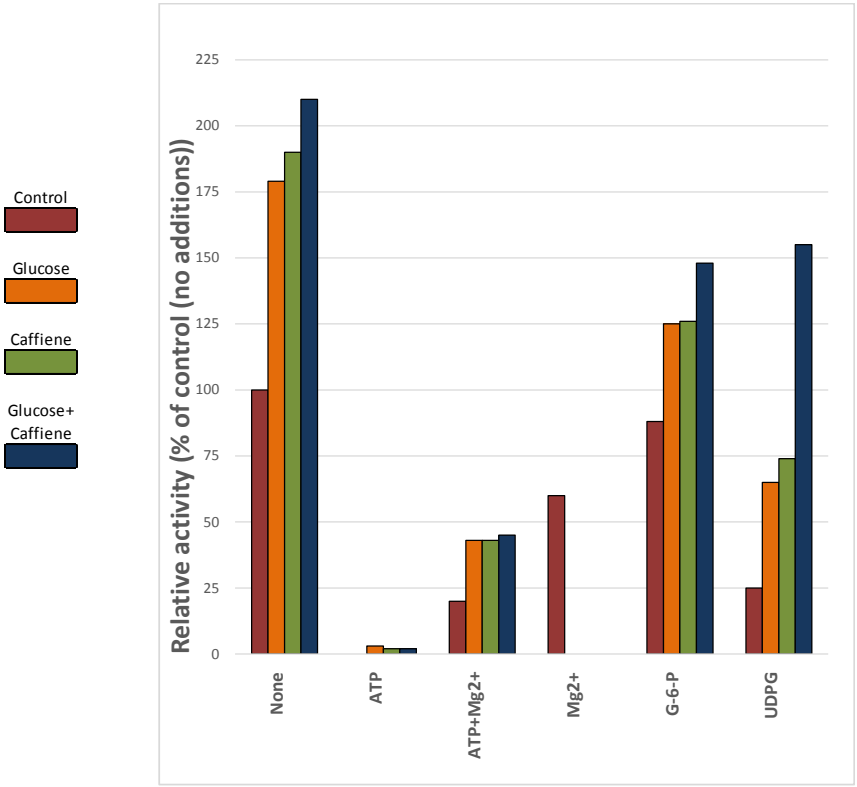


Figure 7. Effects of different combinations of ligands on the activity of 35,000 M_r phosphatase towards rabbit liver ^{32}P-labelled phosphorylase *a*. Final concentrations were: phosphorylase α (40µ*M*), ATP (7.5m*M*), $MgCl_2$ (10m*M*), glucose-6-P (G-6-P)(1m*M*), UDP-glucose (UDPG)(1.86m*M*), glucose (25m*M*), and caffeine (1m*M*). Values are expressed relative to control as 100%.

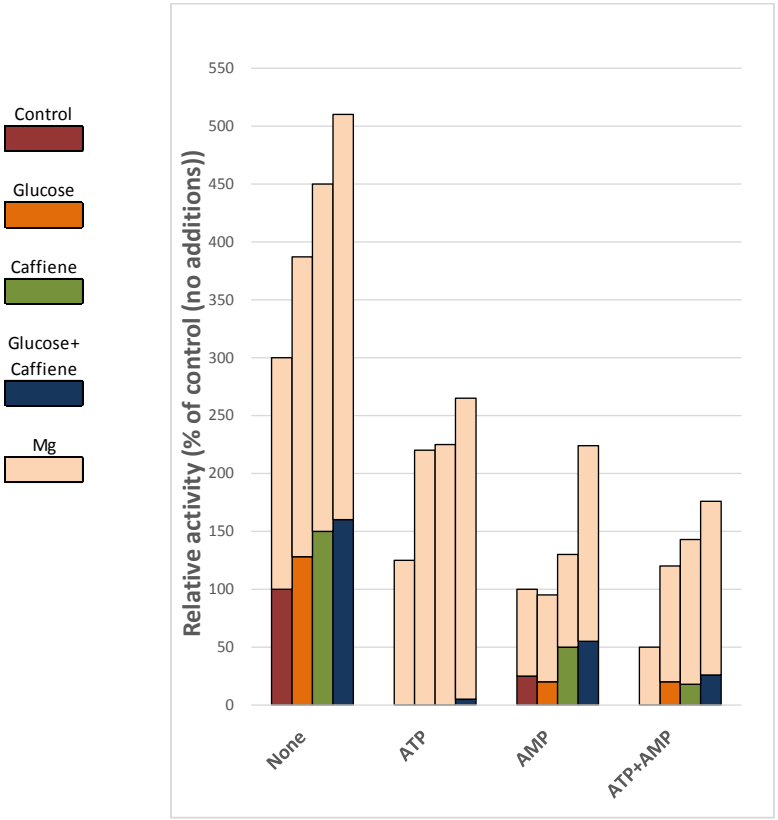


Figure 8. Effects of Mg^{2+}, ATP, AMP, glucose, and caffeine, in different combinations, on the activity of phosphatase $2A_2$ towards rabbit liver ^{32}P-labelled phosphorylase *a*. Final concentrations were: phosphorylase a (40µ*M*), ATP(7.2m*M*), AMP(0.3m*M*), glucose (25m*M*), caffeine (1m*M*), and $MgCl_2$ (2.8m*M*; 9m*M* with ATP). Values expressed relative to control as 100%.

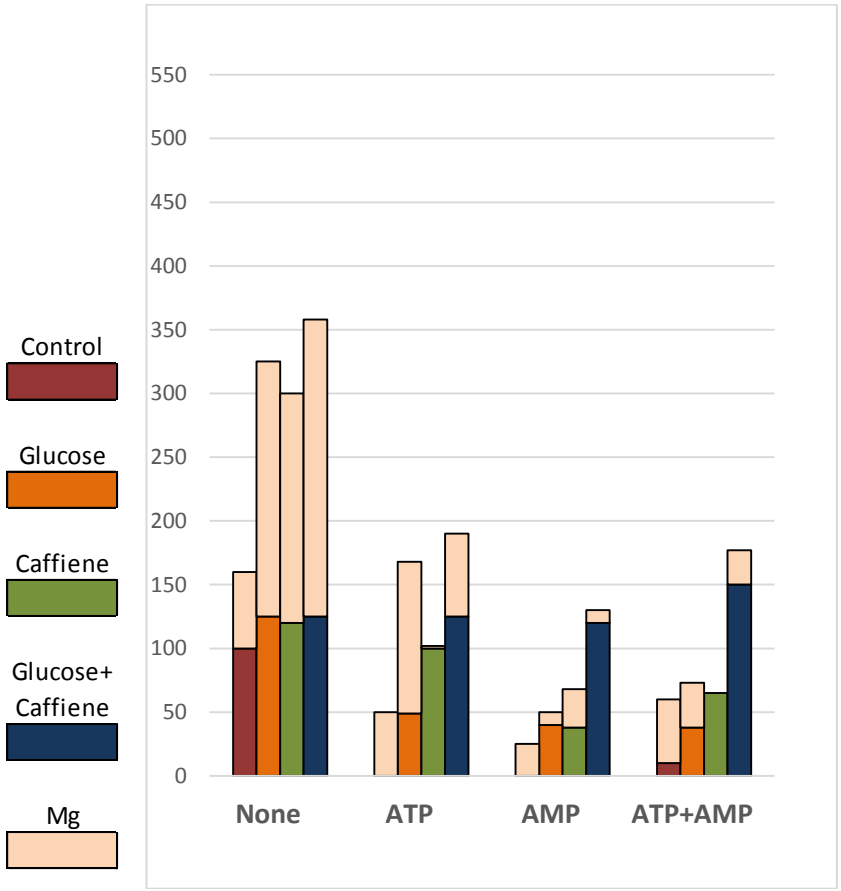


Figure 9. Effects of Mg^{2+}, ATP, AMP, glucose, and caffeine, in different combinations, on the activity of phosphatase $2A_1$ towards rabbit liver ^{32}P-labelled phosphorylase *a*. Final concentrations were: phosphorylase a (40µ*M*), ATP(7.2m*M*), AMP(0.3m*M*), glucose (25m*M*), caffeine (1m*M*), and $MgCl_2$ (2.8m*M*; 9m*M* with ATP). Values expressed relative to control as 100%.

Distinction between substrate- and enzyme-directed effects of modifiers of rabbit liver phosphorylase *a* phosphatises

Having established the synergistic role of glucose and caffeine in modulating the activities of the different phosphatase form, it was necessary to ascertain the mechanism by which these effector molecules exert their effect. There are three possible options: interaction with the protein substrate, the phosphatase itself, or to both. The earlier work, including those from Madsen's laboratory supported the view that glucose and caffeine effect may be substrate-directed effect. To address this aspect of research, investigation was carried out using two alternate substrate forms: ^{32}P-labelled histone and ^{32}P-labelled tetra-decapeptide encompassing the specific Ser-14 phosphorylation site in glycogen phosphorylase *a* (the substrate for the phosphatases). The objective was to 'destroy' the binding sites for glucose and caffeine in the 'normal' substrate for the phosphatase and determining how this would affect the actions of glucose and caffeine on the phosphatase activities. In the course of this research, it was reported (Ingebritsen & Cohen, 1983) that protein phosphatase-1, was the only phosphatase found in glycogen particles, hence investigation of earlier research was extended to this phosphatase form.

The results of effects of ligands on rabbit liver phosphatase-2A_1 activity towards three substrate forms (rabbit skeletal ^{32}P-labelled phosphorylase *a*, ^{32}P-labelled histone and ^{32}P-labelled tetradecapeptide) showed that the stimulating effects by glucose (25 mM), caffeine (1 mM) and Mg^{2+} (1 mM) seen with phosphorylase *a* was not observed with the histone or tetradecapeptide as substrates for the phosphatase. In addition, appreciable inhibition of phosphorylase *a* phosphatase activity for this enzyme form was observed in the presence of AMP (0.3 mM), ATP (7.5 mM), ADP (2.5 mM), Pi (1 mM) and glucose-1-P (1 mM). With ^{32}P-labelled histone as substrate, a small inhibition by P_i was noted, while inhibitions by AMP, ADP and glucose-1-P were not seen. ATP activated the dephosphorylation of histone, an effect that could arise from charged interactions between this nucleotide and the highly charged protein substrate. The dephosphorylation of the tetradecapeptide by this phosphatase form was not affected significantly

by AMP, ADP, ATP, and glucose-1-P, although Pi and Mg^{2+} slightly inhibited the activity.

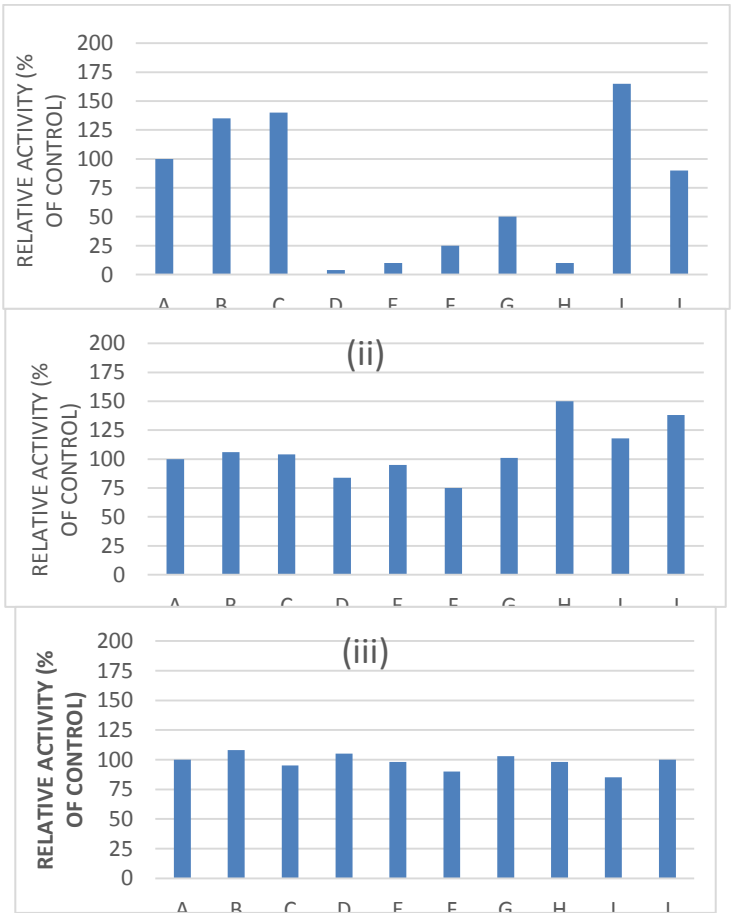
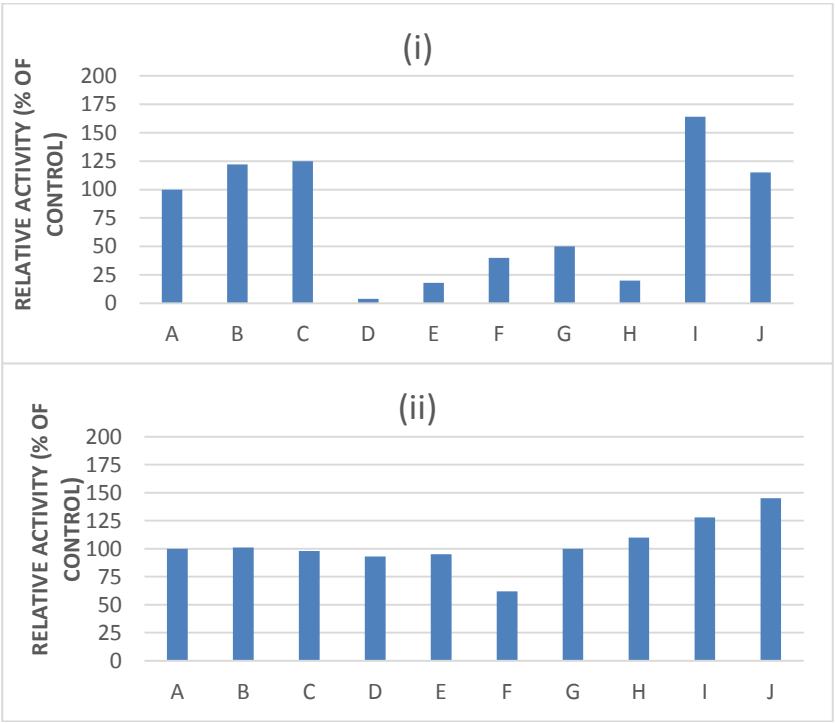


Figure 10. Effects of ligands on rabbit liver phosphatase-$2A_1$ activity towards three ^{32}P-labelled substrate forms (i) rabbit skeletal muscle phosphorylase *a* (40µ*M*), (ii) histone (80µ*M*), (iii) tetradecapeptide (150µ*M*), A (control), B (25m*M* glucose), C (1m*M* caffeine), D (0.3m*M* ATP), E (2.5m*M* ADP), F (1m*M* Pi), G (1mM glucose-1-P), H (7.5m*M* ATP, I (1.5m*M* $MgCl_2$) and J (9m*M* $MgCl_2$+7.5m*M* ATP). Values expressed as relative activity with control set at 100% (*, $p<0.03$).

The activity of phosphatase-$2A_2$ on phosphorylase *a* was activated by glucose, caffeine and Mg^{2+}, but these activations were abolished with phosphorylated his tone or tetradecapeptide as substrates. AMP, ADP, ATP, Pi and glucose-1-P inhibited the phosphorylase *a* phosphatase activity of this enzyme. Some activation was seen in the presence of ATP and Mg^{2+} using phosphorylated histone substrate, while AMP, ADP, and glucose-1-P had negligible effects. Significant inhibition by Pi was recorded. With the tetradecapeptide as substrate, ATP was inhibitory and the additional presence of Mg^{2+} had little effect. This was in contrast with the pattern obtained for phosphatase-$2A_1$. The other ligands, AMP, ADP, Pi and glucose-1-P, had little or no effects.



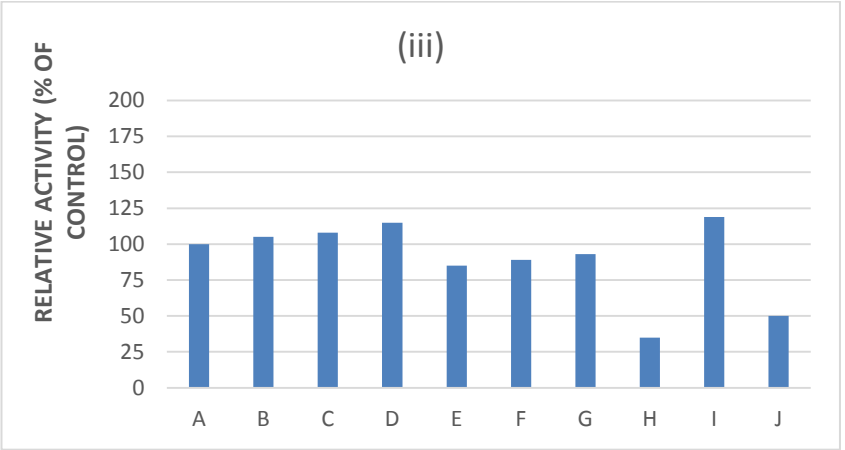
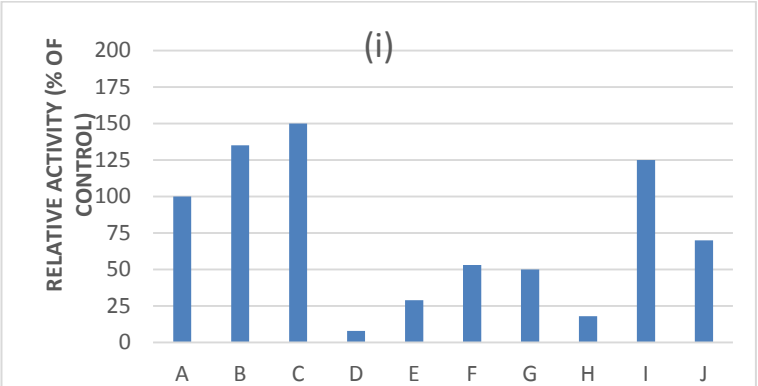


Figure 11. Effects of ligands on rabbit liver phosphatase-$2A_2$ activity towards three ^{32}P-labelled substrate forms. (i) rabbit skeletal muscle phosphorylase a (40µ*M*), (ii) histone (80µ*M*), (iii) tetradecapeptide (150µ*M*), A (control), B (25m*M* glucose), C (1m*M* caffeine), D (0.3m*M* ATP), E (2.5m*M* ADP), F (1m*M* Pi), G (1mM glucose-1-P), H (7.5m*M* ATP, I (1.5m*M* $MgCl_2$) and J (9m*M* $MgCl_2$+7.5m*M* ATP). Values expressed as relative activity with control set at 100% (*, $p<0.03$).

For phosphatase-1, similar patterns were seen for the three substrate forms with ATP inhibition when the phosphorylated tetradecapeptide was the substrate remaining even with Mg^{2+} present in addition. This pattern was similar to the result for phosphatase $2A_2$ against this substrate.


(i)
RELATIVE ACTIVITY (% OF CONTROL)
200
175
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125
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75
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A
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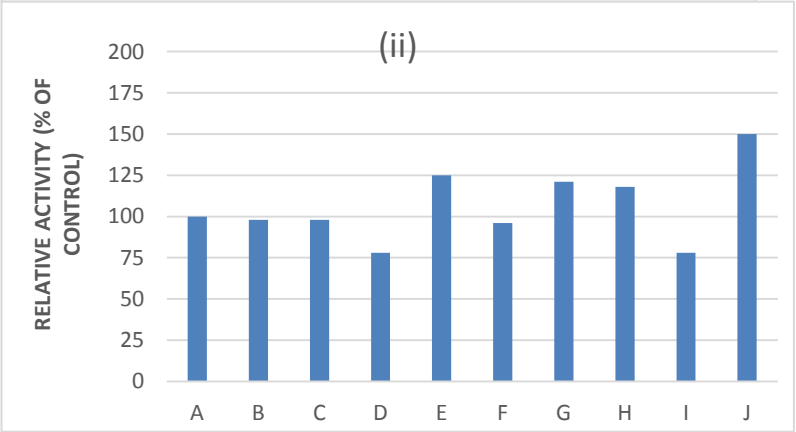
(ii)
RELATIVE ACTIVITY (% OF CONTROL)
200
175
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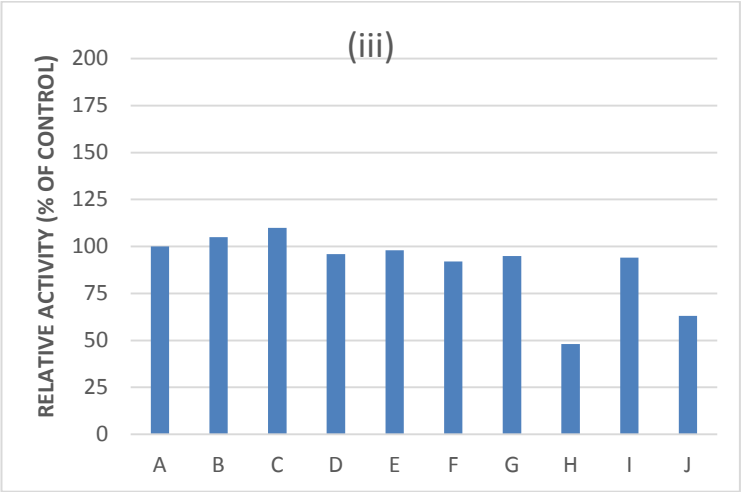

Figure 12. Effects of ligands on rabbit liver phosphatase-1 activity towards three ^{32}P-labelled substrate forms. (i) rabbit skeletal muscle phosphorylase a (40µ*M*), (ii) histone (80µ*M*), (iii) tetradecapeptide (150µ*M*), A (control), B (25m*M* glucose), C (1m*M* caffeine), D (0.3m*M* ATP), E (2.5m*M* ADP), F (1m*M* Pi), G (1mM glucose-1-P), H (7.5m*M* ATP), I (1.5m*M* $MgCl_2$) and J (9m*M* $MgCl_2$+7.5m*M* ATP). Values expressed as relative activity with control set at 100% (*, $p<0.03$).

A major assumption that allows unambiguous interpretation of results in studies elucidating modes of modifier actions using alternate substrate is that the modifier does not interact with the alternate substrate. The results obtained in this study indicated that glucose and caffeine exhibited substrate-directed effects for the phosphatase forms. Also ADP, Pi and glucose-1-P exerted substrate-directed effect. ATP inhibited the phosphorylase *a* phosphatase as well as the phosphorylated tetradecapeptide phosphatase activities of phosphatase-1 and -2A_2 hence showed both substrate- and enzyme-directed effects. For phosphatase-2A_1, only substrate-directed effect was noted.

Effects of glucose and caffeine on the inhibition of glycogen synthase phosphatase by glycogen phosphorylase *a*

The extensive studies by Hers, Stalmans and co-workers established the characteristic latency in the activation of glycogen synthase in rat liver filtrates. Having shown the independent and additive actions of glucose and caffeine on the different protein phosphatases in the earlier work, we decided to investigate what effects glucose and caffeine would have on the latency in glycogen synthase activation in liver filtrates from well-fed New Zealand white rabbits. The findings showed that independently, glucose (20 mM) or caffeine (1 mM) decreased the latency in activation with further decrease occurring in their combined presence. A severe inhibition shown by 0.3 mM AMP was relieved by both ligands acting separately and together. Mg^{2+}.ATP (4 mM and 2.5 mM, respectively) and exogenous glycogen phosphorylase *a* exhibited similar patterns of inhibition and relief. These results are consistent with the view that glucose and caffeine exerted their effects by interactions with glycogen phosphorylase *a*, thus supporting the assertion that the nucleoside binding site on glycogen phosphorylase *a*, probed with caffeine, remains physiologically significant (Monanu and Madsen, 1988).

Studies were carried out to investigate the effect of glycogen phosphorylase a on the dephosphorylation (activation) of glycogen synthase b, phosphorylated by the catalytic subunit of cAMP-dependent protein kinase, by purified rabbit liver protein phosphatases. Protein phosphatase-1 was significantly inhibited whereas the activities of protein phosphatase-$2A_1$ and -$2A_2$, and the M_r 35,000 phosphatase catalytic subunit, were not affected to appreciable extent. No latency was noted in the dephosphorylation of the synthase by any of the phosphatases, either in independent of collective presence of glucose and caffeine. While the findings support the view that protein phosphatase-1 may be the protein involved in the latency in glycogen synthase activation demonstrated with rabbit liver filtrates, the absence of latency in the reconstituted experiment could be a as a result of presence of some 'undefined' natural factors *in vivo* as vis-a-vis the *in vitro* conditions of our study (Monanu and Madsen, 1988).

Nature of the physiological molecule that binds to the nucleoside-binding site

At this point, further research was to be directed to identifying the natural molecule that interacts with the nucleoside-binding site on the glycogen phosphorylase which had been shown to have physiological role in glucose homeostasis. However, it was time for me to complete my postgraduate programme having done sufficient to accomplish the doctoral degree. I had the dream to continue when I got back to Nigeria, in collaboration with Dr. Madsen's laboratory in Canada. Little did I know that the enabling environment and conditions of research would be a serious handicap. I found myself faced with the situation where I could not have access to the sort of laboratories and facilities I experienced while in Canada. This situation at first was very frustrating as I had headed home loaded with all sort of research ideas, with great enthusiasm to help develop our scientific activities, including engaging in production and marketing of biochemical reagents for prime research work. The need to forge ahead dawned on me after some time, though it was difficult to break away from the usual practice among enzymologists of maintaining a monogamous relationship with the doctrine of 'one man-one enzyme'. Consequently, I had to 'flirt' with a number of enzymes to keep the flame of enzyme research going and will highlight some of these in subsequent part of this lecture.

At some point, there was increased interest in examining several aspects associated with the medical condition of Sickle cell syndrome, which afflicts a good number of people of black race. I joined forces with other researchers (Professor Emeritus Anosike, Professor Uwakwe, and Late Professors Ekeke and Ibeh) in the Department to study three key enzymes known to play some part in the metabolism of the human erythrocytes. The objective was to see if there could be any notable variance or otherwise in human erythrocytes from the three genotypes (HbAA, HbAS and HbSS) as well as consider possible molecular mechanisms of ameliorating effects of plant extracts.

Glutathione-S-transferase

Studies had indicated two general categories of functions for the broad group of glutathione-S-transferases, namely, as binding proteins for

such biomolecules as albumin and bilirubin, and as participating in cellular events that protect cellular constituents from electrophilic xenobiotics. Glutathione-S-transferases (GST) have been found to occur in high levels in the liver, these enzymes also occur in a wide variety of tissues, including human erythrocyte (Chasseaud, 1979). While the physiological role of the erythrocyte enzyme has not been fully described, it has been suggested that it could function in the removal of electrophilic xenobiotics. Haemolytic crisis which causes anaemia, is a major complication associated with sickle cell disease. While the genesis of this crisis is yet to be defined at the molecular level, antioxidants such as iron III (Fe^{3+}) and superoxides have been shown to facilitate destruction of red cells (Kuijk *et al*., 1987). In addition, haemin, a protoporphyrin with associated ferric ion, has been demonstrated to produce haemolysis in vitro (Kirshner-Zilber *et al*., 1982). Both liver and erythrocyte forms of GST have the ability to bind haemin. The maintenance of a 'reduced' environment within the erythrocyte is ascribed to the action of reduced glutathione (GSH), the substrate for GST, and depletion of GSH in intact erythrocyte (in vitro) leads to rapid oxidation of large amounts of haemoglobin to methaemoglobin (Anosike *et al*., 1991). The possibility thus exists that the functional status of erythrocyte GST may be related to the haemolytic crisis seen with Sickle cell disease. We therefore undertook comparative studies on the GST of erythrocytes from the three human genotypes.

Our findings showed that the mean activity levels of erythrocyte GST was significantly raised in HbAS and HbSS ($p<0.001$) in comparison with that for HbAA erythrocytes with that for HbSS significantly higher than for HbAS erythrocytes. The Michaelis constant (K_m) for the enzymes in the three genotypes were similar at 1.4 x 10 -4 M, suggesting structural and functional similarities. The variable levels of activity of the order HbSS>HbAS>HbAA could point to increased perturbations of the enzyme in HbSS and HbAS erythrocytes when compared to the normal, HbAA. This increase may be suggestive of higher levels of oxidants (hence electrophiles) such as peroxides, haemin and possibly methaemoglobin. It was suggested that the erythrocyte GST could be acting intracellularly to prevent oxide-

induced haemolysis. It could also be acting generally to curb the toxic effects of erythrocyte electrophiles/oxidants (Tables 2 and 3).

TABLE 2
Activity levels of human erythrocyte glutathione-S-transferase of AA, AS and SS genotypes

Genotype	E (IU/g Hb)	SD	SEM
HbAA	2.75	0.78	0.17
(n = 20)			
HbAS	4.65*	1.09	0.35
(n = 20)			
HbSS	14.61*	3.37	0.77

Results in AS and SS groups compared with those of AA groups by the t index and significance of t values
* $p<0.001$

TABLE 3

Effect of Dithionite (A) and Haemin (B) on GST activity (n = 3)

A) Sample	Enzyme activity (IU/g Hb)		% Increase in activity
	Control	0.3 mM dithionite	
1	16.39	25.95	58.33
2	21.99	37.45	70.30
		29.10	
3	14.03		62.71
			% Increase = 63.70 ± 4.90

B)

Sample	Control	0.3 mM Haemin *	
1	16.39	9.88	39.66
2	21.99	13.06	40.61
3	14.03	8.97	36.07
			% Decrease = 38.98 ± 1.96

* Haemin was solubilized at pH 7.0 before use

The GST activity increased in the presence of methaemoglobin reductant, dithionite, and decreased in the presence of the oxidant, haemin, further supporting the view on the pertubative action of erythrocyte oxidation-reduction status on the enzyme. The implications of our findings is that a criterion of an effective anti-sickling agent should be its ability to reduce to normal, GST activity, and possibly raise levels of erythrocyte electrophiles/oxidants (Anosike *et al*., 1991).

Effects of extract of Cajanus cajan seeds on HbSS erythrocyte Glutathione-S-transferase activity

A number of therapeutic approaches have been employed towards the possible management of Sickle cell disease, and notable are the use of a variety of metabolites and compounds possessing varying degrees of antisickling potency. Some of these include some aromatic amino acids, particularly phenylalanine and tryptophan (Nguchi and Schechter, 1978). About 70 % of the antisickling effect of aqueous extract of the edible beans of *Cajanus cajan* L. miso (Fabaceae) had been ascribed to phenylalanine with the extract containing about 26.3 % of this amino acid in the free state (Ekeke and Shode, 1990). Studies by Russu *et al* (1986) indicated that one binding site, common to all compounds with antisickling activity, is at or near the haem pockets in the alpha and beta chains of both deoxy-HbS and –HbA.

Our studies on GST activity in human erythrocytes of varying genotypes showed higher activity in sicklers than non-sicklers, and it was therefore considered to investigate the effects of the extract of the

beans, *C. cajan*, on erythrocyte GST activity with a view of any impact on the physiological function of this enzyme in HbSS erythrocytes. The results of *in vitro* and *in vivo* experiments indicated dose-dependent inhibition of GST activity by phenylalanine and *C. cajan* extract of HbSS erythrocyte (Table 4 and 5). The inhibition by the extract was more pronounced. These findings were also seen with in vivo experiments in which daily consumption of the extract was undertaken. The monthly decrease in GST activity over 3-month period was significant, and correlated with concomitant improvements in whole blood haemoglobin concentration. Furthermore, overall improvements in health, as reflected by complete arrest of crisis, improved appetite and physical activity, among others, were noted.

TABLE 4
***In vitro* effect of Phenylalanine and *C. cajan* extract on HbSS erythrocyte GST activity**

*(Test substance) mM	% GST activity (X±SD; n =8) +	
	with Phe	with C. cajan extract
0.00 (control)	100.00 ± 0.00	100.00 ± 0.00
0.038	91.29 ± 1.70	84.07 ± 4.95
0.075	86.85 ± 2.65	70.44 ± 6.01
0.150	78.66 ± 3.66	54.64 ± 2.92
0.300	71.59 ± 4.45	25.95 ± 8.62
0.500	41.00 ±+ 5.58	16.00 ± 4.01

+ Percentage decreases in GST activity, relative to control, were in each case significant at 95% confidence level ($p<0.05$). *Amino acid concentration of Phenylalanine or *C. cajan* extract.

TABLE 5
***In vivo* effect of *C. cajan* extract on sickle cell haemoglobin concentration (SCHb) and GST activity**

Consumption time (months)	% Decrease in GST activity (X±SD; n = 8)	% Increase in SCHb (X±SD; n = 8)
0 (control)	0.00	0.00
1	26.89 ± 4.10	2.10 ± 0.55 +
2	54.39 ± 7.62	18.54 ± 5.23 *
3	74.92 ± 8.94	38.43 ± 8.62 **

+ Percentage decreases in GST activity, relative to control, were in each case significant at 95% confidence level ($p<0.05$).
* Percentage increase, relative to control, were significant at 50 % confidence level ($p<0.5$).
**Percentage increase, relative to control, were significant at 95 % confidence level ($p<0.05$).

The conclusion of our findings was that the antisickling nutrients, apart from acting as haemoglobin polymerisation inhibitors, could also be acting, *in vivo*, to reduce the raised oxidant state seen in sickle cell disease. In addition, the improved whole blood haemoglobin concentration following therapy reflects improved haemopoesis and possible red cell survival rate (Uwakwe *et al*., 1996).

Activities of NAD (P)H Diaphorases in erythrocytes from HbAA, HbAS and HbSS subjects

NADH- and NADPH-diaphorase are the main methaemoglobin reducing enzymes of the human erythrocyte (Nagai *et al*., 1980; Yubusi *et al*., 1980). Methaemoglobin is a derivative of haemoglobin in which the iron of the deoxygenated haem complex has been oxidized to the ferric form (Harris and Kellermeyer, 1974). The amount of methaemoglobin in 'normal' erythrocyte is continuously formed and reduced by specific mechanisms such as to retain less than 1 to 2 % of the total pigment. An abnormal quantity of this compound is known to occur under a number of conditions (Kellermeyer, 1974). *In vivo* studies showed that about 16 % of methaemoglobin formed is reduced by

ascorbic acid (Vitamin C) and 12 % by reduced glutathione (GSH). The remainder is reduced enzymatically by the methaemoglobin reductases (NAD(P)H diaphorases). Methaemoglobin can be hydrolysed to release the oxidants, protoporphyrin and haemin (Orji *et al*., 1981; Vearley *et al*., 1984). In large amounts, haemin causes the lysis of malaria parasites (Kirshner-Zilber *et al*., 1982). This high amount of haemin accessible to bind chloroquine in HbSS erythrocytes is offered as an explanation for death of malaria parasites in these erythrocytes. High haemin could also bear a relationship to the redox state and hence the methaemoglobin generating and/or reducing rate of the sickle red cell, and was the subject of our investigation.

Our findings (Table 6) showed different levels of NADH-diaphorase activity in the three genotypes with the following pattern: HbSS>HbAS>HbAA. The NADPH-diaphorase activity showed a similar pattern. Increase in activity was seen for both enzymes in the presence of 0.1 mM haemin, while decreases were obtained for 0.3 mM dithionite, a known reductant of methaemoglobin. The enzymes from the different genotypes had similar Km's suggesting structural and functional similarities. The activity order of HbSS>HbAS>HbAA

TABLE 6

Human erythrocyte NAD (P) H diaphorase activity of HbAA, HbAS and HbSS subjects

	NADH diaphorase activity (iu/g Hb, X±SD)		
Haemoglobin Genotype	***Both sexes***	***Males***	***Females***
HbSS	19.58 ± 4.71 (n=21)	18.28 ± 4.21 (n=13)	21.68 ± 4.73 (n= 8)
HbAS	12.28 ± 2.52 (n=15)	13.14 ± 3.05 (n= 7)	11.53 ± 1.61 (n= 8)
HbAA	9.58 ± 1.76 (n=21)	10.21 ± 1.38 (n= 9)	9.07 ± 1.84 (n=12)
	NADPH diaphorase activity (iu/g Hb, X±SD)		
HbSS	7.87 ± 1.89 (n=22)	8.35 ± 1.76 (n=14)	7.03 ± 1.80 (n= 8)
HbAS	2.58 ± 0.72 (n=17)	2.63 ± 0.62 (n=10)	2.50 ± 0.85 (n= 7)
HbAA	1.62 ± 0.43 (n=20)	1.56 ± 0.49 (n=10)	1.67 ± 0.36 (n=10)

suggests higher level of the oxidant, methaemoglobin, in HbSS and HbAS subjects compared to HbAA genotypes. In addition, it could be suggestive of insufficient level of reductants arising from low levels of NADH and HADPH generation in erythrocytes from HbSS and HbAS individuals, to measure up with the raised oxidant levels. The increased activity, in vivo, of the enzyme seen with the addition of haemin further confirms the pertubative action of the oxidation-reduction status on these enzymes, and/or on the Fe^{3+}:Fe^{2+} haemoglobin ratio. The intermediate values in the activities of the enzymes in HbAS erythrocyte could be due to the 'hybrid' nature of the heterozygous haemoglobin, a feature that had not been previously emphasized (Uwakwe *et al*., 1999).

Activities of Aspartate and Alanine transaminases and Alkaline phosphatase in human erythrocyte of different genotypes

The significance of determining enzyme activity levels in different samples, including body tissues and fluids, as a tool for understanding the molecular basis for several biochemical processes, has been recognised in scientific activities. One important activity relating to amino acid metabolism is transamination, catalysed by transaminases. These enzymes require the coenzyme, pyridoxal phosphate, and reports had been made that erythrocytes accumulate the coenzyme, a phenomenon purported to be dependent on the haemoglobin content of the erythrocytes (Mehansho and Henderson, 1980). Furthermore, erythrocytes of sickle cell patients have been reported to show increased zinc content (Czajka-Narins, 1986). Zinc, a divalent cation, is an important prosthetic group for metallo-enzymes including alkaline phosphates. It was considered relevant to undertake study of comparative levels of the three enzymes - aspartate and alanine transaminases (AST and ALT) and alkaline phosphatase (ALP) in human erythrocytes from three genotypes, to ascertain what roles they could play in the biochemical activities involving the enzymes in erythrocyte metabolism.

TABLE 7
Activity levels of Aspartate transaminases from HbAA, HbAS and HbSS human erythrocytes

	Enzyme activity (U/g Hb)		
	HbAA (n=20)	HbAS (n=20)	HbSS (n=20)
Males (n = 26	6.90 ± 0.98 (n=12)	7.81 ± 1.01 (n=7)	13.81±1.80* (n=6)
Females (n=21)	6.42 ± 1.00 (n=8)	7.56 ± 0.98 (n=8)	13.10 ± 1.51* (n=6)
Average	6.75 ± 0.99	7.48 ± 1.99	13.37 ± 1.78*

*significantly different ($p<0.05$) from normal (HbAA)

Activity levels of Alanine transaminases from HbAA, HbAS and HbSS human erythrocytes

	HbAA (n=20)	HbAS (n=15)	HbSS (n=12)
Males (n= 26)	4.11 ± 1.01 (n=12)	4.10 ± 0.95 (n=7)	6.91 ± 1.30*(n=6)
Females (n=21)	4.15 ± 0.98 (n=8)	4.41 ± 0.99 (n=8)	7.35 ±2.00* (n=6)
Average	4.26 ± 0.24	4.33 ± 0.44	7.24 ±1.49*

*significantly different ($p<0.05$) from normal (HbAA)

Activity levels of Alkaline phosphatase from HbAA, HbAS and HbSS human erythrocytes

	HbAA (n=20)	HbAS (n=15)	HbAS (n=12)
Males (n=26)	70.10 ± 3.90 (n=12)	65.15 ± 8.20 (n=7)	50.10 ± 3.51* (n=6)
Females (n=21)	68.20 ± 4.50 (n=8)	60.95 ± 5.30 (n=8)	41.20 ± 5.20* (n=6)
Average	69.24 ± 8.94	64.26 ± 9.17	48.49 ± 4.35*

*significantly different ($p<0.05$) from normal (HbAA) and HbAS genotypes

Our results (Table 7) showed different levels of activity in these genotypes for the three enzymes with the following order: AST (HbAA>HbAS>HbSS), ALT (HbAA>HbAS>HbSS) and ALP (HbAA>HbAS>HbSS). However, no significant difference was observed for the levels of the three enzymes between HbAA and HbAS erythrocytes. AST and ALT levels in HbSS erythrocytes were significantly higher ($p<0.05$) than for HbAA erythrocyte. ALP showed significantly lower activity level in HbSS than HbAS erythrocytes. The findings thus suggest differences in biochemical activities involving these enzymes in the human erythrocytes studied (Onwubiko et al., 2003).

Studies on aqueous extract of *Garcinia kola* seeds

A number of plant products had gained attention as sources of the active principles in the management of sickle cell disease, and of them is *Garcinia kola* (a popularly consumed seed in Southern Nigeria, commonly called 'bitter kola'). This seed has also been ascribed many trado-medical applications (Willis, 1973; Iwu, 1985). A number of studies were designed to investigate possible roles for the aqueous extract of this seed in the management of sickle cell disease.

Effect on membrane stability of human erythrocytes of three genotypes

Our findings showed that the aqueous extract of *G. Kola* seeds stabilized the membranes from HbAA, HbAS and HbSS erythrocytes by 22, 17, and 25 %, respectively over the stability recorded in its absence (Table 8 & 9). Phenylalanine, a known anti-sickling agent stabilized the HbSS membrane by 17.85 % suggesting a more potent effect by the extract. The HbSS blood viscosity was decreased significantly over HbAA and HbAS blood samples by the extract. In addition, the extract showed pronounced reversion of 2 % sodium metabisulphite-induced sickling. These findings support the view that the extract may have usefulness in the management of sickle cell crisis (Elekwa *et al*., 2003a).

TABLE 8

Median corpuscular fragility (MCF) values and % stabilization for HbAA, HbAS and HbSS erythrocytes in the absence/presence of (A) Phenylalanine (400µmole, final concentration) or (B) aqueous extract of *G. kola* seeds [100g/250 ml, (w/v)].

A:	**MCF expressed as [NaCl] g/l**		
Genotype	***Control***	***+ Extract***	***% stabilization***
HbAA	3.6 ±0.1	4.4 ± 0.1	22.22
HbAS	3.4 ± 0.1	4.0 ± 0.1	17.65
HbSS	2.8 ± 0.2	3.5 ± 0.1	25.00
B:			
HbAA	3.6 ± 0.1	4.3 ± 0.1	19.44
HbAS	3.4 ± 0.1	3.8 ± 0.1	11.77
HbSS	2.8 ± 0.2	3.3 ± 0.1	17.85

TABLE 9: ***Effects of aqueous extract of G. kola seeds [100 g/250 ml (w/v)] and Phenylalanine (400 µM) on the viscosity of blood samples.***

	Viscosity (x 10^{-3}Pa-S)						
		+ G. kola			+ Phenylalanine		
Incubation time (mins)	0	30	60	90	30	60	90
HbAA (n = 5)	1.7	1.5	1.3	1.3	1.6	1.4	1.4
HbAS (n= 5)	2.1	1.8	1.7	1.7	1.8	2.0	2.0
HbSS (n = 5)	3.4	2.5	1.9	1.5	2.6	1.8	1.3

Effect on human erythrocyte adenosine triphoshphatases

Three different adenosine triphoshphatases (Na^+, K^+-, Ca^{2+}- and Mg^{2+}-ATPases) have been described in the human erythrocyte membranes (Drickamer, 1975; Ibeh *et al*., 1992). These enzymes act as ion pumps in the unidirectional active transport of ions, a process that is thought to play major role in maintaining the stability of the erythrocyte membrane. Reports had suggested that pharmacological agents that alter membrane permeability could be beneficial in the management of sickling of erythrocytes, a major manifestation in sickle cell disease. In view of the role of the ATPases in maintaining the stability of the membrane, investigation of the effect of the aqueous extract of *G. kola* was undertaken to survey its potential as an agent in the management of sickle cell disease.

Our results (Table 10) on the activity levels of the different enzymes in the three genotypes showed that for Na^+, K^+-ATPase and Ca^{2+}-ATPase, the activity trend was HbAA>HbAS>HbSS while for Mg^{2+}-ATPase, the trend was HbSS>HbAS>HbAA. The extract increased the activity of Na^+, K^+-ATPase and Ca^{2+}-ATPase in the three genotypes with the trend of HbSS>HbAS>HbAA. Phenylalanine showed similar pattern. In contrast, both the extract and phenylalanine decreased the activity of Mg^{2+}-ATPase in all the genotypes with the trend of HbSS>HbAS>HbAA. The observed similarities inaction between the extract and phenylalanine, a known anti-sickling agent provides support for the possible use of the extract for management of sickle cell disease (Elekwa *et al*., 2003b).

TABLE 10: ***Effect of different concentrations (w/v) of aqueous extract of G. Kola seeds on the activity of erythrocyte 'ghost' membrane (A) Na^+, K^+-ATPase, (B) Ca^{2+}-ATPase and (C) Mg^{2+}-ATPase activities (mean ± SD)***

Extract conc. (g/ml)	*Enzyme activity (µmoles Pi/mg protein/hr x 10^{-3})*		
A:	HbAA (n = 5)	HbAS (n = 5)	HbSS (n = 5)
0.00 (basal)	274.5 ± 2.1	256.3 ± 4.5	132.6 ± 1.3
0.20	310.2 ± 1.5	279.8 ± 3.8	200.3 ± 1.9
0.40	349.8 ± 0.9	310.3 ± 4.1	261.2 ± 2.4
0.80	360.2 ± 2.3	330.1 ± 1.5	270.2 ± 1.5
1.00	360.2 ± 2.3	330.1 ± 1.5	275.7 ± 1.8
B:			
0.0 (basal)	177.6 ± 1.2	257.2 ± 0.9	293.6 ± 3.1
0.20	229.5 ± 1.4	300.1 ± 1.3	350.1 ±1.8
0.40	270.1 ± 1.5	349.8 ± 2.5	389.7 ± 2.3
0.80	300.2 ± 2.3	380.2 ± 1.7	425.1 ± 1.5
1.00	302.3 ± 1.7	390.1 ± 0.8	430.3 ± 0.9
C:			
0.0 (basal)	103.5 ± 2.3	144.2 ± 3.5	216.1 ± 1.1
0.20	102.3 ± 1.8	143.1 ± 3.4	180.3 ± 1.4
0.40	101.4 ± 2.1	140.5 ± 2.8	129.8 ± 1.7
0.80	100.2 ± 2.4	139.2 ± 1.8	125.1 ± 1.9
1.00	95.1 ± 1.5	139.3 ± 1.8	125.7 ± 1.8

Studies on sodium benzoate effect on plasma aspartate amino transferase and alkaline phosphatase of wistar albino rats

A number of preservatives are applied in many products consumed by humans and sodium benzoate which is used at optimum concentration of 0.1 % in preserving products like 'soft' and fruit drinks, margarine and some fish products (Srour, 1988). The allowable upper limits vary in different parts of the world. The implication of the use of this preservative is that it could be assimilated widely by consuming a wide range of food products. While several studies have been done to ascertain short- and long-term effects of consuming products preserved with sodium benzoate, most have investigated organ disposition as well as clinical parameters of experimental animals and human subjects (Toth, 1984; Fujitani, 1993; Fillet and Leonard, 1998).

We therefore decided to investigate the effect of short-term oral administration of defined doses well below the 'safe' limits on the plasma levels of the two enzymes known to be markers of biochemical status of the liver of wistar albino rats. Our findings (Table 11).

TABLE 11: Plasma levels of (a) AST and (b) Alkaline phosphatase (IU/l) following administration of sodium benzoate

Conc. (mg/kg)	*0-day*	*2nd day*	*6th day*	*14th day*
(a) Control	27.74±0.95	27.79±1.25	27.77±1.04	27.78±1.02
30	27.75±1.06	29.75±1.96	30.80±1.13*	37.50±0.70*
60	27.70±0.98	30.50±0.70	33.25±0.35*	38.75±0.53*
120	27.80±0.98	27.26±0.35	30.25±0.45*	36.75±1.05*
(b) Control	114.60±0.40	114.85±0.38	114.77±0.44	114.79±0.42
30	114.75±0.35	115.25±0.36	118.75±0.55[b]	129.75±0.45[b]
60	114.80±0.56	115.25±0.35	119.01±0.70[b]	129.75±0.45[b]
120	114.80±0.70	113.80±0.28	120.70±0.98[b]	129.80±0.28[b]

Values are mean±SD of triplicates and those with * are considered significantly ($p<0.05$) different from each other and control.

showed increased levels of the two enzymes in the plasma, suggestive of possible damages to the liver of the experimental animals. It is remarkable that routinely, the determination of plasma/serum levels of AST and ALP are applied in clinical diagnosis, hence our findings point to cautionary actions when interpreting blood chemistry data, especially as samples drawn from individuals who may have consumed sodium benzoate-containing foods before sample collection could affect the outcome of the determination (Ibekwe *et al*., 2007).

Investigations on the effect of oral administration of ether extract of Cannabis sativa on some liver function enzymes of albino rats

Cannabis sativa (also known as marijuana or Indian hemp in many quarters) contains a unique class of molecular compounds called cannabinoids (Liu *et al*., 2000). The famous route of abuse of *C. sativa* is through inhalation followed by oral ingestion. The molecular mechanism of action of the active ingredient, tetrathydrocannabinoid (THC) has been shown include direct vasodilation and activation of vascular cannabinoid receptors (Gebremedhin et al, 1999). There have been increasing reports of the abusive use of *C. sativa* in many

countries and regions including parts of Nigeria, where even persons of school age have admitted having made use of this plant in various ways. It was thus considered necessary to investigate the effect of the ether-extract, which contains the active principles, on plasma levels of Aspartate and Alanine transaminases (AST and ALT, respectively), liver marker enzymes, to ascertain what impact the extract could have on the experimental animals.

The results (Table 12) indicated increases in the plasma levels of AST and ALT at the different concentrations of the extracts, suggesting negative impact on the liver following ingestion of the extracts (Monago and Monanu, 2007).

TABLE 12. Effects of oral ingestion of ether-extract of C. sativa (Indian hemp) on serum level of liver function enzymes of wistar albino rats

Conc. (mg/kg body wt)	**AST (U/l)**	**ALT (U/l)**	**ALP (U/l)**
0.00 (control)	61.70±1.40	57.50±1.70	56.30±8.50
2.50	63.80±10.90*	58.40±10.10*	59.20±6.10*
5.00	62.60±14.40*	59.20±13.30*	58.80±7.30*
7.50	62.40±5.30*	59.80±5.40*	59.60±6.90*

Values are mean ± SEM of triplicate determinations while * indicates significant ($p<0.05$) difference when compared with control.

Studies on aqueous extract of African mistletoe (Tapinanthus bangwesis)

The use of plant extracts in management of ailments in human health care has continued to gain significance, particularly in developing countries where over 80 % of the world population have been shown to rely on traditional medicines (WHO, 1993). Promising returns have been documented for applications of numerous plant materials (roots, barks and leaves) in the treatment of various ailments (Soforuwa, 1975; Ekeke and Shode, 1990, Gupta, 1994, Michel, 2002, Elekwa *et al*., 2005a & b, Wang *et al*., 2007). African mistletoe (*T. bangwesis*) is the common genera of the hemi-parasitic angiosperm found in West Africa (Onofeghara, 1971) and several medicinal applications have been ascribed to extracts of this plant (Obatomi *et al*., 1994; Anthony *et al*.,

1997; Stein and Berg, 1998); Vehemeyer *et al*., 1998). It had been shown that a single administration of carbon tetrachloride (CCl_4), a hepatotoxin, could produce damages to the liver of experimental animals (Anand *et al*., 1992). There had been reports of protective effects of extracts from plants against CCl_4-induced liver damage (Dahiru *et al*., 2005; Obi *et al*., 2005), and we sought to experiment on the efficacy or otherwise of the aqueous extract of T. bangwesis in this process by determining its effect on serum levels of AST, ALT and ALP in wistar albino rats.

The findings (Table 13) showed increased serum levels of the three enzymes and this was reduced when extracts of T. bangwesis were administered orally on a daily basis after CCl_4-induced liver damage (Table). At high concentration of the extract, the expected increase in effect was not seen suggesting reduced 'efficacy' in restoring the damaged tissues and harmful effects, a finding earlier reported (Spiller *et al*., 1996; Alessi *et al*., 2003). Histological investigations of the liver showed results that correlated with the enzyme level determinations. The observation of ameliorating effect on the CCl_4-induced damage to the liver suggests possible therapeutic role for this plant extract for damaged liver (Omeodu *et al*., 2008).

TABLE 13. Serum (a) AST, (b) ALT and (c) ALP activities (U/l) in rats following CCl_4-induced liver damage and administration of aqueous extract of T. bangwesis.

Treatment	*24hrs post CCl_4*	*7-days post CCl_4*	*14-days post CCl_4*	*21-days post CCl_4*
(a) Control	115.64±2.54	117.42±2.99	116.35±1.25	118.43±3.01
CCl_4	209.32±3.72*	182.37±3.00*	163.47±22.89*	130.40±22.09*
12.5 mg	ND	165.71±3.21^{y}	144.49±2.98^{y}	125.48±2.43^{y}
25.0 mg	ND	151.95±2.87^{y}	139.49±2.79^{y}	121.48±2.79^{y}
37.5 mg	ND	144.48±2.79^{y}	130.42±3.01^{y}	119.28±2.45^{y}
(b) Control	38.47±0.95	39.37±1.00	38.79±0.97	38.65±0.99
CCl_4	104.27±1.45*	94.81±1.79*	83.37±2.00*	79.48±2.01*
12.5 mg	ND	84.79±1.99^{y}	78.27±1.85^{y}	69.25±1.99^{y}
25.0 mg	ND	78.87±2.00^{y}	67.40±2.98^{y}	48.98±2.62^{y}
37.5 mg	ND	52.17±2.01^{y}	48.78±2.00^{y}	40.99±1.48^{y}

(c) Control	187.48±5.21	189.40±4.50	188.80±4.55	187.99±4.87
CCl_4	402.93±8.42*	394.49±8.99*	362.00±9.00*	340.91±8.96*
12.5 mg	ND	367.88±9.10 ^{y}	320.77±8.95 ^{y}	299.78±9.45 ^{y}
25.0 mg	ND	340.75±7.98 ^{y}	310.71±9.02 ^{y}	240.02±9.87 ^{y}
37.5 mg	ND	300.47±7.98 ^{y}	270.78±8.15 ^{y}	220.50±8.01 ^{y}

Values in the column with * are significantly different ($p<0.05$) from the control, while superscript y denote values significantly different ($p<0.05$) from the control and CCl_4-treated. ND: not determined.

Aqueous extract of Carica papaya leaves on liver enzymes

Another plant product that has been applied in trado-medical practice is *Carica papaya*, as various parts (including the leaves) have been reported to be applied to treatment of varied ailments as malaria, dysentery, diarrhoea, and typhoid in various parts of Africa, including Nigeria and Ghana (Agoha, 1981; Morton, 1987). The antimicrobial activity had also been reported (Trease and Evans, 1989). It was therefore considered to investigate possible side effects of aqueous extracts of *C. papaya* and biochemical parameters, including liver enzymes with a view to providing information on the 'safety' or otherwise of such application.

The findings on the activity levels of plasma levels of AST and ALT showed no significant changes in the enzymes levels after 14-days oral administrations of the extracts to wistar albino rats (Table), supporting the view that the application of the extract at the dosage investigated had no adverse effects of the liver of the experimental animals (Nwiloh *et al*., 2009).

TABLE 14. The effects of aqueous extract of C. papaya on serum AST and ALT of wistar albino rats

Treatment	***Days***	***AST (U/l)***	***ALT (U/l)***
Control	7	24.07±2.82	41.57±3.21
	14	24.05±4.80	41.00±3.00
2 ml extract (0.11 g/ml)	7	20.62±1.15	36.02±3.74
	14	20.93±4.24	35.56±7.61

n = 5; AST: aspartate amino transferase, ALT: alanine amino transferase

TABLE 15. The effects of aqueous extract of C. papaya on WBC, lymphocytes, neutrophils and thrombocytes of wistar albino rats

Treatment	*Days*	*WBC* (x 10^9/l)	*Lymphocytes* (%)	*Neutrophils* (x 10^6/µl)	*Thrombocytes* (x 10^5/mm^3)
Control	7	2.50±0.3	58.1±4.34	1.025±7.06	1.20±0.37
	14	2.60±1.7	59.0±0.00	1.061±0.02	1.22±0.00
2 ml extract (0.11 g/ml)	7	3.00±1.7	64.4±3.62	1.041±9.12	1.35±0.46
	14	3.90±1.1	69.7±4.40	1.197±11.2	1.90±0.18

n = 5, WBC (white blood cell)

In vivo studies on Yaji (a complex Nigerian meat sauce containing clove, ginger, garlic and red pepper).

Yagi, a sauce for meat delicacy commonly called 'suya', consists of a complex mixture of groundnut cake powder, additives, spices (cloves, ginger, garlic, red/black pepper), salt and 'maggie cubes'. The delicacy is widely consumed in many parts of Nigeria and beyond. Studies had indicated that excessive consumption of Yaji-spices, especially in combination, was capable of inducing pancreatic, liver and kidney damage (Nwaopara *et al*., 2004, 2008). It was therefore considered relevant to study the effects of the mixture on several biochemical parameters of experimental animals with a view to ascertaining safe use or otherwise of Yaji meat sauce. Pre-determined amounts of the meat sauce were compounded in the diet fed to male wistar albino rats of average weight of 74 g. The feeding was for a period of 21 days and blood samples collected at 7-day interval. The levels of plasma AST, ALT and ALP, known markers of the liver cell, were determined.

Our findings (Table 16) showed that there was no significant change in plasma levels of AST, ALT and ALP, at the adjusted concentration of 4 g spices for a 70 kg adult human (Nwaopara *et al*., 2009). This finding suggests no adverse effect of the meat sauce over the period of the experiment (Okoro *et al*., 2014).

TABLE 16. Activity levels of serum AST, ALT and ALP (IU/l) following intake of different combinations of spices by wistar albino rats (values are mean ± SD)

Group	*Day*	*AST*	*ALT*	*ALP*
	0	12.00±1.41	10.00±1.41	9.35±4.12
A	14	11.00±2.83	9.50±2.12	9.20±0.00
	21	10.00±1.41	9.00±1.41	9.20±0.44
B	14	10.50±4.95	10.00±2.83	9.35±0.65
	21	8.50±0.71	13.00±0.00	11.04±0.44
C	14	11.50±2.12	11.50±0.71	10.27±0.66
	21	8.50±0.71	9.00±2.83	9.97±1.09
D	14	13.00±2.83	10.00±1.41	9.97±1.07
	21	8.50±0.71	8.50±0.71	9.97±0.21
E	14	11.50±2.12	9.50±2.12	10.43±0.44
	21	10.00±4.24	9.50±2.02	9.35±0.21
F	14	11.00±0.00	9.00±0.00	9.66±1.53
	21	10.00±0.00	8.50±0.71	8.58±0.00
G	14	10.00±0.00	10.50±0.71	9.66±1.53
	21	9.50±2.12	8.00±0.00	7.98±0.42

A: Control, B: mixture of clove, ginger, garlic and red pepper, C: yaji, D: clove, E: ginger, F: garlic,
G: red pepper

Studies on some soil biomarkers enzymes following induced remediation of crude oil impacted soil

The contamination of the environment by crude oil and its product has posed serious problem worldwide, and happening is well felt in the Niger-Delta region of Nigeria. Many of the current technologies for remediating crude oil impacted areas include physical, chemical and biological approaches, most of which are either costly or do not completely remove the contaminants (Ting *et al*., 1999; Bachoon *et al*., 2001). The biological methods are gaining ground due to the environmentally friendly nature (Balba *et al*., 1998). Bioremediation employs various materials like bacteria, fungi, alga and plants (Leung, 2004). A large number of enzymes from bacteria, fungi and plants have been shown to facilitate the biodegradation of toxic organic pollutants. The determination of levels of soil enzymes have shown promise as indicators of environmental quality, and have been applied in national and international monitoring programs for crude oil contaminations.

It was considered useful to evaluate the effects of induced remediation of crude oil impacted soils on three key soil enzymes (soil dehydrogenase, peroxidise and phenol oxidase) with a view to evaluating the efficiency of the bioremediation facilitated by the inherent microorganism in the soil. Remediation was induced by biostimulation as well as treatment with enzyme additive and a chemical surfactant.

The results (Table 17-20) revealed varying response of soil dehydrogenase to the additives. While significant increase was observed for SDS-, uric acid- and enzyme-additive treated reaction vessels, significant inhibition was seen for control soil samples (no additive). Soil peroxidase activity was enhanced by the various additives, while phenol oxidase showed patterns similar to soil dehydrogenase. The conclusion of our study was that adverse impact on the enzymatic activities of the three enzymes, an effect overcome by enhance bioremediation initiated by improved growth of microbial organisms with time of remediation, provided a potential monitoring tool for assessing bioremediation (Okoro et al., 2014).

TABLE 17. Experimental subgroups (A = 20 g impacted soil + 7 kg agricultural soil)

Group Name	***Subgroups***		
	I	II	III
SAC	**A** + 200g SDS in 5L water (4% w/v)	**A** + 400g SDS in 5 L water (8% w/v)	**A** + 600g SDS in 5l L water (12 %w/v)
NAD	**A** + 40g Uric acid in 200 ml water (10% w/v)	**A** + 60 g Uric acid in 200 ml water (15% w/v)	**A** + 80 g Uric acid in 200 ml water (20% w/v)
ENZ	**A** + 0.5 L enzyme additive in 1 L river water	**A** + 0.8 L enzyme additive in 1 L river water	**A**+1 L enzyme additive in 1l L river water
SEN	**A** + 0.5 L enzyme additive in 1 L river water+ [40g uric acid + 200g SDS] in 5 L water	**A** + 0.8 L enzyme additive in 1 L river water + [60g uric acid + 400g SDS] in 5 L water	**A**+1 L enzyme additive in 1l L river water + [80g uric acid + 600g SDS] in 5l L water
GS-S	**A**		
S-S	20g impacted soil only		

TABLE 18. Effects of various treatments on soil dehydrogenase activity (µg TPF/g soil)

Groups	*Day 0*	*Week 1*	*Week 5*	*Week 9*
SAC	SAC-I	22.06±1.91	15.26±3.4*	21.93±2.40
	SAC-II	23.28±2.25	19.13±3.02	23.90±2.99
	SAC-III	14.77±1.45	17.83±1.89	21.03±2.78*
NAD	NAD-I	54.67±3.39	52.27±6.04	41.13±2.82*
	NAD-II	52.29±2.64	42.40±3.10*	51.07±5.42
	NAD-III	44.17±4.42	56.27±3.95*	55.47±6.01*
ENZ	ENZ-I	81.42±6.66	88.69±7.22	79.49±4.64
	ENZ-II	78.58±2.94	90.32±6.25*	80.24±3.20
	ENZ-III	81.73±3.92	80.33±3.07	92.53±2.89*
SEN	SEN-I	73.73±7.06	81.56±4.49	87.71±4.08
	SEN-II	73.27±7.06	88.33±3.09*	81.64±5.35
	SEN-III	74.07±5.55	89.33±4.018	82.47±4.91
GS-S	4.10±0.89	10.17±5.07	12.73±2.49*	12.77±1.69*
S-S	2.23±0.40	2.30±0.53	2.93±1.02	2.67±1.07

Values are mean ± SD and * denotes significantly different ($p<0.05$) from Day0/Week 1 values for control & experimental groups respectively

TABLE 19. Effects of various treatments on soil peroxidase activity (µmol/hr/g)

Groups	*Day 0*	*Week 1*	*Week 5*	*Week 9*
SAC	SAC-I	4.46±1.25	7.37±0.27*	4.95±0.21
	SAC-II	5.46±0.48	7.93±0.20*	5.26±0.82
	SAC-III	4.61±0.27	5.11±0.26	4.19±0.91
NAD	NAD-I	4.54±1.09	5.57±1.30	4.43±0.56
	NAD-II	5.31±0.88	4.36±0.69	4.80±0.83
	NAD-III	6.48±1.08	5.57±0.21	5.30±0.82
ENZ	ENZ-I	4.87±0.30	4.37±0.29	5.43±0.64
	ENZ-II	3.73±0.38	5.52±0.66*	5.70±0.23*
	ENZ-III	5.87±0.52	5.63±0.75	5.92±0.01
SEN	SEN-I	6.87±0.87	5.58±0.77	4.53±1.41
	SEN-II	5.26±1.16	4.07±0.56	7.91±0.24*
	SEN-III	9.50±0.21	7.56±1.18*	6.50±0.20*
GS-S	1.90±0.44	3.51±0.21*	1.87±0.71	2.27±0.27
S-S	0.39±0.06	0.50±0.04	0.59±0.04*	0.69±0.05*

Values are mean ± SD and * denotes significantly different ($p<0.05$) from Day0/Week 1 values for control & experimental groups respectively

TABLE 20. Effects of various treatments on phenol oxidase activity (µmol/hr/g)

Groups	*Day0*	*Week 1*	*Week 5*	*Week 9*
SAC	SAC-I	2.97±0.24	3.97±0.19*	3.20±0.49
	SAC-II	5.87±0.55	7.13±0.93	6.58±0.22
	SAC-III	2.91±0.58	3.89±0.69	3.20±0.10
NAD	NAD-I	10.50±0.28	7.63±0.23*	7.21±0.33*
	NAD-II	8.97±0.22	8.63±0.16	7.53±0.77*
	NAD-III	7.53±0.34	6.83±0.43	6.62±0.39
ENZ	ENZ-I	5.24±0.19	6.45±0.30	6.84±0.86
	ENZ-II	5.24±0.20	5.81±0.47	7.06±0.70*
	ENZ-III	5.40±0.18	6.71±0.63*	7.27±0.28*
SEN	SEN-I	9.03±0.17	3.61±0.22*	2.47±0.58*
	SEN-II	10.60±0.62	4.40±0.11*	3.98±0.20*
	SEN-III	10.23±0.25	3.79±0.33*	4.16±0.12*
GS-S	2.33±0.51	1.87±0.14	2.29±0.90	1.89±0.07
S-S	0.90±0.03	0.57±0.16	0.83±0.25	0.70±0.04

Values are mean ± SD and * denotes significantly different ($p<0.05$) from Day0/Week 1 values for control & experimental groups respectively

SIGNIFICANCE OF CONTRIBUTIONS

A cursory look at the studies described would consider them as unrelated investigation of different enzymes. However, a more in-depth consideration will reveal an interwoven relationship of the studies. The studies carried out on the regulation of glycogen phosphorylase *a* phosphatase activity gave a clue to the possible physiological role of the nucleoside-binding site, probed by caffeine, in the overall control of glycogen metabolism. While the natural effector molecule was not identified, it remained an important contribution to the vital biochemical event central to glucose homeostasis.

The subsequent thrust in research provided molecular clues as to the various biochemical events that are associated with the medical conditions which the different plant products were being employed in ameliorating the lesions caused by the defects in the make-up of principal players in the lesions. For instance, by revealing the differences in the activity level of the glutathione-S-transferase, diaphorases, AST, ALT and ALP, of human erythrocytes of different genotypes, the studies provided avenue for exploitation of different

palliative actions that would provide relief to sicklers in crisis. This paved the way for the studies that investigated effects of the extracts of plant products on the various enzymes researched on. The second group of research related to the use of enzyme markers to assess the impact (positive or negative) of various constituents of food consumed by humans on the liver, a key metabolic organ in the human body. Also relevant is the need to assess the safety of the different herbal remedies presently gaining ground in our environment as curative agents for numerous medical conditions known to plague the human population.
Finally, the foray into investigation of enzyme activities in bioremediation portrays the need to consider making impact in the immediate locality of the University of Port Harcourt, being situated in the Niger Delta of Nigeria where crude oil exploration activities have produced the unfortunate negative impact caused by oil spills and attendant devastation of the oil producing lands.

RECOMMENDATIONS/CONCLUSION

From the discuss, I believe that the array of information presented had convinced us that indeed these biological 'wonder' macromolecules deserve our praise! It has been estimated that global market for industrial and medical enzymes respectively stood at US$3.3 billion and US$3.3 billion in 2010, projected to reach US$4 billion and US$7.2 billion in 2015. These 'wonder' macromolecules have played vital roles not only as analytical tools but as biosensors for medical applications.

On future research point of view, there are numerous frontiers yet to be delved in. For instance, the large array of concussions that we see being paraded as wonder drugs with numerous claims of what ailments they can cure need to be investigated with a view to determining their efficacy and safety. This, no doubt, would require inter-disciplinary efforts by such researchers as biochemists (enzymologists) pharmacists, clinicians, botanists, chemists, to mention but a few. Indeed the concept of reverse-pharmacology is relevant here.

However, for meaningful progress there should be steady and consistent effort at providing the enabling environment that would ensure adequate electricity supply to maintain the fine biochemicals and other materials employed in such fine biochemical investigations. There is need for

concerted effort at providing relevant equipment and conditions that will facilitate the 'feel' of students of enzymology since present conditions leave much to talk of. In fact, a good number of graduates in the field of Biochemistry and related fields do not demonstrate any level of understanding of the role, character and practical applications of enzymes. A major difficulty is lack of adequate equipment and reagents for enzyme research. While some efforts have been made inthis University by centralizing equipment through the Central Instruments Laboratory of the University, more input is required to achieve meaningful results. The practice in developed world is for joint ventures by related disciplines in acquisition of equipment of common use since independent units cannot afford to have all that is required for excellent research activities.

Furthermore, I join those who advocate that the system of academic evaluation of multiple authorship publications should be reviewed such that academics are not disadvantaged in their assessment scores since it is clear that multi-disciplinary research has come to be a trend that provide more meaningful outcome to result-oriented research.

With the great economic potential of industrial and medical enzymes in global market, if the environment is improved significantly, Nigerian enzymologist can key into the market with the obvious implications for improved earnings to the country. There are a lot of requirements for these enzymes in our industrial and medical practice. It is however regrettable that the increasing demand and usage of enzymes in industrial and medical practice in Nigeria and Africa is largely skewed in favour of foreign supply. It is hoped that this trend will be reversed in the near future when cogent steps are taken to encourage our scientist in developing this highly profitable venture in science development.

Mr. Vice Chancellor, Sir, and this wonderful audience, I rest my case for the praise of enzymes:

Thank you for listening.

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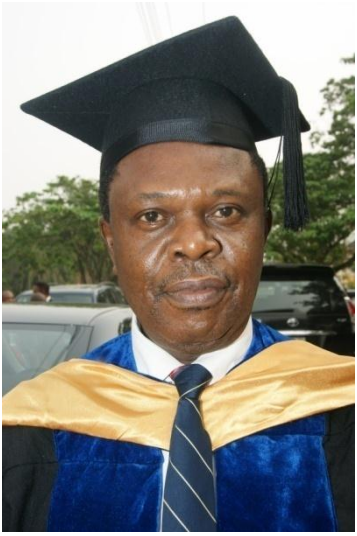

PROFESSOR MICHAEL OKECHUKWU MONANU
B. Sc. (Nigeria), Ph. D. (Alberta)

Born to Late Pa Rufus Onyemaechi and Late Ma Paulina Uzoamaka (nee Okoli) of Igbo-Ukwu town in Aguata LGA of Anambra State on September 29, 1955, Professor Michael Okechukwu Monanu demonstrated quite early in life his academic prowess. He had accelerated promotions in the Primary school (St. Paul's College Practising School, Awka, Anambra State), ending with a credit pass at the 1st School leaving certificate exams in 1966. Professor Monanu subsequently gained admission into Class 1 at the prestigious Government Secondary School, Owerri, Imo State in 1967 but was disrupted by the Nigeria civil war. At the end of the war, he returned to Owerri but had the WASC examinations of Owerri zone cancelled in June of 1973. Prof. Monanu enrolled for a retake of WASC at Agulu Grammar School, Agulu, Anambra State and thus completed his secondary education with Grade 1 (distinction, aggregate 10) in 1974.

Soon after, he was admitted into the University of Nigeria, Nsukka (UNN) to read Biochemistry. Professor Monanu was a Federal Government Scholar for 3 of the 4-year programme and obtained a Second Class (Upper Division) honour degree in June of 1978. Following the NYSC service at Lafia of the then Plateau State, he had a brief postgraduate studies at UNN before securing a Graduate Teaching Assistantship at the University of Alberta, Edmonton, Canada in September 1980 to pursue an M. Sc. degree. During the preliminary stages of the programme he excelled in the course work hence his supervisor, Dr. Neil B. Madsen, a renowned Enzymologist convinced him to go for a straight Ph. D. programme. While on this, Professor Monanu was awarded an Alberta Heritage Trust Fund Fellowship for Medical Research, which provided stipends/research grants that funded his postgraduate training. Prof. Monanu successfully bagged the Ph. D. degree in August, 1986. He was the Secretary of the Nigerian Union of Student, U of A (1982 to 1983).

His quest to make contributions to stem the brain-drain phenomenon in Nigeria, which was at its infant stage in the '80s made Professor Monanu to apply for employment to Nigerian Universities, and was attracted to the University of Port Harcourt, by his lecturer at UNN, Professor Emeritus E. O. Anosike, the then HOD. In fact, ABU, Zaria had almost completed Prof. Monanu employment before the offer from UNIPORT came. Since joining this University as Lecturer II in 1986, Professor Monanu has made meaningful contributions to the growth of the discipline, the Department and the University at large, in many ways, and was promoted to Professor of Enzymology/Protein Chemistry in 2009.

Professor Monanu served as the General Secretary, Biochemical Society of Nigeria (BSN) (1988-1990), Financial Secretary, BSN (1990 - 1994); was an International Union of Biochemistry Travel Fellow (Jerusalem, 1990), Acting HOD (1994-1996), Chairman University Time Table Committee (1994 to 1996); member of several Faculty and University-wide Committees such as Business Manager, Scientia Africana (publication of Faculty of Science, Uniport, 1994 to 1997), University Exams Committee (2008 to 2012), Senate Degree Results

Verification Committee (2004 to 2013), 19th and 20th Convocation Committee (2002 and 2003), and Chairman, Certificate Verification Committee (2010 to date). He was the pioneer Dean, Faculty of Chemical Sciences of the College of Natural & Applied Sciences (2013-2015). He has served as External Examiner (Undergraduate & Postgraduate) for UNIZIK, ABSU, UNICAL, MOUA, NDU and FUTO at various times. Prof. Monanu had served ASUU, Uniport branch as Treasurer and Financial Secretary (1987 to 1994), as Member (2007 to 2010) and the Vice President (2010 to date) of Management Committee, ASUU Cooperative Society. Professor Monanu has taught many post graduate students of which over seven are now Professors of Biochemistry.

Professor Monanu is a Knight of St. Christopher of the Anglican Communion of Nigeria, and is happily married to Dr. (Mrs.) Nkechinyere (nee Aguta). They are blessed with three children (Okechukwu and Chukwudi, both Civil Engineers from UNIPORT, and Chinenye, a 2nd year undergraduate of Geology, UNIPORT). My Vice - Chancellor Sir and distinguished ladies and gentlemen please permit me to introduce an icon of many feathers, an academic per excellence. I present to you Professor Michael O Monanu as our 124th Inaugural Lecturer for the day.

Professor A. A. Uwakwe